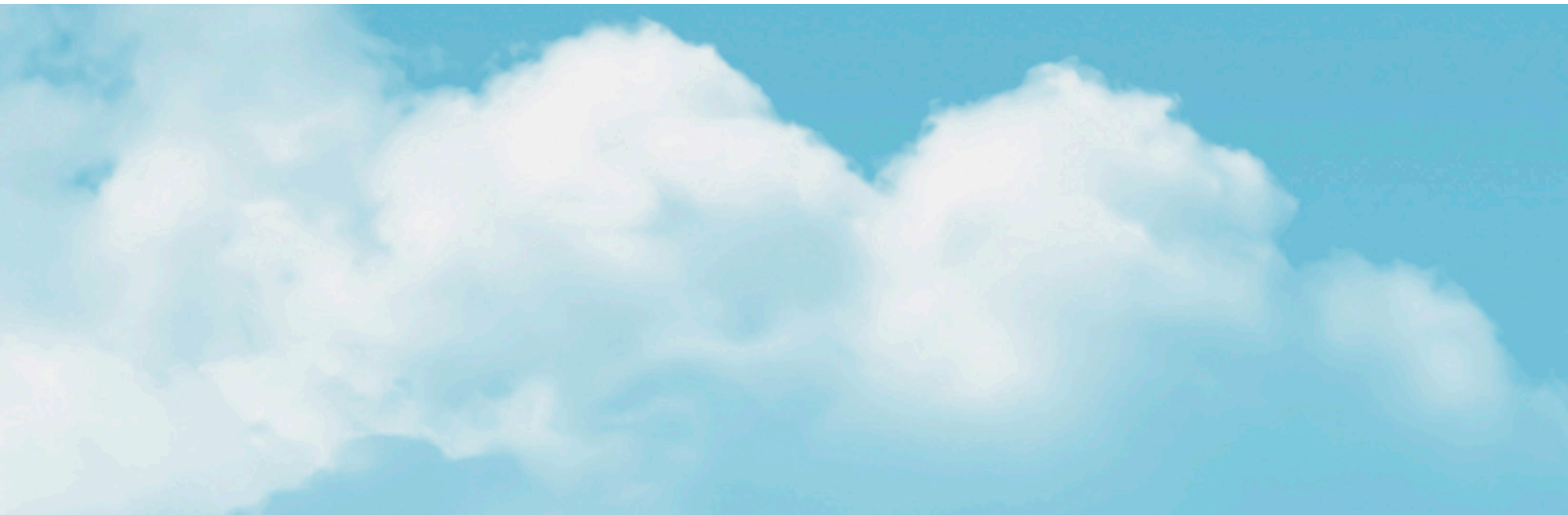
SPANISH NATIONAL CANCER RESEARCH CENTRE, CNIO

SCIENTIFIC REPORT 2012

cnio Centro Nacional de Investigaciones Oncológicas



CNIO SCIENTIFIC REPORT 2012

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Centro Nacional de Investigaciones Oncológicas

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“DURING 2012, THE CNIO HAS SUCCESSFULLY GENERATED NOVEL IDEAS AND ACTIVITIES THAT — AS I STRONGLY BELIEVE — WILL SHAPE THE CENTRE FOR THE NEXT DECADE.”

MARIA A. BLASCO DIRECTOR

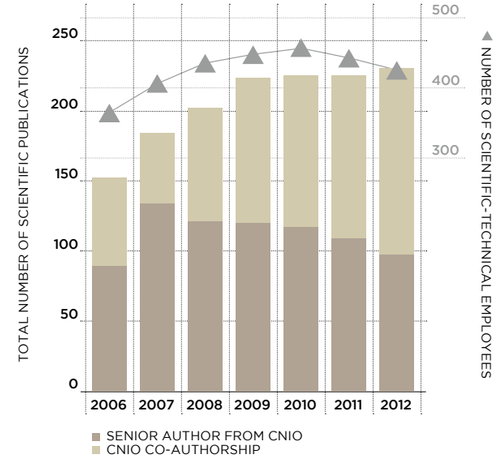
FOREWORD

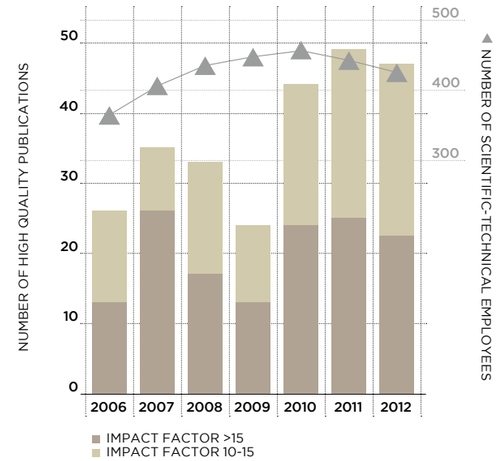
MARIA A. BLASCO DIRECTOR

In every living system, there is much to improve and optimise, but it is in these times of change when improvements should take place. Thus, in a year of economic constraints for Spanish science, and consequently, for the CNIO, I think it is fair to say that the CNIO has successfully faced this challenge by generating novel ideas and activities that – as I strongly believe – will shape the Centre for the next decade. Creativity, optimisation of resources, solidarity, sustainability, and opportunity have been key words for the CNIO during 2012. All of them operating under the umbrella of scientific excellence, and without forgetting that our ultimate goal is to foster translation of scientific breakthroughs into novel and more effective ways to prevent, diagnose and treat cancer.

During 2012, the CNIO has experienced an unprecedented activity in the five strategic areas of the Centre, which include Basic and Translational Research, Innovation, Training and Education, and Communication. 2012 has also been the year for launching exceptional social activities at the CNIO, such as the creation of the **Dean's Office for Academic Affairs**, with María S. Soengas as the Dean, and of the **WISE CNIO Office** (Women in Science Office at CNIO), headed by Mirna Pérez-Moreno and Marisol Quintero. Both activities have been made possible thanks to the volunteer work of CNIO students and staff, showing that – in particular, in times of economic restrictions – CNIO staff is fully committed to improve scientific life at the Centre.

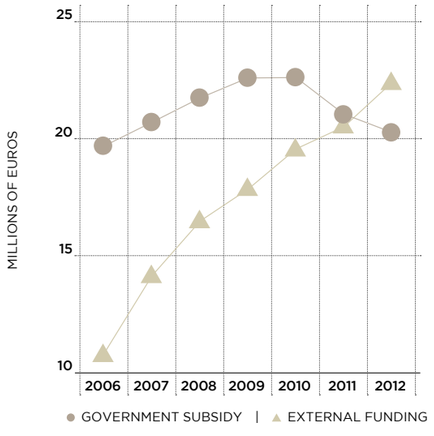
Let us begin with CNIO's scientific performance during 2012. This past year, the CNIO published a total of 229 papers, 47 of which were published in journals with impact factors in the range of 10 to 15 and >15. After initial fluctuations (2006-2009) in the number of high-quality publications per year, from 2010 until now, we have consolidated a top-notch scientific production that could be maintained throughout 2012 despite a 6.3% reduction in scientific personnel, when compared to 2010.





This scenario – maintaining scientific excellence with fewer resources – is indicative that in recent years, the excellence of our researchers is growing steadily. In fact, some of the publications that were built on the participation of CNIO scientists have been considered among the best of 2012. We are particularly proud of scientists from our **Structural Biology and Biocomputing Programme** who have participated in the ENCODE Project, which is considered one of the top 10 medical breakthroughs of the year by ***Time*** magazine. Moreover, a published study on personalised 'OMICs', with the participation of CNIO scientists, was also included among *The Best of 2012* in the journal ***Cell***.

Closely linked to scientific performance is the ability of the Centre to secure external funding. We have successfully managed to increase our external funding by 14.4% as compared to 2010, facing at the same time a 10.4% cut in government subsidies. This year, for the first time in the history of the CNIO, external funding through competitive grants, patronage and scientific services exceeds the annual subsidy we receive from the government.



An indicator of our competitiveness is our capacity to create new positions for junior group leaders in key strategic areas for the CNIO. During 2012, a year characterised by severe government restrictions on the hiring of new staff at the expense of public funds, the CNIO received substantial donations from the **Seve Ballesteros Foundation**, the ***CRIS* Foundation Against Cancer**, and the **Spanish Association Against Cancer (*AECC*)**; this has allowed us to bring two new Junior Groups to the CNIO. Last fall, Massimo Squatrito moved from the **Memorial Sloan-Kettering Cancer Center** in New York to the CNIO, in order to set up the new **Seve-Ballesteros Foundation Brain Tumour Group** in the **BBVA Foundation-CNIO Cancer Cell Biology Programme** directed by Erwin F. Wagner. Around the same time, David Olmos, from the **Institute of Cancer Research and The Royal Marsden NHS Foundation Trust** in the UK, established the new **Clinical Research Unit on Prostate Cancer and Genitourinary Tumours** thanks to the generous support from the *CRIS* Foundation and the *AECC*. Indeed, fundraising will continue being a priority for our future Communication and Innovation activities at the Centre in the years to come.

During 2012, after Keith Ashman's departure back to Australia, we recruited Javier Muñoz as new **Head of the Proteomics Unit**. Javier moved from the **Netherlands Proteomics Centre at Utrecht University** to the CNIO this past summer and he is already running our state-of-the-art proteomics core facility.

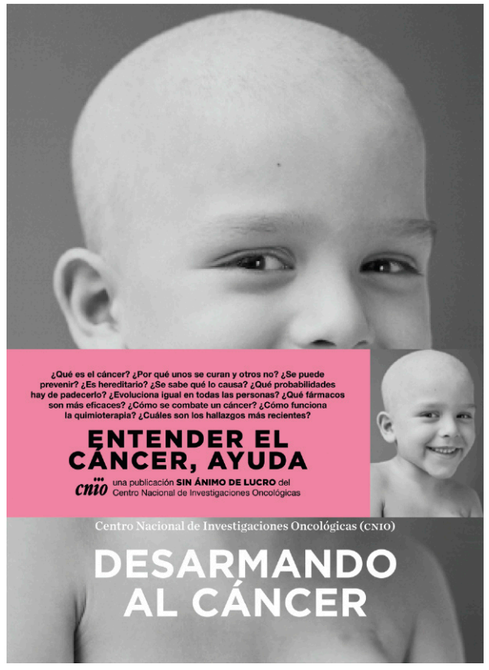
I am also very pleased to announce that we have just recently launched the **Familial Cancer Unit** at CNIO, headed by Miguel Urioste, in collaboration with the ***Hospital Universitario de Fuenlabrada***. In 2012, the Unit has offered genetic counselling to a total of 165 patients and performed more than 1,800 genetic tests.

In this regard, 2012 has been an outstanding year for the CNIO in terms of its translational research activities. In addition to the in-house Clinical Research Units of the **Clinical Cancer Research Programme** directed by Manuel Hidalgo, the CNIO has signed agreements to create **Associated Clinical Units** in four major public Hospitals of the **Regional Government of Madrid**, including our surrounding hospitals ***Hospital La Paz*** and ***Hospital Ramon y Cajal***. These new agreements – together with our previous activity at the ***Hospital Universitario de Fuenlabrada*** and the private ***Hospital de Madrid*** – have permitted the enrolment of more than 300 patients in PhaseI/II clinical trials led by CNIO oncologists. In addition, CNIO oncologists are coordinating two large, country-wide, Clinical Trials Networks in Breast and Prostate Cancer.

During 2012, the CNIO's Clinical Research Programme also launched the **CNIO Personalised Oncology Platform**, which has provided more than 30 patients with the opportunity to have their tumours sequenced and characterised at the molecular level. The platform counts with support from the recently created **Translational Bioinformatics Unit** under the leadership of Fátima Al-Shahrour, who moved to the CNIO a year ago from the **Broad Institute of MIT and Harvard** in Boston. An essential part of the platform is the generation of the so-called ***Avatar mice***, a mouse model based on xenografts derived from the patient's tumour, which – together with the genetic and molecular information obtained from the same patient – are used to decide the most optimised and promising treatment for each individual patient. A total of 14 patients with advanced tumours who had failed to respond to available standard therapies have already been treated using this platform. Eight of these patients responded positively, highlighting the enormous potential of this novel approach.

In this regard, I would like to point out what I consider the success of the Communication Office that we recently created over a year ago. The appearances of CNIO scientists and their discoveries in the media (in particular, on National TV stations) have increased by more than 90% when compared to previous years. Furthermore, recent results from **EurekAlert!**, the online, global news service operated by the **American Association for the Advancement of Science (AAAS)**, reveal that press releases from the CNIO received more than 45,000 visits since March of 2012; thus, majorly boosting CNIO's visibility amongst universities, medical centres, journals, governmental agencies, corporations and other organisations engaged in research all around the World. I would like to highlight the impact of a term coined at the CNIO in the international media, namely, the *Avatar mice* mentioned above. This term was created by our researchers to refer to mice that are implanted tumour biopsies from cancer patients. This allows the physician to test various treatment options in these mice, monitor the response of the tumour, and – based on this observation – to select the best-suited chemotherapeutic agent for a particular cancer patient (http://en.wikipedia.org/wiki/Mouse_avatars). ***The New York Times, The Mayo Clinic***, and ***Nature***, among others, have popularised this term during 2012. Furthermore, a year ago the CNIO created its own Twitter account, attracting more than 2,500 followers from all countries, a number that keeps growing day by day.

This brings me to one of the most pleasant experiences that we have accomplished during 2012 in terms of dissemination of our activities. We published, the book entitled ***Desarmando al Cáncer***, in which the outstanding science journalist Mónica G. Salomone narrates in a lay language what is cancer and what we do at the CNIO to improve our understanding of cancer, its prevention, diagnosis, and treatment. The book that is now for sale at all bookshops of the *Grupo VIPS* in Spain, and also on the web via our recently created CNIO Store (https://store.cnio.es), has become a great success in terms of scientific outreach and visibility, demonstrating our commitment as an institution to the fight against cancer through cancer research of excellence and innovation.



During 2012, the newly created Direction of Innovation has been working on three main strategic areas: consolidation of the **Experimental Therapeutics Programme**, establishment of new alliances with pharmaceutical companies, and fostering new entrepreneurial activities. The Experimental Therapeutics Programme has integrated to the Centre as an additional research programme and will work closely with some of our researchers to validate new therapeutic targets. The projects developed by the Programme in previous years have attracted the interest of several companies that would like to further develop them into clinical candidates. The final confirmation regarding the success of this strategy will come once one of our molecules enters a clinical trial. We believe we are on the right track, however, we will need more time to see the results. Collaborations with industrial partners have also been reinforced and the best example of this is represented by an agreement signed in June, through which the CNIO is now part of the **Roche's Extending Innovation Network**. This strategic collaboration will last from three to five years and both institutions will work together to translate research projects hosted at the CNIO into innovative ideas and products to improve diagnosis, prevention and treatment in the field of cancer. Our objective is to combine public and private funding resources to advance our best projects. The CNIO is the third institution in Europe to be part of this network.

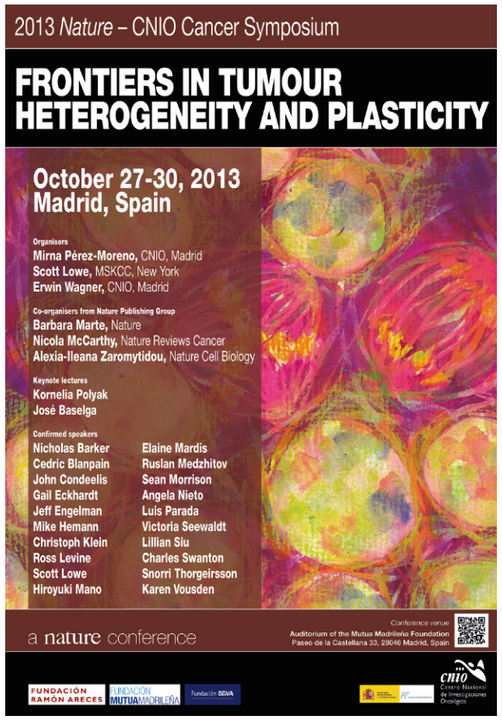
"Roche's Extending Innovation Network".

The programme, *'Managerial Skills for Scientists and Researchers – Linking Science to Business'*, designed in collaboration with the *Instituto de Empresa Business School* in Madrid (one of the world's leading business schools), is at the basis of our ongoing efforts to create an interdisciplinary post-doctoral programme that fills the gap between basic research and knowledge-driven innovation. To make the programme available to CNIO researchers over the next few years, we have applied to the EC for co-funding of Regional, National and International Programmes (COFUND, FP7 Marie Curie Actions – People). Our 2.5 million EUR *CNInnOtrain* proposal was selected amongst the top proposals; co-funding by the EC with 1.0 million EUR is currently under negotiation.

In 2013, we will celebrate the 10th anniversary of the official inauguration of the CNIO. For this occasion, our Vice-Director for Basic Research, Erwin F. Wagner – together with Mirna Pérez-Moreno from CNIO's **Epithelial Cell Biology Group** and Scott Lowe from the **Memorial Sloan-Kettering Cancer Centre in New York** – have been working hard this year to organise the ***Nature-CNIO Cancer Symposium*** on *Frontiers in Tumour Heterogeneity and Plasticity* that will take place in Madrid October 27-30, 2013. Keynote lectures by Kornelia Polyak and José Baselga, plenary talks by 20 of the world's leading experts, poster sessions and short talks, will set the scene for the exchange of novel ideas and discussions on emerging molecular mechanisms of tumour heterogeneity and its clinical implications.

To finish, I am happy to announce that we have a **new CNIO External Scientific Advisory Board** (SAB). In 2012, Joan Massagué, who was appointed as new Chair of the CNIO SAB by the CNIO Board of Trustees in June 2011, has led the renewal of its members. The strategy has been to keep a few historic members, those who have served on the Board for almost 10 years now, and to include new members, all of whom are experts in the different areas of research at the CNIO. I am particularly happy to see that the new SAB is now more gender balanced.

One more year, I would like to thank visual artist Amparo Garrido for the fabulous photographs of CNIO scientists that she has produced for this report. The subject (scientists) may not be very usual in art, but Amparo's work certainly is! ■



ORGANISATION OF RESEARCH

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ERWIN F. WAGNER VICE-DIRECTOR OF BASIC RESEARCH

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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

BBVA FOUNDATION-CNIO CANCER CELL BIOLOGY PROGRAMME

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Erwin F. Wagner
Genes, Development and Disease Group

Mirna Pérez-Moreno
Epithelial Cell Biology Junior Group

Nabil Djouder
Growth Factors, Nutrients and Cancer Junior Group

Massimo Squatrito
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Guillermo Montoya
Macromolecular Crystallography Group

Francesco L. Gervasio
Computational Biophysics Junior Group

Daniel Lietha
Cell Signalling and Adhesion Junior Group

Santiago Ramón-Maiques
Structural Bases of Genome Integrity Junior Group

Ramón Campos-Olivas
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Bioinformatics Core Unit

Alfonso Valencia
National Bioinformatics Institute Core Unit

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Melanoma Group

Francisco X. Real
Epithelial Carcinogenesis Group

Marta Sánchez-Carbayo
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HUMAN CANCER GENETICS PROGRAMME

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Javier Benítez
Human Genetics Group

Juan C. Cigudosa
Molecular Cytogenetics Group

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Núria Malats
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Anna González-Neira
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Manuel Hidalgo
Gastrointestinal Cancer Clinical Research Unit

Christopher Heeschen
Stem Cells and Cancer Group

Miguel Quintela-Fandino
Breast Cancer Junior Clinical Research Unit

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

Luis J. Lombardía
Molecular Diagnostics Clinical Research Unit

Fátima Al-Shahrour
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MARISOL QUINTERO DIRECTOR OF INNOVATION

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Orlando Domínguez
Genomics Core Unit

Sagrario Ortega
Transgenic Mice Core Unit

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Monoclonal Antibodies Core Unit

Marta Cañamero
Histopathology 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

Isabel Blanco (Charles River Laboratories)
Animal Facility

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Sonia Martínez
Medicinal Chemistry Section

Carmen Blanco
Biology Section

Susana Velasco
Lilly-CNIO Cell Signalling Therapies Section

TECHNOLOGY TRANSFER AND VALORISATION OFFICE

Marisol Quintero Head of Office

“MY MAIN MISSION, AS VICE-DIRECTOR FOR BASIC RESEARCH, IS TO STRENGTHEN CNIO’S SCIENTIFIC EXCELLENCE BY FURTHER IMPROVING THE CENTRE’S INTERNATIONAL STANDING, ENHANCING SCIENTIFIC COMMUNICATION, AND MOST IMPORTANTLY, HELP TRAIN OUR YOUNG FACULTY.”

ERWIN F. WAGNER VICE-DIRECTOR OF BASIC RESEARCH

VICE-DIRECTION OF BASIC RESEARCH

ERWIN F. WAGNER VICE-DIRECTOR

MOLECULAR ONCOLOGY PROGRAMME

MANUEL SERRANO PROGRAMME DIRECTOR

Early this year, I had the honour of being appointed as the Director of the Molecular Oncology Programme. My first words to show my appreciation are for the former Director, Maria A. Blasco, and to the other Programme Group Leaders, whom I thank for entrusting me with this responsibility that I will endeavour to uphold with professionalism and enthusiasm.

The Molecular Oncology Programme has been, and continues to be, the flagship of CNIO's scientific excellence. At present, the Programme consists of 7 Groups that cover some of the most active areas of research in cellular oncology. These areas include DNA and chromosome stability (Maria A. Blasco, Óscar Fernández-Capetillo, and Ana Losada), oncogenes and cell cycle kinases (Mariano Barbacid), DNA replication (Juan Méndez), mitosis (Marcos Malumbres), and tumour suppressors (Manuel Serrano). The top-level quality of the research conducted by each of these Groups is detailed in the following pages. Still, I would like to highlight a few especial facts: this year the Programme has authored a total of 10 papers in Cell journals (*Cell* 2×, *Cancer Cell* 4×, *Molecular Cell* 2×, *Cell Stem Cell*, and *Cell Metabolism*); this trend continues with *EMBO J* 4×, *The Journal of Experimental Medicine* and *The Journal of Clinical Investigation*. Also, during this year, researchers from the Programme have filed several patents, some of which have been licensed, and a spin-off company has been created.

All the above achievements give us reason to be proud, but also motivate us to continue along this road of progress upon which we have embarked. Over the past few years, each of the CNIO Groups have been steadfastly improving in quality, productivity, originality, and international recognition and this will not stop in the foreseeable future. Some of the credit for our success should go to the mindset of collective interaction that all of us foster. Ideas, expertise, and reagents flow freely among our Groups, and collaborations flourish after each seminar. If we have arrived at this stage, by working hard together with shared enthusiasm, then we can achieve even more in the future. ■

"THE MOLECULAR ONCOLOGY PROGRAMME IS CNIO'S FLAGSHIP PROGRAMME. PART OF OUR SUCCESS LIES IN OUR COLLECTIVE GOAL TO MAKE OUR RESEARCH INNOVATIVE AND EFFICIENT. MANY OF THE DISCOVERIES MADE IN OUR PROGRAMME ARE ALREADY IN TRANSLATIONAL PHASES AND ARE, THUS, CONTRIBUTING TOWARDS THE IMPROVEMENT OF CANCER TREATMENT."

TUMOUR SUPPRESSION GROUP

Manuel Serrano
Group Leader

Staff Scientists
Manuel Collado (until May), Luis Enrique Donate, Han Li, Susana Llanos, Antonio Maraver, Cristina Pantoja

Post-Doctoral Fellows
María Abad, Timothy Cash, Pablo J. Fernández-Marcos (since February), Cian J. Lynch, Daniel Muñoz, Sandrina Nóbrega, Daniela Piazzolla

Graduate Students
Katharina Hess (since September), Elena López-Guadamillas, Lucía Morgado, Lluc Mosteiro, Ana Ortega (until August), Adelaida Palla


Manuel Serrano ESP


Han Li CHN


Susana LLanos ESP


Antonio Maraver ESP


Cristina Pantoja ESP


María Abad ESP


Timothy Cash USA


Pablo J. Fernández-Marcos ESP


Cian J. Lynch IRL


Daniel Muñoz ESP


Sandrina Nóbrega PRT


Daniela Piazzolla ITA


Katharina Hess DEU


Elena López-Guadamillas ESP


Lucía Morgado ESP


Lluc Mosteiro ESP


Adelaida Palla VEN

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, either accidentally or through the action of mutations. In this manner, the affected cells become partially unprotected from cancer and, in combination with additional mutations in other genes, can potentially give rise to the development of a tumour.

Tumours are stressfull environments, and an important feature of tumour cells is their capability to survive and multiply under stress. One of the most common responses to stress is the permanent arrest of cell proliferation (senescence). Understanding how tumour suppressor genes work may help us to design drugs that block cancer growth. Also, we manipulate the mouse genome to create novel alterations that increase or decrease tumour suppression potency.

"OUR FINDING THAT LUNG CARCINOMAS ARREST AFTER TREATMENT WITH GAMMA-SECRETASE INHIBITORS OPENS A NOVEL THERAPEUTIC STRATEGY TO TREAT LUNG CANCER; THE LEADING CAUSE OF CANCER-RELATED DEATHS IN THE WORLD."

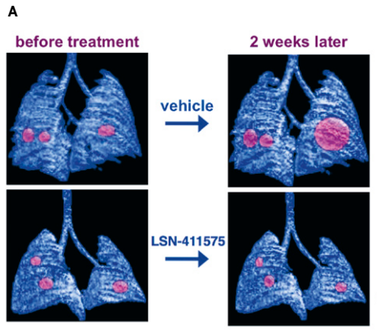
The goals of our Group are: i) to understand the mechanisms of tumour suppression and identify new tumour suppressor regulators; ii) to study the interplay between tumour suppression and ageing; iii) to analyse the involvement of tumour suppressors in the regulation of metabolism and protection from metabolic damage; iv) to characterise cellular senescence as a tumour suppression mechanism; and v) to elucidate the involvement of tumour suppressors in the regulation of nuclear reprogramming of differentiated cells to induced pluripotent stem (iPS) cells.

RESEARCH HIGHLIGHTS

A new therapeutic strategy for non-small-cell lung carcinoma (NSCLC)

We have deciphered one of the molecular pathways behind lung cancer and used this knowledge to identify an experimental drug that blocks lung cancer growth in mice.

Deregulation of the Notch cell-signalling pathway is implicated in cancer. Depending on the cellular type, it can be either oncogenic or tumour suppressive. In the case of NSCLCs, high Notch pathway activity is observed in up to 50% of the cancers, and this correlates with poor prognosis; moreover, gain-of-function mutations are present in a small percentage of patients.



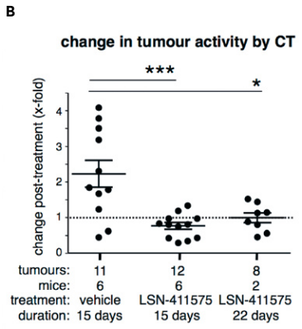


Figure 1 Pharmacological inhibition of gamma-secretase arrests NSCLCs. (**A**) Representative PET/CT lung images at the beginning and end of treatment with vehicle (top) or LSN-411575 (bottom). (**B**) Change in tumour activity detected by PET after treatment with vehicle or LSN-411575.

By using a mouse model – developed by Mariano Barbacid at the CNIO – in which oncogenic *Kras* is induced in adult mice to produce NSCLC, we demonstrate that the concomitant loss-of-function of the Notch pathway and activation of the *Kras* oncogene abolishes the generation of NSCLCs. Oncogenic *Kras*-driven lung carcinogenesis is therefore strictly dependent on the presence of a functional Notch pathway. Gamma-secretase inhibitors (GSIs) were previously known to inhibit the Notch pathway. As shown in the figure (FIGURE 1), we have now found that murine *Kras*-driven NSCLC arrested their growth after 15 days of treatment with a GSI (Eli Lilly compound LSN-411575). A co-clinical trial (parallel treatment of mice and humans) is currently under way at the CNIO, in collaboration with Manuel Hidalgo, to test the effect of another Notch inhibitory drug in human patients.

Impact of the tumour suppressor PTEN on metabolism and longevity

The tumour suppressor PTEN is one of the four most potent anti-cancer genes. We generated transgenic mice expressing twice the standard levels of PTEN and found that PTEN confers a number of health benefits by protecting against cancer, boosting longevity and combating obesity. *Pten* transgenic mice were far more resistant to cancer as compared to controls, lived an average of 12% longer, and, very surprisingly, they were significantly thinner – by 28% on average – even though they were eating more. We found that the ability of PTEN to activate brown fat and increase energy expenditure explained the thinness of the mice carrying extra copies of the gene. Interestingly, and subsequent to our work, other investigators found that PTEN also regulates obesity in humans. These findings open the door to novel therapeutic options against cancer, obesity and even the ageing process. The CNIO Experimental Therapeutics Programme has developed a small compound that mimics the activity of PTEN. Treatment of mice with this compound produces the same anti-obesity benefits observed in *Pten* transgenic mice.

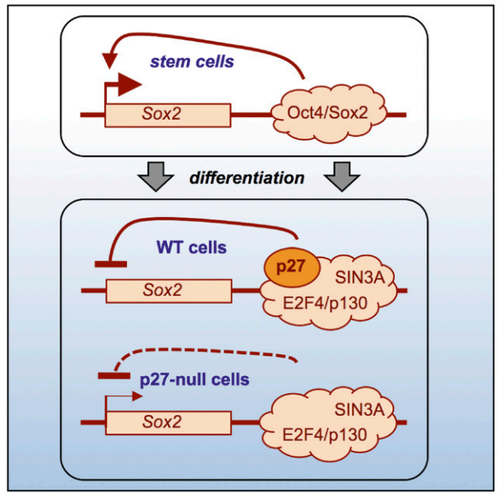


Figure 2 The tumour suppressor p27 represses the pluripotency gene *Sox2*. Upon differentiation of stem cells, a repressive complex containing p27 and p130-E2F4-SIN3A assembles at the critical SRR2 enhancer downstream of the *Sox2* gene. In the absence of p27, repression is incomplete, allowing abnormally high levels of SOX2, which can result in tumour development.

A connection between p27 and SOX2

Tumour cells frequently undergo loss of differentiation and acquisition of stem cell features. Thus, it is important to understand how pluripotency genes are kept in a repressed state in normal differentiated cells and how this repression is lost in cancer cells. We have unveiled an unprecedented mechanistic connection between p27 and *Sox2* relevant for cancer, in which p27 binds and represses the expression of *Sox2*.

The clue to this connection derived from our studies on reprogramming. Differentiated cells can be converted into induced pluripotent stem cells (iPSCs) through the combined action of the transcription factors OCT4, KLF4 and SOX2. We had previously shown that certain tumour suppressors oppose reprogramming and limit the efficiency of this process. We studied cells lacking the gene *p27,* noticing that these cells can be reprogrammed into iPSCs without ectopic expression of the gene *Sox2*. This led us to explore the potential link between two previously unrelated proteins; the tumour suppressor p27 and the transcription factor SOX2. We demonstrated that p27 contributes to the transcriptional repression of *Sox2* (FIGURE 2). Absence of p27 leads to defective repression of *Sox2* in several types of tissues, which leads to an increased incidence of tumours. We characterised p27 as a novel transcriptional regulator of *Sox2* together with a repressive complex formed by p130, E2F4 and SIN3A at a critical enhancer responsible for *Sox2* expression. ■

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OVERVIEW

Our laboratory is primarily interested in developing genetically engineered mouse models (GEMMs) of cancer that faithfully reproduce the natural history of the human disease with the ultimate goal of using them to validate targets of therapeutic value, mostly, but not exclusively, by genetic means. Currently, we are focusing on 2 of the tumour types with the lowest survival rate – K-Ras oncogene-driven non-small cell lung carcinoma (NSCLC) and pancreatic ductal adenocarcinoma (PDAC) – for which there are no efficacious medicines. Hence, the results derived from our work should be of significant value to the development of novel therapeutic approaches.

Specifically, we have interrogated the therapeutic value of each of the individual components of the Raf/Mek/Erk signalling cascade as well as of the interphase cell cycle kinases in a GEMM of NSCLC. Targets found to be essential for tumour initiation have been further evaluated in a second generation GEMM of tumour progression, in which we can separate tumour initiation from target ablation. We have also evaluated the role of the epidermal growth factor receptor (EGFR) in the development of K-Ras oncogene-driven PDAC; this was based on a study that reported some clinical responses to the EGFR inhibitor Erlotinib, in spite of the fact that the EGFR is supposed to signal upstream of K-Ras. The laboratory is also working on identifying the signalling molecules that are responsible for mediating normal Ras signalling beyond the Raf/Mek/Erk cascade and in understanding the role of the Cdk1 and Cdk7 cell cycle kinases in normal homeostasis.

"OUR STUDIES HAVE LED TO THE IDENTIFICATION OF C-RAF AS A CRITICAL MEDIATOR OF K-RAS ONCOGENIC SIGNALLING IN BOTH NSCLC AND PDAC TUMOURS. THESE RESULTS HAVE PROMPTED SEVERAL PHARMACEUTICAL COMPANIES TO DEVELOP C-RAF SELECTIVE INHIBITORS, SOME OF WHICH HAVE ALREADY ENTERED EARLY PHASE CLINICAL TRIALS."

RESEARCH HIGHLIGHTS

Last year, we interrogated the role of individual members of the Raf/Mek/Erk cascade in the onset of K-Ras oncogene-driven NSCLC. In those studies we showed that ablation of Erk1, or Erk2, in K-*Ras* oncogene expressing lung cells had no significant effect due to compensatory activities. Yet, elimination of both Erk kinases completely blocked tumour development. Similar results were obtained with Mek kinases. Likewise, ablation of B-*Raf* had no significant effect on tumour development. However, we determined that c-Raf expression was absolutely essential for the onset of NSCLC. Interestingly, concomitant elimination of c-*Raf* and B-*Raf* in adult mice had no deleterious consequences for normal homeostasis. These results indicate that c-Raf plays a unique role in mediating K-Ras oncogenic signalling and makes it a suitable target

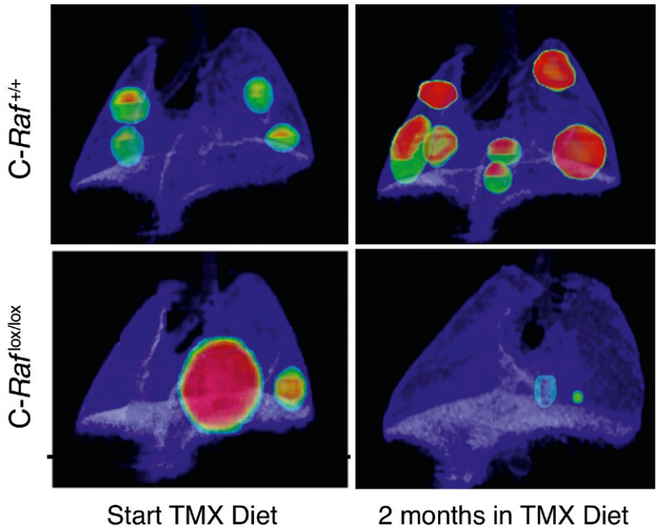

Figure 1 c-Raf expression is essential for progression of PET-positive K-Ras oncogene-driven NSCLC tumours in K-*Ras*$^{+/FSFG12V}$ mice.

for therapeutic intervention. Yet, these observations only demonstrated that c-Raf was essential for tumour initiation, but not for tumour progression. This year we have extended these findings using a GEMM for NSCLC tumour progression. In brief, we have generated a K-*Ras*$^{+/FSFG12V}$ mouse strain in which expression of a resident K-*Ras*^{G12V} oncogene requires recombination of an frt-STOP-frt cassette mediated by Flpse activity, which can be achieved via adenoviral vectors through tracheal infection. This GEMM has been crossed to mice carrying c-*Raf* conditional floxed alleles and a transgene encoding an ubiquitously expressed Cre recombinase (Ub-CreERT2); the activity of which can be induced by systemic exposure to tamoxifen. These mice were allowed to develop large CT/PET+ tumours before the c-*Raf* floxed alleles were ablated by the CreERT2 recombinase. We have observed that tumours rapidly decrease in size and become PET negative. Thus, indicating that c-Raf kinase activity is also essential for K-Ras driven tumour progression.

Previous clinical studies have suggested a therapeutic benefit of Erlotinib – an EGFR inhibitor – in pancreatic ductal adenocarcinoma patients. During 2012, we showed that these observations may have a mechanistic base. Clinical evidence indicates that mutation/activation of EGF receptors (EGFRs) is mutually exclusive with the presence of K-RAS oncogenes in lung and colon tumours. We have validated these observations using GEMMs. However, PDACs driven by K-Ras oncogenes are totally dependent on EGFR signalling. Similar

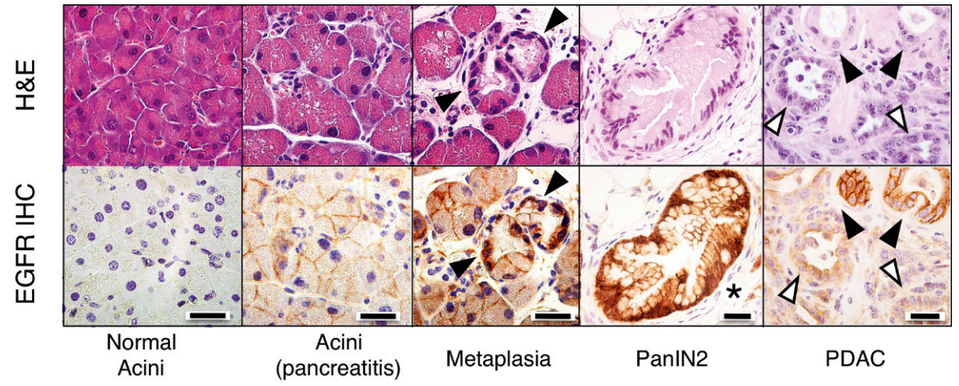

Figure 2 Expression of EGFRs in a normal pancreas as well as in the pancreases of *ElasK-Ras*^{G12V} mice displaying pancreatitis, metaplasia, PanIN lesions and PDAC tumours.

results were obtained when using human pancreatic tumour cell lines. EGFRs were also essential, even in the context of pancreatic injury and absence of p16Ink4a/p19Arf. Only loss of p53 made pancreatic tumours independent of EGFR signalling. Additional inhibition of PI3K and STAT3 effectively prevented proliferation of explants derived from these p53–defective pancreatic tumours. Thus, successful treatment of advanced human pancreatic tumours may require inhibition of at least 4 distinct signalling cascades, including those driven by K-RAS, EGFRs, PI3K and STAT3. These findings may provide the bases for more rational approaches to treat pancreatic tumours in the clinic.

During 2012, we have also studied the role of two cell cycle kinases: Cdk1 the mitotic kinase, and Cdk7, the catalytic subunit of the Cdk-activating kinase complex that has been implicated in the control of cell cycle progression and of RNA polymerase II -mediated transcription. To study their roles in normal homeostasis and in tumour development we have generated conditional mutant mice for each of these kinases. Whereas *Cdk1*$^{lox/lox}$ mice are undergoing characterisation at this time, mice carrying a conditional *Cdk7* locus have already been characterised. Genetic inactivation of the *Cdk7* locus revealed that, whereas Cdk7 is completely dispensable for global transcription, it is essential for the cell cycle via phosphorylation of Cdk1 and Cdk2. *In vivo*, Cdk7 is also indispensable for cell proliferation except during the initial stages of embryonic development. Interestingly, widespread elimination of Cdk7 in adult tissues with low proliferative indexes had no phenotypic consequences. However, loss of Cdk7 in tissues with elevated cellular turnover led to their efficient repopulation with Cdk7-expressing cells derived from adult stem cells that escaped inactivation of their targeted *Cdk7* alleles. This process, a physiological attempt to maintain tissue homeostasis, led to the attrition of adult stem cell pools and the appearance of age-related phenotypes including telomere shortening and early death. Next year, we plan to interrogate the role of these cell cycle kinases in K-Ras driven tumour development. ■

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AWARDS AND RECOGNITION

- **Member** (Foreign), **National Academy of Sciences of the USA.**
- *V de Vida* Award, *Asociación Española Contra el Cáncer* (AECC), Spain.
- *I Premio Internacional de Investigación Oncológica Ramiro Carregal,* Spain.
- Keynote Speaker, 2012 Inflammation-induced Cancer Conference, Israel.

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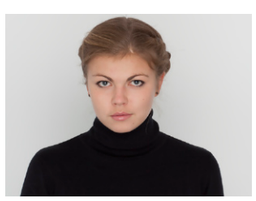
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OVERVIEW

We study the mechanisms by which tumour cells become immortal, and normal cells remain mortal. The immortality of cancer cells is one of their most universal characteristics. The enzyme telomerase is present to high levels in more than 95% of all types of human cancers but is less abundant in healthy 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 key drivers of age-related pathologies, including cancer, is the progressive shortening of telomeres associated with organismal ageing. When the length or integrity of telomeres is altered, adult stem cells have a maimed regenerative capacity.

"OUR NEWLY-GENERATED MOUSE MODEL FOR APLASTIC ANAEMIA ASSOCIATED WITH SHORT TELOMERES MAY AID IN THE DESIGN AND TESTING OF NOVEL THERAPEUTIC STRATEGIES, AS WELL AS PROVIDE NOVEL INSIGHTS INTO OTHER PROCESSES LINKED TO TELOMERE LENGTH, SUCH AS CANCER."

Our research activities aim: i) to understand the biology of telomeres and telomerase by generating mouse models to probe the role of telomeres and telomerase in cancer and ageing; ii) to decipher the interplay between telomeres and DNA repair pathways; iii) to characterise the role of telomeric heterochromatin; iv) to develop strategies for telomerase activation; and v), to elucidate 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

A successful telomerase gene therapy against ageing-associated decline without increasing cancer

A major goal in ageing research is to increase the length of a disease-free life, also known as 'health span'. Telomeres have been linked with ageing and disease; in mice, genetic manipulations that shorten or lengthen telomeres result, respectively, in decreased or increased longevity. Therapies that impact on telomere length are thus expected to have an impact on health span. We tested the effects of a telomerase gene therapy in adult (1-year) and old (2-year) mice. Treatment of both groups with a viral vector (AVV9) of efficient viral


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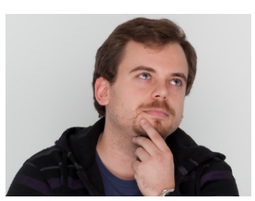
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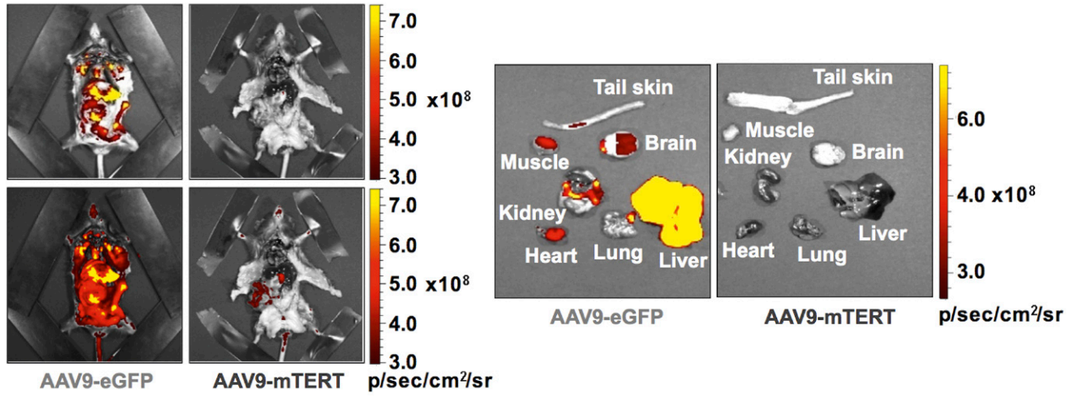

Figure 1 Efficiency of AAV9 infection. Direct GFP measurements in the indicated organs of female mice (n=3) treated with either AAV9-eGFP or AAV9-mTERT vectors.

infection (FIGURE 1) and wide tropism expressing mouse telomerase demonstrated beneficial effects on health and fitness, improving several molecular biomarkers of ageing. Telomerase-treated mice did not develop more neoplasias as compared to controls; thus, the known tumourigenic activity of telomerase is markedly decreased when expressed in adult or old organisms by using this strategy. Re-introduction of telomerase in both 1-and 2-year old mice increased their median lifespan by 24% and 13%, respectively. Our results constitute a proof-of-principle for a role of telomerase in delaying physiological ageing, improvement of health span, and extension of longevity in normal mice. Our telomerase-based gene therapy represents a novel type of therapeutic intervention against various age-related diseases.

Combined telomere dysfunction and deficient DNA repair lead to hyper-activation of DNA damage response pathways

Depletion of telomeric protein TRF1 results in telomere fusions as well as accumulation of DNA damage and activation of both the ATM- and ATR-mediated DNA damage response (DDR) pathways. The protein 53BP1, present at dysfunctional telomeres, is an ATM target that accumulates at DNA double-strand breaks and favours non-homologous end-joining (NHEJ) repair over ATM-dependent resection and homology-directed repair (homologous recombination, HR). We generated mice lacking TRF1 and 53BP1 to address the role of 53BP1 at dysfunctional telomeres. 53BP1 deficiency significantly rescued telomere fusions in mouse embryonic fibroblasts (MEFs) lacking TRF1, but they showed evidence of a switch from the NHEJ-to HR-mediated repair of uncapped telomeres. Concomitantly, double-mutant MEFs showed evidence of hyper-activation of the ATR-dependent DDR. In intact mice, combined 53BP1/TRF1 deficiency in stratified epithelia resulted in earlier onset of DNA damage and increased phosphorylation of the kinase CHK1 during embryonic development, leading to aggravation of skin phenotypes (FIGURE 2).

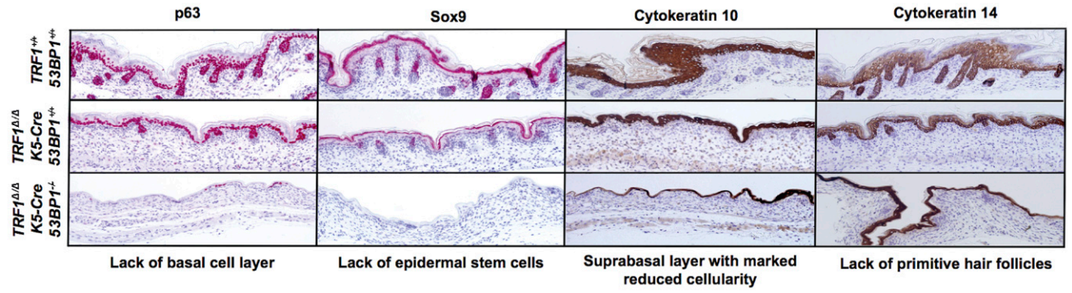

Figure 2 Deleterious genetic interactions between TRF1 and 53BP1 *in vivo*. Representative images of back skin sections stained for p63, Sox9, cytokeratin 10, and cytokeratin 14.

A link between telomeres and bone marrow failure

Ablation of the telomeric protein TRF1 induces chromosome and telomere alterations associated with telomere fragility. *Dyskeratosis congenita*, a human disease paradigmatic of premature ageing syndromes, is characterised by the triad of bone marrow failure (BMF), skin abnormalities and increased risk of cancer, and by the presence of short telomeres because of mutations in genes related to telomere maintenance. Subsets of patients showing mutations in the TRF1-interacting protein TIN2, have a more severe phenotype and the presence of very short telomeres despite normal telomerase activity. We generated a mouse model with conditional TRF1 deletion in the haematopoietic system to investigate a possible role for TRF1 dysfunction in BMF. Chronic TRF1 deletion results in increased DNA damage and cellular senescence in bone progenitor cells, leading to severe aplasia. We observed increased compensatory proliferation of bone marrow stem cells, associated to rapid telomere shortening, and further increase in senescent cells *in vivo*, providing a mechanism for the very short telomeres of patients with mutations in TIN2. Our unique mouse model for aplastic anaemia may also offer insights into other processes linked to telomere length, such as ageing and cancer. ■

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AWARDS AND RECOGNITION

- Honorary Ambassador of the Spain Brand *(Embajadora Honoraria de la Marca España)*, Leading Brands of Spain Forum (*FMRE*).
- Member of the Board of Trustees and President of the External Advisory Board, The Spanish National Centre for Research on Ageing (*Centro Nacional de Investigación en Envejecimiento, CNIE*).
- Member, *Faculty of 1000* (Ageing Section).

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OVERVIEW

The Cell Division and Cancer Group aims to understand the relevance of mitotic regulators, not only during the cell cycle, but also in physiological processes in different tissues. Mitosis is mostly regulated by post-transcriptional mechanisms such as protein phosphorylation or protein degradation. We are therefore interested in the study of mitotic kinases and phosphatases, as well as regulatory complexes involved in ubiquitin-dependent degradation of proteins. The control of proper chromosome segregation by these regulators is essential to prevent genomic instability and may have critical implications in the generation of aneuploidy; an imbalance in the number of chromosomes. In addition, we are interested in understanding how small, non-coding RNAs are involved in the control of the cell cycle and cell proliferation. Our research also focuses on the regulatory mechanisms that control asymmetric cell division in progenitor/stem cells and their relevance to development, tissue homeostasis and cancer. The Group's main areas of focus include: i) understanding the basic control mechanisms of the mammalian cell cycle; ii) characterising the physiological and therapeutic consequences of cell cycle deregulation *in vivo*; iii) characterising the function of microRNAs in cell biology and tumour development; and iv), understanding how progenitor cells and cancer stem cells control their self-renewal and proliferative properties.

"DURING 2012, WE HAVE REPORTED THAT DEREGULATION OF TPX2, A PROTEIN INVOLVED IN MICROTUBULE DYNAMICS, MAY LEAD TO CHROMOSOMAL INSTABILITY AND TUMOUR DEVELOPMENT *IN VIVO*. WE HAVE ALSO ANALYSED THE RELEVANCE OF THE UBIQUITIN LIGASE APC/C AS WELL AS OF CYCLIN-DEPENDENT KINASES AS TARGETS FOR CANCER THERAPY."

Oncogene-induced mitotic stress

We have recently proposed the presence of an oncogene-induced mitotic stress response that may alter the proper regulation of mitosis under specific oncogenic mutations. This is based on the fact that multiple mitotic regulators are E2F targets that are induced by oncogenic events. Loss of pRB or p53, for instance, results in increased levels of the spindle assembly checkpoint (SAC) protein MAD2, and, as a consequence, MAD2 is frequently overexpressed in tumour cells, possibly resulting in deregulated SAC and chromosomal instability (CIN; FIGURE 1). Many other mitotic regulators (such as TPX2, PRC1, FOXM1, CDK1, TOP2A, PCNA, and UBE2C) are co-overexpressed with MAD2 in CIN tumours. Out of all these genes, TPX2 displays the highest correlation with chromosomal instability. We have recently reported that TPX2 is haplo-insufficient to maintain genomic instability seeing as TPX2-heterozygous mice develop tumours in significantly higher ratio than their wild type counterparts. As expected, these tumours are highly aneuploid, suggesting a causal connection between deregulated expression of TPX2, aneuploidy and cancer. As demonstrated for other models, it is likely that the overexpression of TPX2 may result in similar defects in spindle dynamics and chromosome segregation. The concomitant overexpression of MAD2 in the same tumours is likely to disrupt a normal function of the SAC, thus facilitating CIN in these conditions (FIGURE 1).

The Anaphase-Promoting Complex

The requirements for the Anaphase-Promoting Complex, also called cyclosome (APC/C) during the cell cycle have led to different studies that evaluate the relevance of this complex as a cancer target. The suggestion that targeting mitotic exit is a relevant therapeutic strategy has been confirmed by our Group through the use of mice deficient for the cell-division cycle protein 20 (Cdc20); the first APC/C inhibitor is currently under preclinical studies. Elimination of APC/C-Cdc20 activity results in metaphase arrest due to the lack of degradation of cyclin B1; this strategy is more efficient for killing cells than current microtubule poisons such as taxanes. The possible inhibition of the alternative APC/C cofactor, Cdh1, has been considered as a potential undesired effect since partial inactivation of Cdh1 may contribute to increased levels of cell cycle regulators and tumourigenesis. Contrary to these expectations, we have now demonstrated – using genetic models – that ablation of Cdh1 results in replicative stress *in vivo,* as well as in a general anti-proliferative response that is not p53-dependent (FIGURE 2). Thus, putative APC/C inhibitors are unlikely to generate proliferative responses, even in the case of unspecific inhibition of Cdh1 and with independence of the p53 status of tumour cells.

Mitosis and mitotic cell death

We, and others, have shown that activation of protein phosphatase 2 (PP2A) during mitotic exit is achieved by inactivation of the kinase Mastl; a kinase recently identified in humans as the functional orthologue of Greatwall kinase. RNA interference studies have shown that robust depletion of Mastl results in a significant delay in G2, which can be rescued by PP2A inhibition. To understand the relevance of Mastl in mitosis, we have recently generated a mouse model with a loss-of-function mutation in the murine *Mastl* gene. We have observed that Mastl is required for embryonic development; the detailed analysis of the phenotype is currently in progress. We have also observed that Mastl is overexpressed in several tumour types and the consequences of its expression and targeted downregulation are now under investigation. We have also generated mice deficient in B55alpha; the PP2A regulatory subunit inhibited by Mastl and involved in dephosphorylation of mitotic phosphoproteins. Since mitosis is a critical target of cancer drugs such as microtubule poisons, we are also analysing the molecular pathways that determine mitotic cell death in the presence of these drugs. Current studies in our laboratory suggest the involvement of multiple cell death pathways with possible implications in cancer therapy. ■

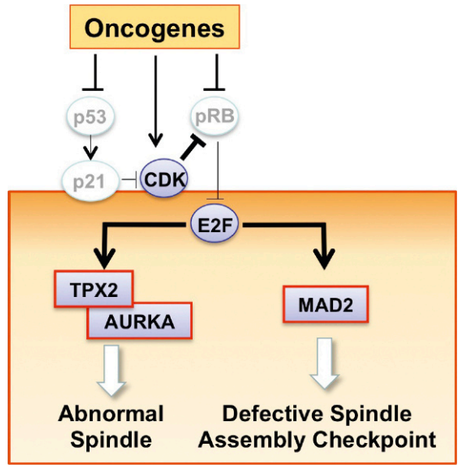

Figure 1 Oncogene-induced mitotic stress. The activation of oncogenes or loss of tumour suppressors, such as pRB or p53, results in the overexpression of E2F targets such as MAD2 and possibly TPX2. These alterations are probably involved in the chromosomal instability that is frequently observed in tumours.

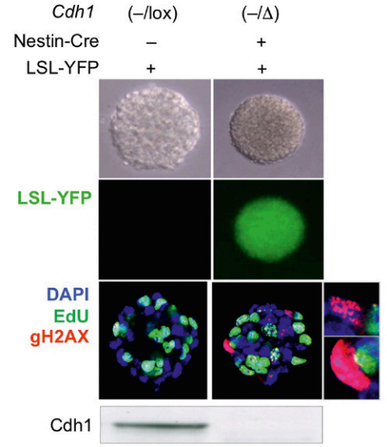

Figure 2 Inactivation of the APC/C cofactor Cdh1 [*Cdh1*(–/Δ)] results in replicative stress (γ-H2AX signal) in neurospheres. The activity of the Cre-recombinase is monitored by the presence of the yellow fluorescent protein (YFP) signal and lack of the Cdh1 protein (bottom panel).

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OVERVIEW

DNA damage is the source of pro-cancerous mutations. In addition, recent evidence suggests that the reverse connection might also exist; namely, that oncogenes can promote the generation of DNA damage. However, the nature of the damage 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 arises unavoidably each time a cell replicates its DNA, and that is mainly prevented by ATR and Chk1 kinases. Unfortunately, the essential nature of these kinases imposes important limitations on their investigation, particularly at the organismal level. In order to overcome these limitations, a significant part of our work over these last few 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 RS in cancer and ageing, and have resulted in drugs that can be used to test our conceptual approaches to cancer therapy. All in all, our main goal is to understand how genome maintenance is safeguarded – particularly during replication – and to exploit this knowledge in the fight against cancer.

"OUR RESEARCH IN 2012 REVEALED THAT CHK1, A WELL-KNOWN TUMOUR SUPPRESSOR, MIGHT ALSO FACILITATE TRANSFORMATION BY LIMITING ONCOGENE-INDUCED DNA DAMAGE. WE PROPOSE THAT CHK1 LEVELS COULD BE USED AS A BIOMARKER TO IDENTIFY TUMOURS THAT MIGHT BE SENSITIVE TO THERAPIES THAT INHIBIT THE REPLICATIVE STRESS RESPONSE."

Chk1: A tumour suppressor and an oncogene?

The activation of certain oncogenes can promote a promiscuous S-phase entry that leads to the generation of RS and subsequent DNA damage. This DNA damage activates a DNA damage response initiated by ATM, ATR, Chk1 and Chk2 kinases. We previously showed that inhibition of ATR is particularly deleterious for tumours presenting high loads of RS, such as Burkitt lymphomas (Murga *et al., Nat Struct Mol Biol,* 2011). In 2012, in collaboration with the group of Eric J. Brown (University of Pennsylvania), we provided further proof of these concepts in additional tumour models. However, both studies were based on observations made with a mouse model for the ATR-Seckel Syndrome that confers high levels of RS and premature ageing (Murga *et al., Nat Genet,* 2009). The reduced lifespan and severe symptoms of ATR hypomorphic mice limit their use in cancer studies. In order to circumvent these limitations, we decided to generate a novel mouse model that would be selectively protected from RS.

In 2012, we reported that mice carrying a supra-physiological gene dosage for *Chk1* (Super-Chk1) are selectively protected from RS. Cells from these mice present lower levels of RS when exposed to RS-inducing agents such as hydroxyurea or aphidicolin. *In vivo*, an extra *Chk1* allele was able to partially rescue the viability and enhance the lifespan of ATR-Seckel mice, providing the first example of a genetic context that could limit the severity of the disease. Strikingly, we found that Super-Chk1 mouse embryonic fibroblasts (MEFs) were more easily transformed by oncogenes than their wild type counterparts (FIGURE 1). This observation was puzzling, given that Chk1 is generally considered a tumour suppressor. The explanation for this conundrum comes from the finding that increased levels of Chk1 limit the amount of DNA damage generated by oncogenes, thereby limiting the toxicity associated to the transformation process. Our idea is that Chk1 could work as an 'RS-buffer', facilitating the viability of cells suffering from RS. This model could explain why Chk1 levels are frequently upregulated in human cancers, in contrast to the lower levels that would be expected from the action of a tumour suppressor. We propose that Chk1 levels would indeed be a predictive biomarker of tumour-associated RS; this could help to identify tumours that would be most sensitive to ATR or Chk1 inhibitors.

A proteomic view of the human replication fork 'neighbourhood'

A proper control of DNA replication is essential to preserving genome integrity. To achieve this, the replisome is enriched in accessory factors that facilitate DNA replication and coordinate it with other cellular processes such as transcription or chromatin remodelling. We believe that a proper identification of these factors provides an opportunity for exploring novel synthetic lethal interactions that could be used in chemotherapy. By coupling a protocol that isolates proteins on nascent DNA chains with mass spectrometry, we have performed, in collaboration with the Proteomics Core Unit of the CNIO, a comprehensive proteomic characterisation of the human replisome and replisome-associated factors. Our analysis identified known components of the replisome, including DNA polymerases, primases and helicases, among others (FIGURE 2). In addition, we found a broad list of proteins and activities that are located in the vicinity of the replisome, some of which were not previously associated to replication. We believe that this resource provides a panoramic view of the proteins that concentrate in the surroundings of the replisome; this should facilitate future investigations on DNA replication and genome maintenance. We are currently exploiting this technology in order to identify factors that are recruited to the replisome in conditions of RS. ■

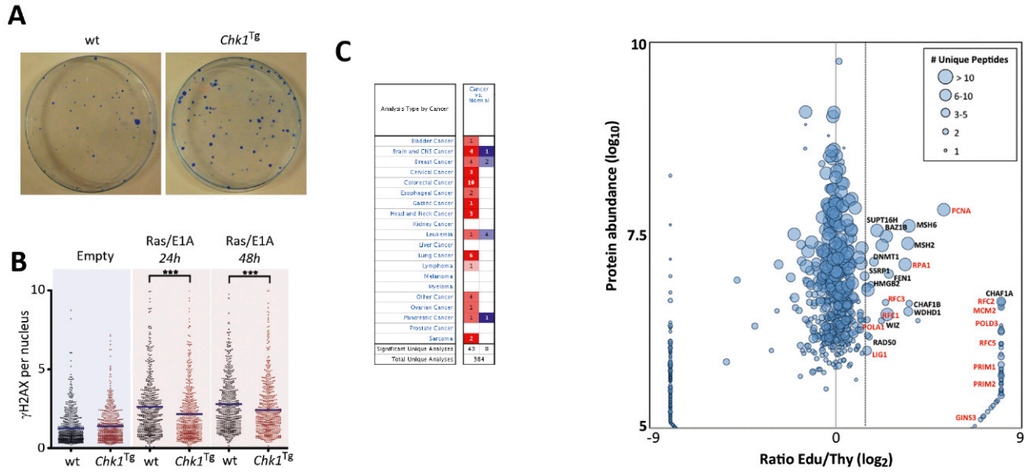

Figure 1 Chk1 facilitates transformation by oncogenes. (**A**) Representative pictures of the number of colonies found in wild type (wt) and Chk1 transgenic (*Chk1*^{Tg}) MEFs 10 days after transformation with Ras and E1A oncogenes. (**B**) Levels of RS, quantified by high-throughput microscopy as the nuclear signal of phosphorylated histone H2AX, in wt and *Chk1*^{Tg} MEFs after transformation with Ras and E1A oncogenes. (**C**) Data extracted from Oncomine (www.oncomine.org) that indicate the frequent overexpression of Chk1 found in human tumours.

Figure 2 A proteomic view of the mammalian replisome. The figure illustrates the outcome of a typical experiment, in which proteins that are enriched in the vicinity of the replisome are found towards the right hand side of the chart. The position of some known replisome components is indicated in red, and that of proteins that are known to be in close proximity to the replisome, in black.

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AWARDS AND RECOGNITION

- Howard Hughes Medical Institute (HHMI) International Early Career Award, USA.

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OVERVIEW

Proper development of a multicellular organism entails two major processes. One is proliferation; i.e. the cell duplicates its genetic material and divides into two 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 each of these two processes. On the one hand, cohesin mediates sister chromatid cohesion and thereby ensures faithful DNA repair by homologous recombination and proper chromosome segregation during cell division. On the other hand, cohesin contributes to the spatial organisation of the genome by promoting or stabilising the formation of chromatin loops. Mutations in cohesin and its regulatory factors have been identified in a class of human syndromes, collectively known as cohesinopathies, as well as in several tumour types. 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 epigenetic inheritance of centromeres mediated by the histone H3 variant CENP-A, another essential aspect of chromosome segregation.

"WE HAVE IDENTIFIED A SPECIFIC ROLE OF COHESIN-SA1 IN TELOMERE COHESION AND DESCRIBED A NOVEL MECHANISM DRIVING ANEUPLOIDY IN SA1-DEFICIENT CELLS AND EMBRYOS. ANEUPLOIDY MOST LIKELY CONTRIBUTES TO THE INCREASED CANCER SUSCEPTIBILITY OF SA1 HETEROZYGOUS MICE."

Cohesin-SA1, aneuploidy, and cancer

Vertebrate somatic cells have two versions of cohesin; namely, cohesin-SA1 and cohesin-SA2, that contain Smc1, Smc3, Rad21 and either SA1 or SA2. As yet, the functional specificity of these two variants has been largely ignored. The characterisation of an SA1 knock-out mouse model has allowed us to demonstrate that cohesin-SA1 mediates cohesion at telomeres, which is required for their replication. Due to their repetitive nature and peculiar structure, telomeres pose a challenge to the replication machinery, causing frequent stalling of replication forks. Homologous recombination-mediated mechanisms are required for fork restart and/or repair of DNA breaks generated by fork collapse; such mechanisms are promoted by cohesion. Incompletely replicated telomeres could be the cause of the lagging chromosomes and anaphase bridges observed in SA1 null cells (FIGURE 1). These aberrant anaphases often end in cell death or in cytokinesis failure; the latter causes tetraploidy or formation of micronuclei, both of them known pathways to aneuploidy. Increased aneuploidy could explain the earlier onset of spontaneous tumourigenesis in mice heterozygous for SA1. Of note, these animals develop pancreatic cancers that are extremely infrequent in mice. Whether or not pancreatic cells are more sensitive to telomere dysfunction and the ensuing genomic instability is currently under investigation. We are also exploring the contribution of altered transcription to the tumourigenic effect of SA1 deficiency.

Cohesin-SA1, transcription, and Cornelia de Lange Syndrome

In collaboration with CNIO's Bioinformatics Core Unit, we have analysed the genome-wide distribution of cohesin-SA1 and cohesin-SA2 by means of chromatin immunoprecipitation (ChIP) sequencing with 4 different antibodies. Cohesin-SA1 has greater propensity to localise to gene promoters and gene-associated regions. More striking, in the absence of SA1, cohesin is driven away from promoters and relocates to intergenic regions. The peak intensity of these new sites is clearly lower, which most likely reflects a more random genomic distribution of cohesin. In addition, the new cohesin sites show reduced co-localisation with the chromatin insulator CTCF. As a consequence, gene expression is altered and affects biological processes related to the most prevalent cohesinopathy, the Cornelia de Lange Syndrome (CdLS). The presence of cohesin-SA1 at several gene promoters positively regulates expression of the respective genes. Clear examples are the genes coding for Myc and Protocadherins, essential proteins for proliferation and neuronal connectivity, respectively. Their down-regulation could contribute to the growth and mental retardation observed in CdLS patients. The absence of cohesin-SA1 also alters cohesin binding patterns along some gene clusters and leads to dysregulation of genes within these clusters. We speculate that partial deficiency of the cohesin loader Nipped-B-like protein – the most common cause of CdLS – may restrict cohesin loading to an extent that allows for proper cohesion, but affects the transcription of genes that are critical for proper development. We are currently testing this hypothesis.

The role of Shugoshin-2 in spindle assembly

Shugoshins (Sgo) are conserved proteins that act as protectors of centromeric cohesion in mitosis and meiosis. Most vertebrates contain two Sgo proteins, but their specific functions are controversial. We have addressed this issue using the *Xenopus* egg cell-free extracts; a powerful system for the study of chromosome dynamics. We found that Sgo1 protects centromeric cohesin from the prophase dissociation pathway whereas Sgo2 promotes spindle assembly (FIGURE 2). While Sgo1 is required for proper localisation of the Chromosomal Passenger Complex (CPC) containing Aurora B, Sgo2 positively regulates its activity and the subsequent phosphorylation of key substrates for spindle assembly. Finally, the observed functional specificity of Sgo1 and Sgo2 could rely on their association with different regulatory subunits of phosphatase PP2A. ■

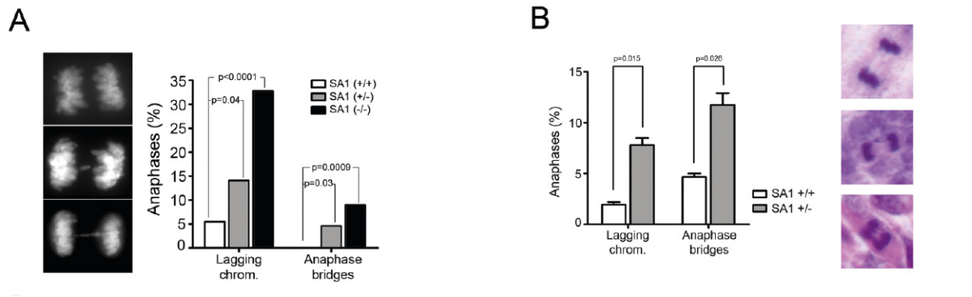

Figure 1 Cohesin-SA1 deficiency leads to chromosome segregation defects. Impaired telomere replication in the absence of cohesin-SA1 ultimately results in defective chromosome segregation during mitosis, as indicated by the increased frequency of aberrant anaphases in cultures of mouse embryonic fibroblasts (**A**) and embryo skin sections (**B**) with a complete or partial deficiency in cohesin-SA1. Examples of a normal anaphase (top panel), a lagging chromosome (middle panel) and an anaphase bridge (bottom panel) are shown. At least 50 anaphases per embryo from 2 independent embryos per genotype were counted.

Figure 2 Sgo2 is required for proper spindle assembly. Metaphase spindles were assembled in *Xenopus* egg extracts that had been mock-depleted or, using specific antibodies, depleted of Sgo1, Sgo2 or both of them. Sperm DNA (stained with DAPI, in blue) and rhodamine-labelled tubulin (red) were added to the extracts. The observed spindle structures were classified into the categories indicated and quantified for each experimental condition. Data represent mean values obtained from more than 200 structures in 3 independent experiments.

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OVERVIEW

Through the process of DNA replication, our dividing cells maintain a complete copy of their genome. Even in healthy cells and tissues, the genome-copying process introduces sporadic mutations, albeit with low frequency, which eventually promote cancer by activating oncogenes or inactivating tumour suppressors. Exposure to chemicals, UV light, or ionising radiation, interferes with the operation of the replisome; the large protein machinery that drives DNA replication. In recent years, the notion that 'replicative stress' is a causal factor of carcinogenesis has attracted a great deal of attention. Multiple mechanisms have evolved to counteract replication stress, including factors that stabilise stalled replication forks or facilitate fork re-start. Our previous research has identified one of such responses to replication stress, namely, the activation of 'dormant' replication origins that serve a back-up function upon the collapse of paused replication forks.

The molecular mechanisms underlying genomic duplication and the cellular responses to replicative stress have been extensively studied *in vitro*, and we are still contributing to this effort by analysing the biochemical properties of several replisome components. In parallel, we have devised *in vivo* strategies to evaluate the effect of replicative stress in mammalian organisms.

"WE HAVE CONTRIBUTED TO DEFINING THE MOLECULAR ORGANISATION OF DNA REPLICATION FACTORIES IN HUMAN CELLS. WE HAVE ALSO GENERATED GENETICALLY-MODIFIED MOUSE STRAINS TO STUDY REPLICATIVE STRESS AND DNA OVER-REPLICATION; TWO KNOWN SOURCES OF GENOMIC INSTABILITY COMMONLY OBSERVED IN CANCER CELLS."

Mechanism of action of the minichromosome maintenance (MCM) helicase

DNA replication occurs in specialised 'factories' that can be visualised as defined nuclear foci after the incorporation of nucleotide analogues into newly synthesised DNA. Major replisome components such as PCNA, RPA or DNA polymerases have been detected immunologically at these structures, whereas the presence of the DNA helicase MCM has remained elusive. In the past year, using a cell culture synchronisation protocol coupled to high-throughput confocal microscopy, we have shown that MCM complexes are indeed accumulated at the chromatin structures bound to become replication factories, but their levels drop drastically after the initiation of DNA synthesis. We have proposed that the amount of active MCM acting at replication factories is strongly regulated to prevent promiscuous origin activity and DNA re-replication.

We have previously reported that human MCM interacts with Cdc45 and GINS proteins to assemble a larger-order complex called hCMG. One of our long-term goals, in collaboration with CNIO's Macromolecular Crystallography Group, is the elucidation of the molecular mechanisms used by hCMG to promote DNA unwinding. This poses a significant biochemical challenge because hCMG is formed by 11 different polypeptides. As we progress towards this goal, we have extended our studies to include a model MCM protein complex encoded in the genome of *Bacillus cereus* (BcMCM). Structure-function studies in BcMCM support the notion that ring-shaped hexameric MCM helicases operate by translocation on ssDNA and 'steric extrusion' of the other strand. Interestingly, BcMCM also contains DNA primase and polymerase activities, suggestive of a role in replication re-start at stalled forks.

New mouse strains that allow for the inducible overexpression of DNA replication genes *CDC6* and *CDT1*

Previous work from our laboratory and those of others, has shown that the amount of chromatin-bound MCM complexes affect the availability of replication origins. We have generated knock-in mouse strains for the inducible expression of CDC6 and CDT1; the two main factors responsible for MCM loading onto DNA. Some types of cancer, particularly non-small cell lung carcinoma, show abnormally high levels of these two factors. We are characterising the *in vivo* effects of CDC6 and CDT1 overexpression, either individually or in combination. In embryonic fibroblasts, we have already observed that CDC6 overexpression is sufficient to alter the patterns of DNA replication, triggering more origin activity and reducing the fork progression rate (FIGURE 1).

A mouse strain expressing a hypomorphic allele of *MCM3*, reveals hypersensitivity of haematopoietic cell precursors to replication stress

We have also generated a conditional knock-out *MCM3* mouse strain that carries loxP insertions and a GFP-luciferase marker at the 3' UTR of the *MCM3* gene. These modifications result in hypomorphic expression of the $MCM3^{GFPLuc\text{-}loxP}$ allele (FIGURE 2) that does not carry any mutation in the coding region. Heterozygous $MCM3^{+/GFPLuc\text{-}LoxP}$ mice are alive and fertile, while $MCM3^{GFPLuc\text{-}LoxP/GFPLuc\text{-}LoxP}$ embryos die *in utero* at late stages of gestation. The embryos are smaller and paler, suggestive of incomplete erythropoiesis in the foetal liver. Indeed, we have detected an accumulation of haematopoietic stem cells (HSCs) and erythrocyte primitive progenitors, coupled to a deficiency in mature erythroblasts, in the liver of mutant embryos. Our hypothesis is that specific S-phase requirements of some of these populations make them hypersensitive to replicative stress during development. We are now investigating the dynamics of S-phase in HSCs and other haematopoietic precursors. The partial loss of MCM3 expression also predisposes to cancer, as both $MCM3^{+/GFPLuc\text{-}LoxP}$ and $MCM3^{+/-}$ adult mice display a high frequency of early-onset lymphomas and other mesenchymal tumours. ■

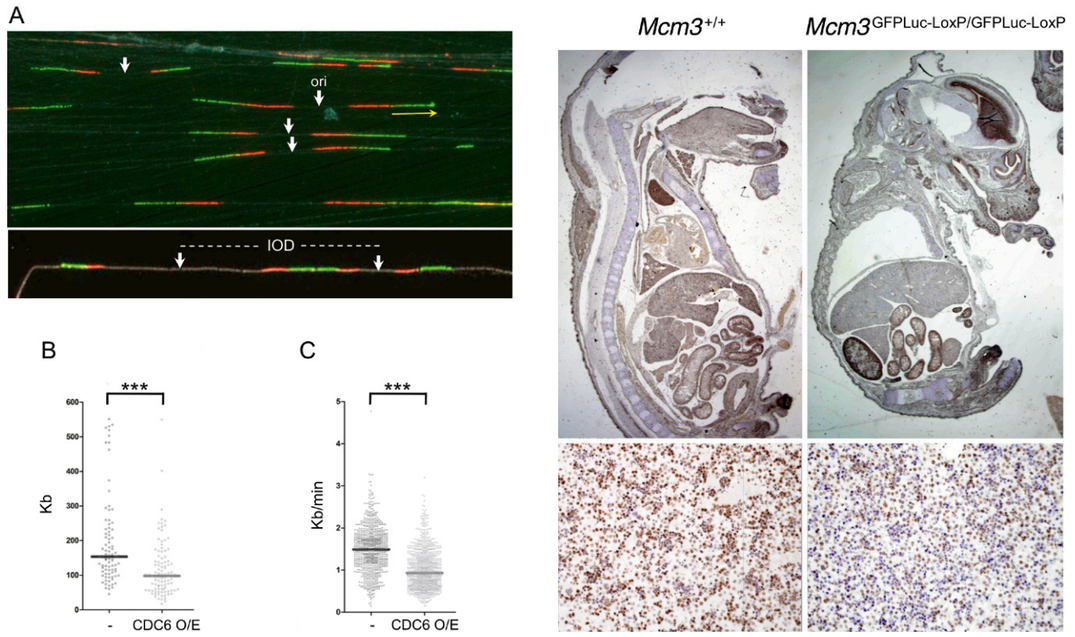

Figure 1 CDC6 overexpression affects inter-origin distance and replication fork progression. (**A**) Stretched DNA fibres labelled with CldU (red) and IdU (green). Origins are indicated by white arrows. A molecule containing 2 adjacent origins is shown. IOD, inter-origin distance. (**B**) Fork progression rates and (**C**) IOD in embryonic fibroblasts with or without CDC6 overexpression.

Figure 2 Hypomorphic expression of MCM3 in the $MCM3^{GFPLuc\text{-}LoxP/GFPLuc\text{-}LoxP}$ mouse strain. Top, immunohistochemical detection of Mcm3 protein in E16.5 embryos. Bottom, details of a foetal liver.

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BBVA FOUNDATION-CNIO CANCER CELL BIOLOGY PROGRAMME

ERWIN F. WAGNER PROGRAMME DIRECTOR

The overall strategic goals of the BBVA Foundation-CNIO Cancer Cell Biology Programme are to achieve a better understanding of the events leading to cancer development and to discover molecular mechanisms that could provide a basis for novel therapies. Our Programme investigates how a tumour can grow as an 'extrinsic organ'. Our research covers various aspects of tumour cell biology, 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, hypoxia, as well as cell adhesion, metabolism and metastasis. Powerful state-of-the-art mouse genetic models, human cellular systems and biochemical tools, as well as patient-derived materials, are employed. At present, these aspects are covered and integrated by the complementary research areas of 1 Senior and 3 Junior Groups.

My own Research Group focuses on understanding the role of the transcription factor complex AP-1 (Fos/Jun) and its upstream stress kinases JNK and p38 in physiological and pathological processes. Our studies focus on bone homeostasis and osteosarcomas, liver fibrosis, inflammation and cancer, and also aim to molecularly define the causes of skin cancer and inflammatory skin diseases, such as psoriasis. Mirna Pérez-Moreno's Junior Group concentrates on the role of cell adhesion, inflammation and cellular signalling in normal skin physiology and cancer development. Nabil Djouder's Junior Group aims to dissect the contribution of nutrient and growth factor signalling pathways to cancer development.

With the establishment of a third Junior Group in October 2012, in part financed by the Seve Ballesteros Foundation with the aim of supporting translational brain tumour research, we have introduced a completely new area of research to the CNIO. The new Group, headed by Massimo Squatrito who moved from the Memorial Sloan-Kettering Cancer Institute in New York to the CNIO, studies how brain tumours, mainly glioblastomas and medulloblastomas, develop and how they respond to therapy. ■

"WE AIM TO MAKE CNIO A MORE INTERNATIONAL INSTITUTION. WITH THE STRONG SUPPORT FROM THE BBVA FOUNDATION WE RECRUITED –BESIDES MY GROUP– 3 FOREIGN GROUP LEADERS, ONE OF WHICH RECEIVES FUNDING FROM THE SEVE BALLESTEROS FOUNDATION. WE HAVE 18 DIFFERENT NATIONALITIES IN OUR PROGRAMME AND ARE HOSTING ONE VISITING PROFESSOR WITH SUPPORT FROM THE *JESÚS SERRA* FOUNDATION."

GENES, DEVELOPMENT AND DISEASE GROUP

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OVERVIEW

Our research interests lie in breaking new ground in the study of mechanism-based functions of the transcription factor complex AP-1 (Fos/Jun) in the whole organism. The aim is to obtain a more global perspective on AP-1 in human physiology and in diseases, such as inflammatory skin diseases and cancer.

Specifically, the functions of AP-1 in regulating cell proliferation, differentiation and cell death are being investigated by employing genetically engineered mouse models (GEMMs) and human samples. The ultimate goal is to define the molecular pathways leading to disease development in organs such as the skin, bone and liver, and to identify novel therapeutic targets (FIGURE 1). The major research goals of our Group are: i) to elucidate a causal link between AP-1 expression, inflammation and cancer, using cell type-specific switchable GEMMs; ii) to develop and characterise new GEMMs for cancer and human diseases, such as osteoporosis, fibrosis/osteosclerosis and psoriasis, and apply these to preclinical studies; iii) to use large-scale genomic or proteomic approaches to compare mouse models of disease to human disease and to identify therapeutically relevant targets; and iv), to establish methods for efficient manipulation and differentiation of mouse Embryonic Stem (ES) cells, as well as human ES and induced Pluripotent Stem (iPS) cells, into bone, cartilage and endothelial cells for tissue regeneration and gene expression studies.

"GENETICALLY-ENGINEERED MOUSE MODELS ARE COMPARED TO HUMAN DISEASES, SUCH AS CANCER AND PSORIASIS, WITH THE AIM OF IDENTIFYING RELEVANT TARGETS THAT CAN BE DEVELOPED INTO NOVEL THERAPIES BY UTILISING CUTTING-EDGE TECHNOLOGIES, SUCH AS PROTEOMICS, EXPRESSION PROFILING, RNA-SEQUENCING AND CHIP-SEQUENCING."

RESEARCH HIGHLIGHTS

Fos /AP-1 in bone development and sarcomas

Fos proteins – c-Fos, FosB, Fra-1 and Fra-2 – are key regulators of bone development. GEMMs with tet-switchable *Fos* alleles were generated. In osteoblasts, c-Fos expression leads to a reversible increase in bone mass and tumour-like lesions, while manipulating Fra-2 affects systemic metabolism and immune homeostasis. The underlying molecular mechanisms (e.g. the function of the c-Fos target TGF-beta-induced gene, TGFBI) and the regulation of circulating molecules by Fra-2, such as osteocalcin, adiponectin and cytokines, are being dissected.

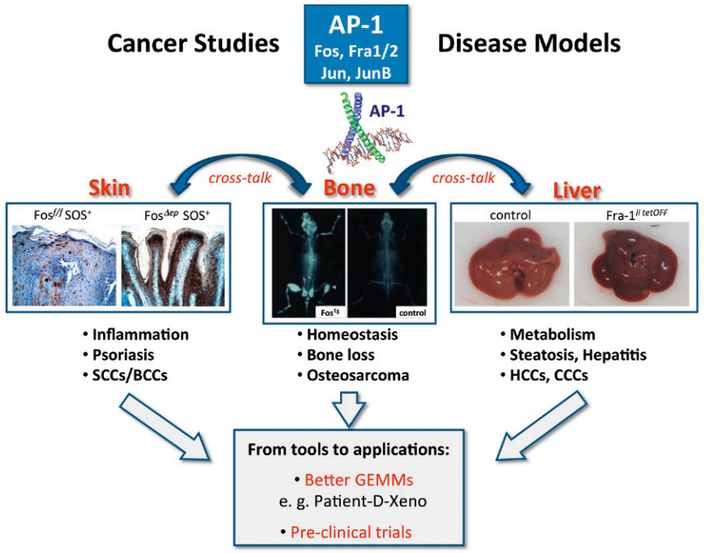

Figure 1 We have generated tet-switchable AP-1 transgenic mice to study the phenotypic consequences of ectopic expression of each specific AP-1 monomer or dimer in the liver, skin and bone. We are utilising cutting-edge technologies, such as proteomics, expression profiling, RNA-sequencing and ChIP-sequencing, to compare mouse models of disease to human disease and identify relevant targets that can be developed into therapies. We are planning preclinical studies in our AP-1-dependent mouse models, using compounds that target the identified molecules and pathways, to determine the potential of translating our findings to treating human disease.

In addition, AP-1 contribution to lung carcinogenesis is being studied in collaboration with the Experimental Oncology Group at CNIO and with Prof. A. Hartmann (Erlangen, Germany). Potential long-range interactions between tumour and host metabolism are also being assessed in cancer GEMMs.

Skin cancer suppression by c-Fos/AP-1

We have unravelled a link between epidermal c-Fos and the Notch pathway. Loss of c-Fos induces p53 expression, thereby activating TACE/ADAM-17, a novel p53 target, and Notch1 leading to skin tumour suppression through induction of differentiation.

Increased c-Fos expression was found in Squamous Cell Carcinomas (SCCs), but not in Basal Cell Carcinomas (BCCs), implying that different signalling networks operate in these common skin cancers. We are modelling SCC development in GEMMs with inducible c-Fos expression and have identified an essential role in modulating immune cell recruitment to the skin, thereby contributing to tumourigenesis.

Epidermis and AP-1-dependent cytokine expression

Epidermal keratinocytes are key decision makers for immune cell functions in the skin. Interestingly, epidermal deletion of Fra-2 leads to enhanced production of the pro-inflammatory cytokine TSLP, thereby inducing skin inflammation and metabolic changes. Mice lacking JunB in the epidermis have inflammation and bone loss (Meixner *et al., Nat Cell Biol,* 2008). We are investigating the crosstalk between skin and bone. Preliminary analyses revealed that JunB represses the expression of pro-inflammatory cytokines, thereby affecting osteoblast activity. We are presently exploring, together with Prof. G. Schett (Erlangen, Germany), the skin-to-bone crosstalk in patients with skin inflammatory conditions.

Genetic and molecular dissection of Psoriasis in mice and humans

Several novel approaches, including genetic – by employing S100A9-deficient mice – and biochemical analyses by proteomics of mouse and human skin samples, were performed to uncover novel pathways and molecules for targeted therapies (reviewed in Wagner *et al., Nat Rev Rheumatol,* 2010; Schonthaler *et al., Ann Rheum Dis,* 2011). Furthermore, the potential role of specific microRNAs in psoriasis pathogenesis is being investigated in collaboration with Dr. E. Daudén (Madrid, Spain) and with Santaris Pharma (Copenhagen, Denmark).

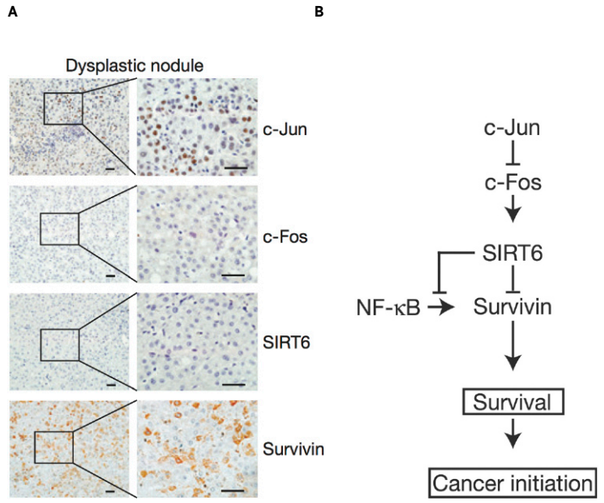

Figure 2 (**A**) c-Jun, c-Fos, SIRT6 and Survivin immunohistochemistry of human liver dysplastic nodules. (**B**) Schematic model of the regulatory network operating at the initiation stage of mouse and human liver cancers. Cell survival, during liver cancer initiation, is controlled by a c-Jun, c-Fos, SIRT6 and Survivin cascade. Survivin expression is regulated by SIRT6, which modulates histone acetylation, and NF-κB signalling at the Survivin promoter. SIRT6 expression is transcriptionally regulated by c-Fos, and c-Jun suppresses c-Fos expression. This pathway does not operate in advanced HCCs. Antagonising this pathway could provide preventive strategies to target pre-cancerous liver lesions.

Role of AP-1 in liver disease and cancer

Gain and loss of function GEMMs are being used to investigate Fos proteins in fibrosis and cancer. In the liver, while expression of Fra proteins had no overt consequences, c-Fos or Fos-containing dimers trigger inflammation, fibrosis and cancer. Conversely, c-Fos deletion in hepatocytes protects from chemically-induced liver cancer.

Using GEMMs, specific for liver cancer initiation, we identified a connection between AP-1, the histone deacetylase SIRT6 and the apoptotic modulator Survivin, which appears relevant to human early-stage Hepatocellular Carcinomas (HCCs); (FIGURE 2). We provide evidence of how AP-1 affects early stages of liver cancer and are suggesting a therapeutically relevant regulatory network. We are currently also dissecting the role of individual AP-1 proteins in hepatitis and steatosis; 2 conditions predisposing to liver cancer.

Stem cell differentiation and tissue regeneration

Human ES and iPS cells are employed to investigate chondro/osteo-progenitor and endothelial cell development. We performed expression profiling of endothelial-like cells, differentiated from human ES cells. Validation of identified genes and functional studies are ongoing in collaboration with the groups of Prof. E. Hofer (Vienna, Austria) and Prof. H. Augustin (Heidelberg, Germany). ■

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- Min L, Ji Y, Bakiri L, Qiu Z, Cen J, Chen X, Chen L, Scheuch H, Zheng H, Qin L, Zatloukal K, Hui L, Wagner EF (2012). Liver cancer initiation is controlled by AP-1 through SIRT6-dependent inhibition of survivin. *Nat Cell Biol* 14, 1203-1211.
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- Gurzov EN, Barthson J, Marhfour I, Ortis F, Naamane N, Igoillo-Esteve M, Gysemans C, Mathieu C, Kitajima S, Marchetti P, Orntoft TF, Bakiri L, Wagner EF, Eizirik DL (2012). Pancreatic ß-cells activate a JunB/ATF3-dependent survival pathway during inflammation. *Oncogene* 31, 1723-1732.
- Cuello S, Ximénez-Embún P, Ruppen I, Schonthaler HB, Ashman K, Madrid Y, Luque-Garcia JL, Cámara C (2012). Analysis of protein expression in developmental toxicity induced by MeHg in zebrafish. *Analyst* 137, 5302-5311.

EPITHELIAL CELL BIOLOGY JUNIOR GROUP

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OVERVIEW

In adult skin, epithelial progenitor cells have been identified as the cells of origin of skin carcinomas; the most common cancers in the world. Epithelial progenitor cells reside in the basal proliferative layer of the epidermis, whereas in the hair follicle they localise to a restricted area known as the bulge. Using skin as a model system, our Research Group aims to understand how the interactions between epithelial progenitor cells contribute to the formation and maintenance of tissue architecture. In addition, we are exploring how epithelial cells interact with their surrounding microenvironment and sustain tissue homeostasis. The results of our research will allow us to understand how these processes are regulated during homeostasis and tissue repair, and how they become disrupted in skin diseases, including cancer.

"OUR RESEARCH IS EXTENDING THE UNDERSTANDING OF THE MECHANISMS THAT CONTROL SKIN ARCHITECTURE, SELF-RENEWAL AND REPAIR UPON INJURY. OUR WORK UNCOVERS NOVEL ASPECTS UNDERLYING TUMOURIGENESIS AND PAVES THE WAY FOR THE FUTURE DEVELOPMENT OF REGENERATIVE AND ANTI-CANCER THERAPIES."

RESEARCH HIGHLIGHTS

Role of cell adhesion and polarity complexes in the maintenance of cell polarity and epidermal architecture

Adherens junctions control the interactions between cells through their connections with the cytoskeleton. As yet, it remains unclear which molecules are responsible for the interaction of adherens junctions with the microtubule cytoskeleton in the skin. We have found a novel interaction between the adherens junction protein p120-catenin and the microtubule binding protein CLASP2, which is required to sustain proper cell-cell adhesion and skin architecture. In addition, in collaboration with the Cell Division and Cancer Group of Marcos Malumbres at the CNIO, we have identified a previously unrecognised role for the mitotic protein Aurora A kinase in the control of the polarised epidermal cell architecture and potentially also in the regulation of the orientation of mitotic divisions of progenitor basal cells in the epidermis.

Role of cell adhesion proteins in the regulation of cell migration and inflammation during tissue repair

The adherens junctions protein p120-catenin can regulate the transmission of signals from epithelial cells across the surrounding macro-environment of the skin. Our recent findings have revealed that p120-catenin controls epithelial cell migration through its crosstalk with the immune response, and that loss of p120-catenin leads to chronic inflammation and cancer.

Role of immune cells in the maintenance of skin progenitor cells in skin homeostasis and disease

Skin epithelial stem cells function within a complex macro-environment that orchestrates their lifetime regenerative properties. However, their interplay with immune cells is not well understood. We have identified a novel link between macrophages and skin progenitor cells, which modulates their stem cell properties and regenerative potential. This is an important step to decipher how such connections are implicated in cancer. ■

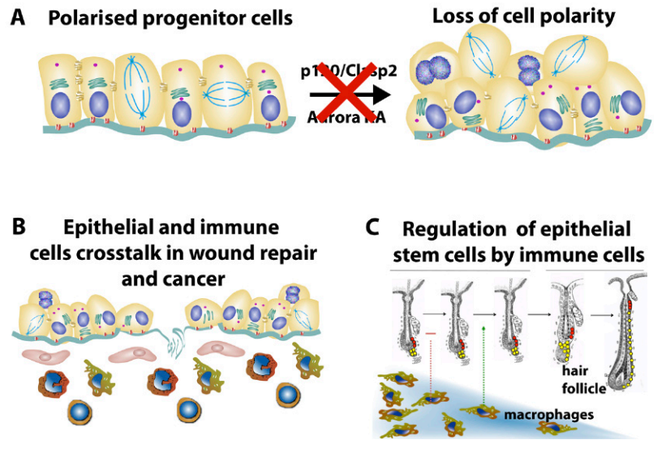

Figure Mechanisms involved in the control of skin architecture, self-renewal and repair. (**A**) Cell adhesion and polarity in epidermal architecture. (**B**) p120 in cell migration and inflammation during skin repair. (**C**) Crosstalk between macrophages and skin progenitor cells in skin homeostasis and disease.

PUBLICATIONS

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- Epifano C, Pérez-Moreno M (2012). Crossroads of integrins and cadherins in epithelia and stroma remodeling. *Cell Adh Migr* 6, 261-273.

GROWTH FACTORS, NUTRIENTS AND CANCER JUNIOR GROUP

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"WE ANTICIPATE THAT THE GENERATION OF NEW MECHANISTIC MODELS FOR URI FUNCTION WILL FACILITATE THE DEVELOPMENT OF INNOVATIVE AND MECHANISM-BASED THERAPEUTICS FOR THE TREATMENT OF METABOLIC DISEASES AND CANCER."

OVERVIEW

Malnutrition has been cited by the World Health Organization (WHO) as the greatest single threat to global public health. Inadequate amounts and/or diversity of food represent the most important risk factor for illness and death. Ever since Western society has shifted to a higher caloric diet and sedentary lifestyle, the incidence of metabolic syndromes and cancer has increased to epidemic proportions.

We are interested in better understanding the contribution of growth factors and nutrients in development of metabolic disease-associated cancer and in elucidating molecular mechanisms that impact on disease patho-physiological states.

In this regard, we aim to decipher the mammalian target of rapamycin (mTOR)/ribosomal protein S6 kinase 1 signalling pathways that integrate growth factor and nutrient signalling inputs. Thus, a major part of our work focuses on URI, a downstream effector of mTOR/S6K1 axis.

RESEARCH HIGHLIGHTS

We are studying diseases associated to liver, intestine and pancreas dysfunctions. As the three organs are physiologically interconnected and influenced through their exocrine and/or endocrine functions, nutrients overload dysregulate their functions affecting the balance of the whole body energy metabolism and leading to severe metabolic disorders-associated cancer.

In vivo URI function and regulation

We have generated 2 knock-in mouse models for wild-type URI and an S371A URI mutant that allow switchable ectopic gene expression, as well as a URI conditional knock-out mouse. Using these mice, we are currently studying URI function and regulation *in vivo*. Finally to understand the contribution of nutrients and growth factors on URI phosphorylation, we are devoting a lot of effort to analyse the interplay between the post-translational modifications O-GlcNAcylation and phosphorylation. We demonstrated a strong correlation between O-GlcNAcylation and URI expression in Hepatocellular Carcinoma (HCC) samples.

Structural analysis of the URI prefoldin complex

We are collaborating with CNIO's Macromolecular Crystallography Group to determine the crystal structure of the URI prefoldin complex.

Novel components of growth factor and nutrient circuits

Since a dynamic exists between phosphorylation of URI and binding of PP1, we created a URI-based fluorescence resonance energy transfer (FRET) system containing the 14-3-3 Ser/Thr phospho-recognising domain. The URI-based FRET system will represent a new biological tool to interrogate spatial and temporal mTOR/URI activation. Through different high-throughput cellular screening, this approach will allow us to identify new components upstream of the mTOR pathway.

In vivo function and regulation of microspherule protein 1

We also discovered microspherule protein 1 (MCRS1) as a novel regulator of mTOR. We gathered evidence that MCRS1 is highly expressed in human colon cancer and that its expression correlates with poor prognosis. We generated MCRS1 mouse models (loss-and gain-of-function) to obtain insights into its role in colon cancer. ■

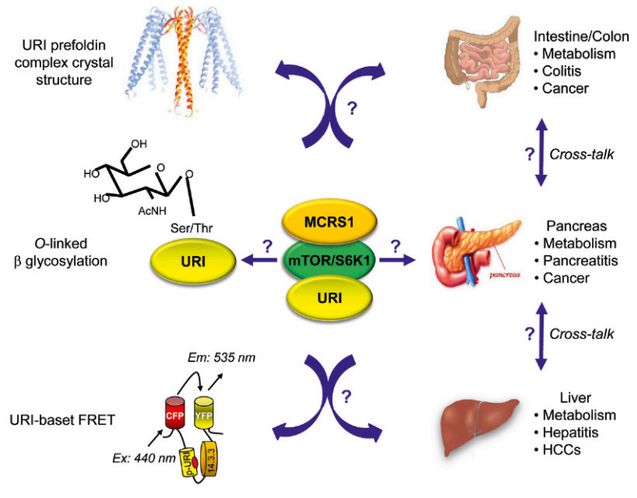

Figure Scheme illustrating present and future research of our Group at the CNIO, with the aim to better understand URI/MCRS1 function and regulation in the liver, colon and pancreas.

SEVE BALLESTEROS FOUNDATION-CNIO BRAIN TUMOUR JUNIOR GROUP

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(since October)
Junior Group Leader

Graduate Student
Carolina Almeida (since October)

Massimo Squatrito ITA

Carolina Almeida PRT

OVERVIEW

Gliomas are a large group of brain tumours. Glioblastoma Multiforme (GBM), the highest grade of malignant astrocytomas (WHO grade IV), is the most common and lethal primary central nervous system tumour in adults. Despite recent advances in treatment modalities, GBM patients generally respond poorly to all therapeutic approaches and prognosis remains dismal.

Maintenance of genomic integrity is essential for embryonic development and adult tissue homeostasis. Defects in the DNA-damage response (DDR) machinery, a network of protein complexes capable of detecting DNA lesions and signalling to downstream effector pathways, are linked to numerous pathological states including cancers. In particular, the nervous system is often greatly affected by DDR deficiency (mainly in the DNA repair machinery), which can lead to neurodegeneration, microcephaly or brain tumours.

"THE MAIN FOCUS OF OUR GROUP IS TO UNCOVER THE GENETIC ALTERATIONS PRESENT IN GBM PATIENTS THAT MIGHT ALSO BE RESPONSIBLE FOR THE MODEST TREATMENT RESPONSE OF THIS TUMOUR TYPE, WITH PARTICULAR ATTENTION PAID TO THE IDENTIFICATION OF THE GENETIC DEFECTS THAT LEAD TO THE MODULATION OF THE ACTIVITY OF THE DNA DAMAGE RESPONSE."

RESEARCH HIGHLIGHTS

53BP1 tumour suppressor activity in Glioblastoma Multiforme

We recently showed that p53-binding protein 1 (53BP1), a key element of the DDR, is heterozygously lost in approximately 20% of human GBM specimens, primarily of the proneural subtype; moreover, low 53BP1 expression levels were associated with poor prognosis. 53BP1 behaves as a haplo-insufficient tumour suppressor in a mouse model of PDGF-induced gliomagenesis. Very low levels of 53BP1, as found in 53BP1 null gliomas, or robust 53BP1 gene silencing in glioma cell lines (but not 53BP1 heterozygous tumours or partial gene knock-down) sensitises glioma cells to ionising radiation, both *in vitro* and *in vivo*. This is due to the modulation of the non-homologous end-joining (NHEJ) DNA repair pathway. Our data suggest that either 53BP1 or other NHEJ components may be key molecular targets for pharmacological treatment in GBM in combination with standard therapies.

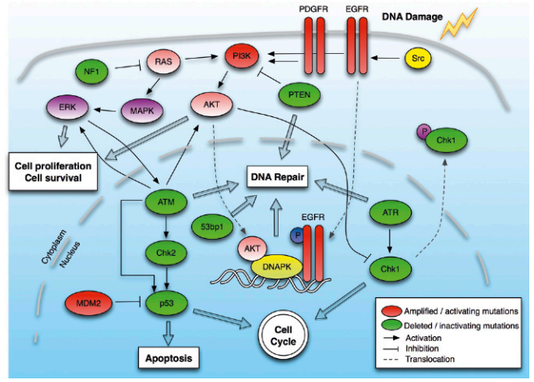

Figure DNA damage response in Glioblastoma Multiforme.

Myeloid Elf-1 like factor promotes stemness in the pathogenesis of gliomas

GBMs are very heterogeneous tumours that contain both neoplastic and non-neoplastic cells, including astrocytes, endothelial, stromal and inflammatory cells. A fraction of cells within the tumour, identified as glioma stem-like cells (GSCs), share some common features with normal neural stem cells (NSCs); they are multipotent and have the property of self-renewal. Indeed, acquisition of stem-like characteristics most likely contributes to the malignant nature of high-grade gliomas and may be responsible for the initiation, growth and recurrence of these tumours. We have shown that MEF (myeloid Elf-1 like factor, also known as ELF4), a member of the ETS family of transcription factors, contributes to gliomagenesis and promotes stem-like characteristics. MEF is highly expressed in both human and mouse GBMs, and GBM patients with low levels of MEF show a significantly better overall survival. Our data suggest that MEF plays a role in promoting stem-like traits, which might reflect changes in the stem cell signature of both neoplastic and non neoplastic cells. ■

PUBLICATIONS

Publications at other institutions

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- Squatrito M, Vanoli F, Schultz N, Jasin M, Holland EC (2012). 53BP1 is a haploinsufficient tumor suppressor and protects cells from radiation response in glioma. *Cancer Res* 72, 5250-5260.
- Fernández-L A, Squatrito M, Northcott P, Awan A, Holland EC, Taylor MD, Nahlé Z, Kenney AM (2012). Oncogenic YAP promotes radioresistance and genomic instability in medulloblastoma through IGF2-mediated Akt activation. *Oncogene* 31, 1923-1937.
- Katz AM, Amankulor NM, Pitter K, Helmy K, Squatrito M, Holland EC (2012). Astrocyte-specific expression patterns associated with the PDGF-induced glioma microenvironment. *PLoS One* 7,e32453.

Article in press

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STRUCTURAL BIOLOGY AND BIOCOMPUTING PROGRAMME

ALFONSO VALENCIA PROGRAMME DIRECTOR

The objective of the Structural Biology and Biocomputing Programme is to decipher the mechanisms behind the molecular systems that are of key importance for cancer. The strength of our Programme resides in its capacity to combine computational, biochemical, biophysical and structural approaches. Our Programme is deeply involved in collaborations with basic and translational Research Programmes at the CNIO, as well as in a number of international large-scale research consortia.

Our 2 main research goals are: i) understanding – at the mechanistic level – the role of protein complexes in cell cycle control, DNA repair, genomic stability and growth factor signalling, and ii), predicting the consequences of mutations associated to cancer at the levels of protein stability, conformational dynamics, allosteric regulation and enzymatic function. Our recent research efforts focus on single and compensatory mutations as well as on the study of alterations in splicing patterns.

Research highlights of this year range from genomic/proteomic to protein dynamic studies. In particular, the Programme has contributed to exploring the consequences of splicing and trans-splicing and the association of these events with cancer. Additionally the Programme contributed to the combined biochemical and structural analysis of the activation of Focal Adhesion Kinase (FAK) by phosphatidylinositol 4,5-bisphosphate (PIP2), the binding of the transcription activator-like effector AvrBs3 to its DNA target, and the function of the MuB AAA+ ATPase in targeted recombination. Finally, it is worth mentioning our work pertaining to the modelling of conformational flexibility of oncogenic non-receptor tyrosine kinases, such as c-Src and c-Abl, and the exploration of the implications for drug binding. ■

"THE WORK OF OUR PROGRAMME – ON THE TRANSLATION OF BIOMEDICAL OBSERVATIONS INTO DETAILED MODELS THAT CAPTURE THE MECHANISM OF THE UNDERLYING MOLECULAR INTERACTIONS – CONSTITUTES ESSENTIAL SCIENTIFIC PROGRESS AND A FUNDAMENTAL CONTRIBUTION TO FUTURE MEDICAL APPLICATIONS."

STRUCTURAL COMPUTATIONAL BIOLOGY GROUP

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OVERVIEW

The main interest of our Group lies in the mechanistic understanding of cancer progression by combining molecular and evolutionary approaches. Our research focuses on the problem of functional specificity and selective molecular interactions in the context of cancer genome research. The strategic goals of the Structural Computational Biology Group are: i) to analyse the function, structure and specific interactions of proteins related to cancer; ii) to develop novel methods and software platforms for the extraction, integration and representation of cancer genomic data, including the statistical analysis of molecular, genomic, epigenomic and phenotypic information in collaboration with cancer genome projects; and iii), to design the next generation of computational methods for the interpretation of personalised cancer genome information.

"OUR RESEARCH IN 2012 HAS PROVIDED A FIRST IN-DEPTH OVERVIEW OF THE LANDSCAPE OF PROTEIN ISOFORM PRODUCTS OF SPLICING AND TRANS-SPLICING, POINTING TO THEIR POSSIBLE BIOLOGICAL FUNCTIONS AND THEIR IMPORTANCE IN CANCER. THE ANNOTATION OF SPLICING ISOFORMS IS AN ESSENTIAL COMPONENT OF THE ENCODE PROJECT THAT AIMS TO GENERATE A HIGH-RESOLUTION MAP OF THE HUMAN GENOME."

RESEARCH HIGHLIGHTS

Splicing and trans-splicing

The splicing of messenger RNA exons (mRNA splicing) is a crucial part of the process by which genomic information is translated into proteins. The inclusion (or not) of exons from unprocessed messenger RNA into the mature RNA transcript is regulated by a process called alternative splicing. This combination of exons can generate a wide range of mature RNA transcripts that may, in turn, be translated into a wide range of functionally different proteins. There is compelling evidence that virtually all genes are capable of expressing multiple distinct RNA variants. The deregulation of splicing and production of chimeric transcripts has been associated with cancer in a very recent study of the Spanish consortium that explores the genome of Chronic Lymphocytic Leukaemia (CLL) within the framework of the International Cancer Genome Consortium (ICGC).

This year, our Group has continued the analysis of the extension and functional significance of splicing. We have published the first version of the APPRIS system for the annotation and evaluation of splice isoforms. APPRIS is used for the annotation of the human genome in collaboration with the HAVANA annotation team at the Sanger Institute, and it is part of the ENCODE/GENCODE NIH-funded project. The results of our system have been published as a contribution to this consortium. The complete ENCODE project is considered as one of the world's main scientific highlights of 2012, if not 'the' main one.

In a complementary research effort, we have analysed the actual presence of the proteins corresponding to splice isoforms by scanning in-depth current mass spectrometry databases. This effort has resulted in the realisation of the largest current collection of genes for which protein evidence is available (85% of the genome) and the first complete set of genes for which there is evidence of the existence of 2 or more protein isoforms. The analysis of these isoforms shows that only very similar isoforms are simultaneously expressed, pointing to a specific selection of the expressed isoforms. These results have been highlighted by the *Science* magazine as the main conclusion of our systematic findings.

While splicing can be considered a classical subject in molecular biology, we have had the opportunity of entering into a newer and more exciting genomic perspective of RNAs containing information from more than one gene. These chimeric RNAs could be the result of chromosomal rearrangements or, perhaps, trans-splicing events.

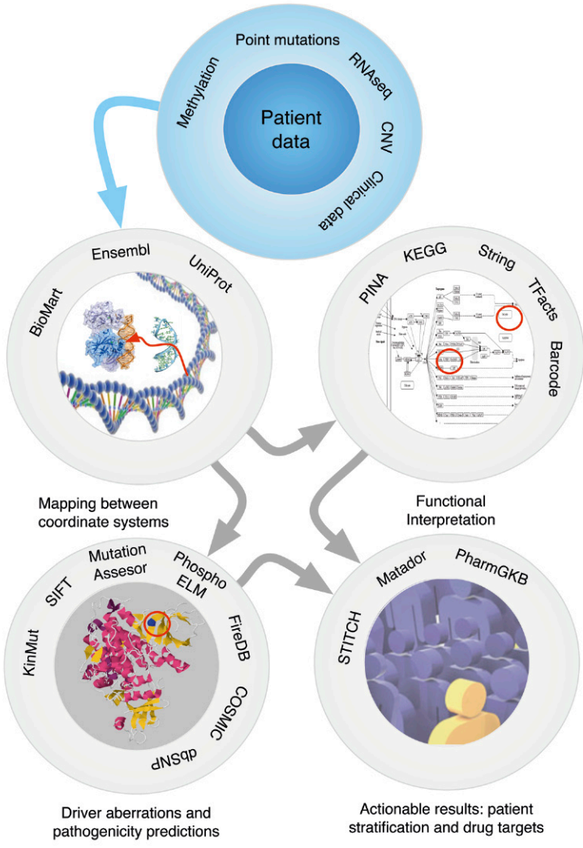
The complementary *ChiTaRS* database contains the, as yet, largest collection of chimeric RNAs: 16,000 chimeric RNAs, with links to the supporting experimental information (confirmed by RNA-seq reads and mass spectrometry data) and references to the cytogenetic breakpoints as well as cancer-related information. This information opens new doors for research on trans-splicing at the genomic scale as well as for the follow-up of new chimeras associated to specific cancer types that could be of clinical relevance.

Cancer genome analysis

In 2012, we have continued developing ground-breaking methods for the analysis of complex cancer genome data. In particular, we have developed novel approaches to improve the use of molecular networks for the functional interpretation of variation data, taking into account the organisation of such interaction networks.

On the application side, we have extended our platform for the systematic analysis of cancer genomes. The system includes novel rules for the prediction of the effects of mutations, an integrated statistical system for the prediction of altered pathways and molecular networks, and a new system for the comparative analysis with previous studies. In collaboration with the Spanish CLL-ICGC consortium, we have applied this analysis framework to the study of CLL. Furthermore, the system is in use for the analysis of the cancer genomes of patients included in the CNIO personalised cancer treatment initiative spearheaded by the Clinical Research Programme. ■

Figure Components of our cancer genome analysis platform. The platform has been designed to support in-depth analysis of cancer genomes by providing an integral view ranging from the genome information to medical actionable items. The beta version, released in 2012, has been used in the context of the Spanish CLL-ICGC project and is a core component of the CNIO personalised cancer treatment initiative led by the Clinical Research Programme. In this schematic view of the system, the first circle corresponds to input genomic information. In the next step, this information is embedded in the context of the main genomic and cancer-specific resources. The following steps include the interpretation at the level of the consequences of individual mutations and their possible impact on pathways, networks and regulatory systems. Finally, the affected genes and systems are used to highlight potentially related drugs, using resources that connect genes, proteins and signalling pathways with information on drugs and clinically relevant observations.



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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. The focus of our Group is 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 in an unparalleled view into the design principles of living systems at levels that span from the 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 AND THE MOLECULAR UNDERSTANDING OF THEIR INTERACTIONS HELP US TO DECIPHER THE MECHANISMS OF THE ONCOGENIC PROCESS, PAVING THE WAY FOR NEW THERAPEUTIC APPROACHES.”

S-Phase and replication

Proper DNA replication is indispensable for reliable inheritance of the genome during each cell division. To which extent this process uses similar mechanisms in bacteria and complex organisms is still under debate. Higher eukaryotic organisms add extra complexity as they require supplementary factors to cope with larger genomes, the fidelity of genome duplication and a wide range of different cell fates. To become duplicated, 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 become active during each cell cycle. To initiate replication, a number of protein complexes are assembled 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 required 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 (FIGURE 1). This study has helped us to propose a working mechanism for the helicase that may have important implications for our understanding of 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.

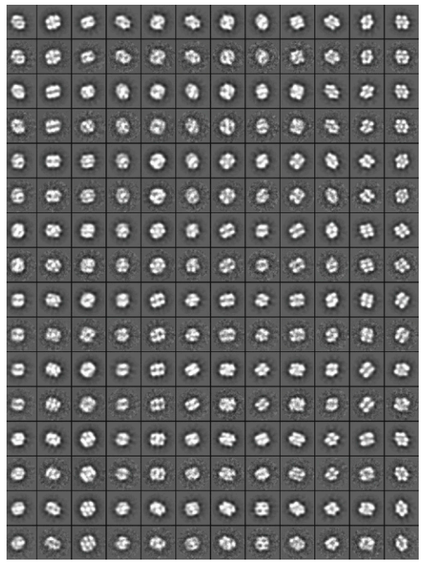

Figure 1 Electron microscopy: 2D averaged views of a 750 kDa macromolecular machine involved in chromosome replication. Data were collected using a FEI-Technai12 equipped with a CMOS camera recently installed at CNIO.

Mitotic complexes

Cellular growth and division are regulated by an integrated protein network that ensures the genomic integrity of all eukaryotic cells during mitosis. These processes involve a completely different set of genes that serve diverse functions ranging from cell motility to cell growth, genome replication, genome maintenance, etc. However, all these genes are interconnected through cellular crossroads and share common cellular homeostatic mechanisms. A protein complex that performs such homeostatic function is CCT (also known as TriC); a eukaryotic macromolecular complex that controls the folding of many essential mitotic regulators such as Cdc20, Cdh1, PP2A regulatory subunits, CyclinE, tubulin, and many more. Upon solving the structure of this macromolecular machine at 5.5 Å in complex with tubulin – one of its main substrates – we have proposed a mechanism for this molecular machine. Our future goal is to obtain high-resolution information regarding the atomic structure of this protein complex and to dissect its regulation by using site-directed mutagenesis. We have already expressed and crystallised the human complex.

Structural design of protein-DNA interactions for gene targeting

We have extended our previous work on homing endonucleases to a new protein-DNA binding scaffold: the TALE (Transcription Activator-Like Effector) proteins that contain a DNA binding domain constituted by 33-35 nucleotide tandem repeats. The assembly of several repeats by redesigned TALE that recognise new DNA targets has confirmed the modularity of these DNA binding domains. Their heterodimeric binding mode to adjacent DNA target sites in specific chromosomal loci, together with the fusion of these scaffolds with the catalytic domain of FokI, eventually generates a double-strand break that can be repaired through homologous recombination. These scaffolds can present new perspectives for a wide range of applications, such as the correction of mutations linked with monogenic inherited diseases. Our Group has solved the crystallographic structures of different variants, revealing the molecular basis of new target DNA recognition domains (FIGURE 2). In addition, we have shown that the repair of the damaged gene can be done at its locus in human cells, opening up new avenues to possible therapeutic applications. ■

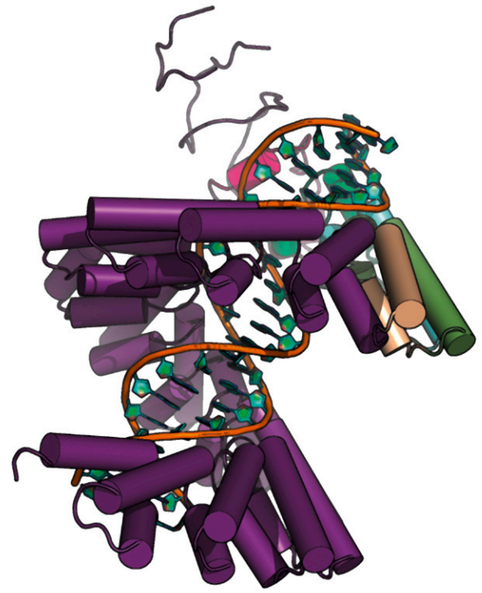

Figure 2 Crystal structure of a protein-DNA complex revealing a new protein DNA binding domain. The protein forms a solenoid that specifically recognises the forward DNA strand sequence.

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OVERVIEW

Our Group is a key player in the field of kinase dynamics as well as in the development of computational methods to calculate the free energy landscapes that are associated with conformational change, ligand binding and allosterism. The use of advanced nuclear magnetic resonance (NMR) techniques, together with convergent free energy calculations, sheds light on the functional consequences of oncogenic mutations in terms of changing the conformational landscape and favouring active *versus* inactive states of kinases in the context of cancer. This in-depth knowledge is useful to rationally design inhibitors that selectively target mutant kinases, and to understand the mode of action of allosteric kinase inhibitors.

"WE PLAYED A KEY ROLE IN ELUCIDATING THE MODE OF ACTION OF THE FIRST GENERATION OF ALLOSTERIC RECEPTOR TYROSINE KINASE INHIBITORS AND CONTRIBUTED TO THE DESIGN OF MORE POTENT DERIVATIVES, AIMED AT INTERCEPTING KEY PATHWAYS IN MANY TYPES OF CANCER."

RESEARCH HIGHLIGHTS

In collaboration with Sanofi-Aventis and several other academic partners, we elucidated the mode of action of the first allosteric inhibitor of a receptor tyrosine kinase.

Using a combination of computer simulations – NMR and mutagenesis – we demonstrated the role of flexibility in the thermodynamic penalty of Imatinib binding to the proto-oncogene tyrosine-protein kinase cSrc and in the emerging Imatinib resistant mutants of the Abl kinase fusion gene, causative of Chronic Myeloid Leukaemia (CML).

In a successful collaboration with CNIO's Experimental Therapeutics Programme, we have shown that, in the case of the phosphatidylinositol 3-kinase (PI3K) isoform PI3K-γ, molecular recognition of drug-like inhibitors can be better described as a combination of large scale conformational selection and locally induced-fit mechanisms. This finding has fundamental consequences, both for our understanding of molecular recognition processes, and for the rational design of PI3K inhibitors.

Obtaining accurate free energy profiles of ligand-target association along realistic association pathways is a long-sought after goal in rational drug design. Its importance stems from the fact that from these profiles, both kinetic and thermodynamic information can be obtained. There are many challenges, ranging from the difficulty of sampling the flexibility of the target, to the need of a good model for water, and to making the approximations for designing the ligand and target force-fields. We have shown, for the case of the mitogen activated protein kinase p38, that our recently developed free energy approaches overcome the most important problems and provide useful information for the design of novel inhibitors. ■

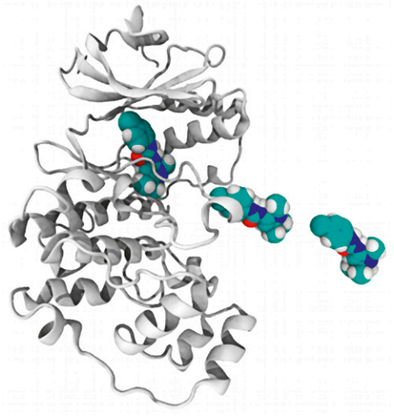

Figure Representation of the most likely path of ligand binding to the p38 mitogen-activated protein kinase.

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OVERVIEW

Our Group is interested in molecular mechanisms of cell signalling, in particular, those triggered by growth and adhesion cues. Such signalling events control important cellular programmes such as cell proliferation, growth, adhesion and survival. We use X-ray crystallography in combination with biochemical techniques to understand these signalling events at atomic resolution. The signalling components involved are important cancer targets, hence we utilise our structural information for the structure-based discovery of anti-cancer therapeutics.

Many of these signalling events occur at the plasma membrane. We focus on three related systems: i) how does Phosphatidylinositol 4,5-bisphosphate (PIP_2) activate focal adhesion kinase (FAK); ii) how does Phosphatidylinositol (3,4,5)-trisphosphate (PIP_3) induce activation of the serine/threonine kinase Akt/protein kinase B (Akt/PKB); and iii), how are the SH2-domain-containing inositol 5-phosphatases (SHIP1, SHIP2) regulated to reduce PIP_3 levels in the plasma membrane.

"WE HAVE MADE SUBSTANTIAL PROGRESS IN UNDERSTANDING HOW THE CANCER TARGETS FAK, AKT/PKB AND SHIP ARE REGULATED. FOR FAK WE HAVE DESIGNED A SCREENING METHOD TO DISCOVER ALLOSTERIC FAK INHIBITORS, AND WE HAVE PERFORMED FRAGMENT SCREENING TO OBTAIN SMALL MOLECULES INTERACTING WITH ALLOSTERIC SITES IN FAK."

RESEARCH HIGHLIGHTS

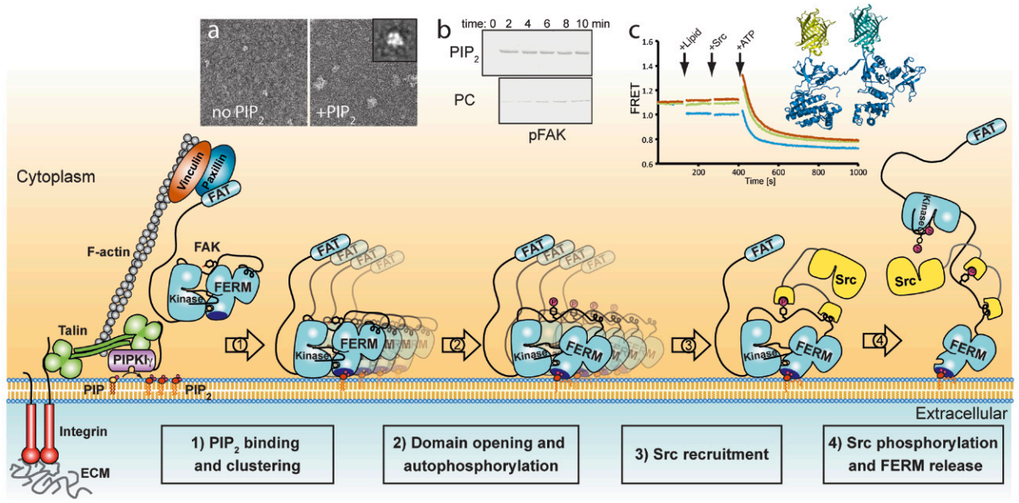

Figure Integrin signalling results in formation of focal adhesions and local generation of PIP_2 (left). PIP_2 induces a multistep activation mechanism of FAK (steps 1-4). Upper insets are electron micrographs of FAK clusters (**a**), FAK autophosphorylation blots (**b**), and conformational changes monitored by FRET (**c**).

Focal Adhesion Kinase

Focal Adhesion Kinase (FAK) is activated by integrin and growth factor receptors, and provides signals for cell migration and survival. In cancer, FAK is heavily implicated in disease progression and metastasis. Auto-inhibited FAK adopts a closed conformation with the kinase bound to the regulatory protein 4.1-ezrin-radixin-moesin (FERM) domain. Employing a highly interdisciplinary approach, including cell biology, electron microscopy, crystallography, biochemistry, *in vitro* Fluorescence Resonance Energy Transfer (FRET) and molecular dynamics simulation, we describe the following FAK activation mechanism (FIGURE). Cell adhesion via integrin receptors results in local production of the phosphoinositide PIP_2. PIP_2 then binds to a basic patch on the FAK FERM domain and thereby induces clustering of FAK on the cell membrane. In these clusters, FAK adopts a partially open conformation, in which the linker that contains the autophosphorylation site is released. Together with clustering, this event promotes highly efficient trans-autophosphorylation. The autophosphorylation site provides a docking site for the proto-oncogene tyrosine-protein kinase Src, and recruited Src phosphorylates the FAK activation loop. Src phosphorylation results in release of the FERM domain and full activation of FAK.

Having a sound structural understanding of FAK regulation, we are now pursuing the discovery of allosteric FAK inhibitors. In one approach we have developed a screening assay for allosteric FAK inhibitors using a conformational FRET sensor (which we also used in mechanistic studies to probe the FAK conformation). In an alternative approach, we have performed nuclear magnetic resonance (NMR)-based fragment screening. We are now analysing several positive hits by X-ray crystallography.

SH2-domain-containing inositol 5-phosphatases

SH2-domain-containing inositol 5-phosphatases (SHIP) remove the 5-phosphate from PIP_3 to produce phosphatidylinositol (3,4)-bisphosphate (PI (3,4) P2) and are therefore important regulators of the PI3K-Akt/PKB signalling pathway. We have solved a crystal structure of a SHIP fragment containing the catalytic and a C2 domain. We are currently performing biochemical analysis which, together with the crystal structure, aims to reveal the regulation mechanism of this enzyme. ■

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OVERVIEW

Safeguarding genome integrity is essential in order to maintain correct cell function and to prevent cancer. At the same time, genome integrity can be an Achilles heel of tumour cells; exacerbating the problems derived from a fast replication rate owing to nucleotide pool depletion, replicative stress or aneuploidy, may provide novel opportunities to fight tumours. Our Group is interested in deciphering molecular mechanisms that affect the integrity of the genome, such as the production of pyrimidine nucleotides, site-specific DNA recombination or the maintenance of chromatin architecture. These processes depend on the assembly of large and dynamic macromolecular complexes. We combine protein engineering, X-ray crystallography and single-particle electron microscopy, together with biochemical and functional studies, to understand the architecture, catalysis and regulatory mechanisms of these protein-protein and protein-DNA complexes.

"WE DETERMINED THE ATOMIC STRUCTURES OF HUMAN DIHYDROOROTASE AND ASPARTATE TRANSCARBAMYLASE, PROVIDING A DETAILED VIEW OF THE CELLULAR MACHINERY FOR PYRIMIDINE SYNTHESIS. OUR FINDING THAT MUB PROTEIN IS AN AAA+ ATPase THAT FORMS HELICAL FILAMENTS ON THE DNA, INCREASES OUR UNDERSTANDING OF DNA TARGET SELECTION FOR RECOMBINATION."

RESEARCH HIGHLIGHTS

Deciphering CAD, the masterpiece in the biosynthesis of pyrimidine nucleotides

Pyrimidine nucleotides are essential building blocks for nucleic acid synthesis and DNA repair. In resting cells, the need for pyrimidines is largely covered by recycling preformed nucleotides, whereas in rapidly proliferating tumour cells *de novo* synthesis of pyrimidines is essential to cover the increased demand of DNA and RNA synthesis, converting this biosynthetic pathway into a potential target for anti-tumoural drugs. In animals, pyrimidine biosynthesis is controlled by CAD, a ~243 kDa multifunctional polypeptide that harbours the first 3 enzymatic activities of the pathway: glutamine-dependent carbamyl phosphate synthetase (GLN-CPS), aspartate transcarbamylase (ATC) and dihydroorotase (DHO).

Until now, there was no direct structural information on CAD, except that it associates forming a homohexamer of ~1.5 MDa that, for unclear reasons, shuttles between nucleus and cytoplasm and is under a strict allosteric control through phosphorylation. Now, we have determined the crystal structures of the DHO and ATC domains of human CAD. These 3D structures, combined with mutagenesis and biochemical studies, allow us to understand one half of the structure of CAD and 2 out of 3 reactions catalysed by the complex. Our future work aims to solve the structure of the GLN-CPS domain in order to reconstruct the architecture of the large CAD oligomers, and to exploit such detailed structural knowledge for the development of potential anti-tumoural compounds.

Basic mechanisms of DNA recombination

We have extended our work on V(D)J recombination to other DNA translocation systems, and in particular, to the mechanism by which MuB protein selects DNA for recombination. We combined bioinformatic, mutagenic, biochemical and electron microscopy techniques to characterise the structure and function of MuB and how it binds to DNA. We discovered that MuB is a new member of the AAA+ ATPase family and identified critical residues for ATPase activity, DNA binding and polymerisation. Indeed, we found that MuB forms helical filaments that coat the DNA, and reconstructed the 3D structure of the nucleoprotein filament. These findings led us to propose a model that explains how an AAA+ ATPase can impose an intricate control on DNA capture for recombination. ■

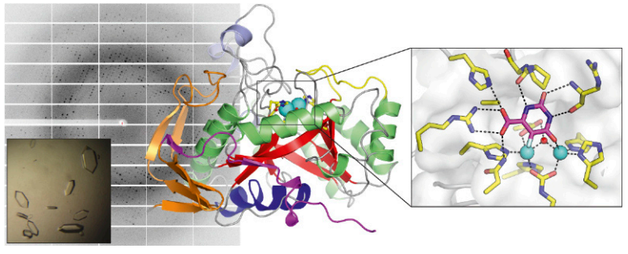

Figure Structure of the DHO domain of human CAD determined by X-ray crystallography. The 3D model is built from X-ray diffraction data collected from protein crystals at synchrotron particle accelerators. On the right, a detailed view of an inhibitor bound to the active site of the protein.

PUBLICATIONS

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SPECTROSCOPY AND NUCLEAR MAGNETIC RESONANCE CORE UNIT

Ramón Campos-Olivas
Core Unit Head

Technician
Blanca López (since March)

Ramón Campos-Olivas ESP

Blanca López ESP

OVERVIEW

The Unit was created in 2008 to unify the technical and scientific management of Nuclear Magnetic Resonance Spectroscopy (NMR) and other biophysical instrumentation available at the Structural Biology and Biocomputing Programme. It provides CNIO researchers with instrumentation and technical support for a variety of optical spectroscopy and other biophysical techniques. This includes NMR for the *in vitro* characterisation of biomolecules (proteins in particular) and their interactions with other biomolecules, as well as with small molecules that could represent initial hits in the drug discovery process or serve as research compounds for biophysical and functional studies. Furthermore, we use NMR to characterise the metabolic profiles of biofluids, cell growth media and intracellular extracts, as well as of intact cells and tissues from both animal models for cancer and human samples.

"WE HAVE IMPLEMENTED PROTOCOLS FOR THE CHARACTERISATION OF METABOLITES THAT PROVIDE ESSENTIAL INFORMATION FOR THE UNDERSTANDING OF METABOLIC REPROGRAMMING IN CANCER. OUR WORK CONTRIBUTES TO THE IDENTIFICATION OF METABOLIC ENZYME TARGETS FOR DRUG DISCOVERY AND METABOLIC MARKERS FOR CANCER DIAGNOSIS AND PROGNOSIS."

RESEARCH HIGHLIGHTS

Our Core Unit incorporates a broad range of instrumentation for the biophysical characterisation of biomolecules and their interactions, including spectrophotometers, a fluorimeter, isothermal titration and differential scanning calorimeters, a circular dichrograph, a surface plasmon resonance machine, analytical ultracentrifugation, and a recently installed multiple-angle light scattering apparatus. Research groups mostly from, but not limited to, the Structural Biology and Biocomputing Programme have extensively used these technologies throughout 2012.

In addition, the Unit has 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 screening of small molecule protein binders (in collaboration with the CNIO's Structural Biology and Biocomputing and Experimental Therapeutics Programmes), as well as for metabonomics measurements that are performed in collaboration with the Lilly-CNIO Cell Signalling Therapies Section, the Growth Factors, Nutrients, and Cancer Junior Group, the Tumour Suppression Group, and the Breast Cancer Junior Clinical Research Unit (from the Experimental Therapeutics, Cancer Cell Biology, Molecular Oncology, and Clinical Research Programmes, respectively). Together with these groups, we have implemented sample preparation protocols and developed spectroscopic and analysis technology to characterise the metabolites present in different biological samples. As part of *REDLAB* – a research laboratory network in the Autonomous Community of Madrid – the Unit also offers all the above mentioned research technologies to the wider research community.

To illustrate the research activities of the Core Unit in 2012, the FIGURE shows the identification of a small fluorinated molecule binding to the Src kinase catalytic domain (SrcKD), a *bona fide* cancer target; the regulation of SrcKD is relevant to multiple aspects of tumour biology. ■

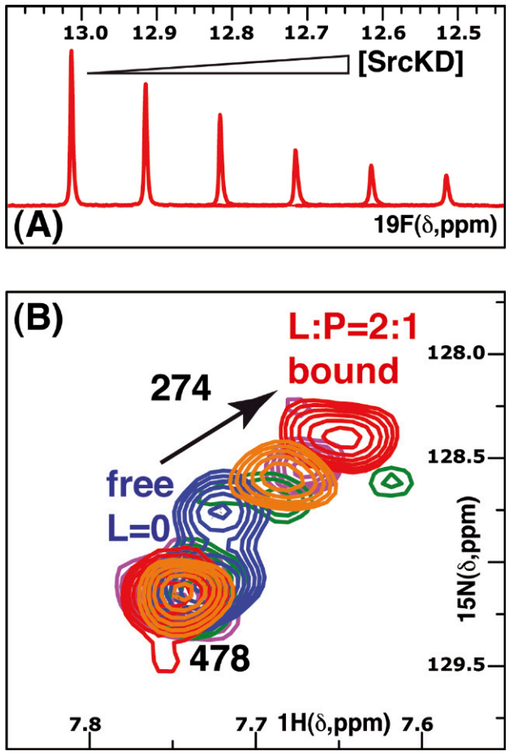

Figure Ligand binding to SrcKD. (**A**) 1D ^{19}F NMR spectra show how ligand signal intensity decreases upon protein addition. (**B**) Superposition of a region of the ^{15}N-^{1}H 2D spectra of SrcKD at increasing amounts of compound show shifts in the backbone amino groups of specific protein residues (274 here, but not 478).

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BIOINFORMATICS CORE UNIT

David G. Pisano
Core Unit Head

Technicians
Eduardo Andrés, Ángel Carro,
Gonzalo Gómez, Osvaldo Graña

David G. Pisano ESP

Eduardo Andrés ESP

Ángel Carro ESP

Gonzalo Gómez ESP

Osvaldo Graña ESP

"THE UNIT CONTRIBUTES TO CNIO'S EFFORTS OF ESTABLISHING A FRAMEWORK FOR GENOMICS-BASED PERSONALISED MEDICINE BY PROVIDING SYSTEMS AND PROTOCOLS FOR THE INITIAL ANALYSIS OF TUMOUR GENOMES. WE ALSO CONTRIBUTE TO DATA MANAGEMENT AND ANALYSIS OF INTERNATIONAL LARGE-SCALE CONSORTIA, FOCUSING ON THE CHARACTERISATION OF CANCER GENOMES (ICGC) AND EPIGENOMES (BLUEPRINT)."

OVERVIEW

In close liaison with researchers from Alfonso Valencia's Structural Computational Biology Group, the National Bioinformatics Unit, and the newly created Translational Bioinformatics Unit led by Fátima Al-Shahrour, our Unit aims to bridge the gap between the quantitative analysis of cancer biology and its actual interpretation in the context of disease aetiology. In practical terms, this implies: helping CNIO researchers with the analysis and interpretation of their data by consulting and assessing computational methodologies; designing and maintaining the scientific computing infrastructures that enable the launch of massive calculation pipelines; and also, training students to perform and analyse their own *in silico* experiments with bioinformatics tools.

RESEARCH HIGHLIGHTS

Bioinformatics as a tool to understand the molecular biology of cancer

We helped CNIO researchers to understand the biological mechanisms underlying cancer. Our Unit uses statistical and computational approaches to generate massive amounts of data that reveal interesting trends and yield enriched functions or biomarkers. We also performed statistical analysis to help Manuel Serrano (CNIO) decipher the role of the Notch-pathway in non-small-cell lung carcinoma and to discover a novel function of the tumour suppressor PTEN in energy expenditure. Together with M.S. Cespedes (IDIBELL), we showed that re-expression of the transcription activator BRG1 in lung cancer cell lines restores the signature of normal lung, pointing to an antagonistic functional relation between BRG1 and MYC. With Juan C. Cigudosa and Sara Álvarez (CNIO), we described the effects of downregulation of specific miRNAs in multiple myelomas, and contributed to the understanding of the role of the AML1-ETO fusion protein on its target genes and in chromatin modification.

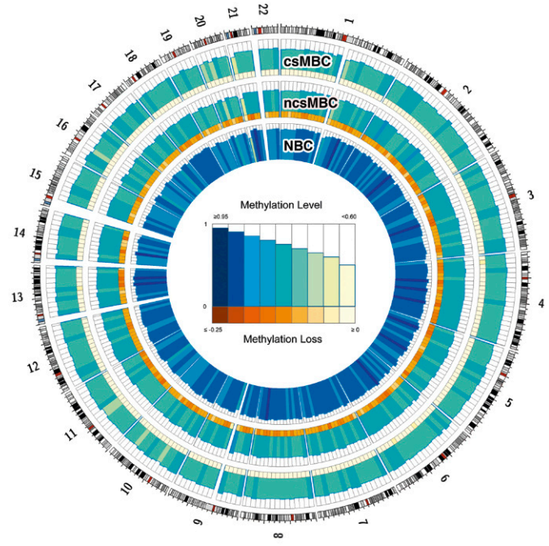

Figure Circular representation of DNA methylation levels for naïve B-Cells (NBCs), non-class switch Memory B-Cells (ncsMBCs), and class switch Memory B-Cells (csMBCs) (Kulis *et al.*, *Nat Genet*, 2012).

Next-generation sequencing and cancer genomes: a genetic cartography of cancer

For the detailed characterisation of cancer genomes, we had the opportunity to collaborate with A. Ramiro (CNIC) to unveil the contribution of uracil-N-glycosylase to the resolution of DNA lesions induced by activation-induced deaminase, using ultra deep sequencing. With Ana Losada (CNIO), we explored novel roles for the distinct subunits of the cohesin complex in the regulation of gene expression, using ChIP-seq. We also helped M.A. Piris (IFIMAV) and M.S. Beato (University Hospital *Puerta de Hierro*) to identify new mutations in chronic lymphatic leukaemia (CLL), using target enrichment and deep re-sequencing. We collaborated with M. Esteller (IDIBELL) to determine the DNA methylation fingerprint of hundreds of human samples, including human tumours, and to pinpoint differences between epigenomes of a newborn and a centenarian. As a participant of the International Cancer Genome Consortium (ICGC), we contributed to the unveiling of new methylation signatures that allowed for the classification of novel clinical CLL subtypes (FIGURE). ■

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NATIONAL BIOINFORMATICS INSTITUTE CORE UNIT

Alfonso Valencia
Core Unit Head

Technicians
Christian Blaschke (until October), Andrés Cañada, Guillermo Comesaña, Victor de la Torre, José M. Fernández, Allan Orozco (until March), José M. Rodríguez


Alfonso Valencia ESP


Guillermo Comesaña ESP


Victor de la Torre CUB


José M. Fernández ESP


José M. Rodríguez ESP

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 distributed 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 main objectives of the INB Core Unit are to:

→ Generate and supply bioinformatics solutions to genomics projects with particular emphasis on 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 in the context of the European Infrastructure for Bioinformatics (ELIXIR) (FIGURE).

"DURING 2012, THE INB BUILT THE FOUNDATIONS OF ITS PARTICIPATION IN THE EUROPEAN BIOINFORMATICS INFRASTRUCTURE (ELIXIR) AND, IN THIS CONTEXT, DEVELOPED THE PARTNERSHIP WITH THE EUROPEAN BIOINFORMATICS INSTITUTE (EBI-EMBL) FOR CO-HOSTING THE EUROPEAN GENOTYPE-PHENOTYPE ARCHIVE (EGA)."

RESEARCH HIGHLIGHTS

During this year, our Core Unit has focused on the following bioinformatics analyses and support systems:

→ The Unit contributed to the development of APPRIS; an integrated system for the annotation of splice isoforms and the detection of principal isoforms. The Unit has further contributed to the development and implementation of the system, and maintains the associated database. APPRIS is being developed in the context of the INB's collaboration in the ENCODE/GENCODE project; in 2012, the ENCODE Project Consortium published the results of this collaboration in the journals *Nature* and *Genome Research*.

→ The Unit built basic components of the platform for the analysis of cancer genomes, and supported the necessary infrastructure for the analysis of Chronic Lymphocytic Leukaemia (CLL) genomes as part of its participation in the CLL-ICGC (International Cancer Genome Consortium) project. The Unit also designed and implemented the database for the BLUEPRINT project; BLUEPRINT is part of the International Human Epigenome Consortium (IHEC). ■

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Article in press

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Figure ELIXIR is the European Infrastructure for Bioinformatics; a distributed organisation coordinated by the EBI-EMBL that acts as the central hub, and of which the INB will be one of the nodes. The goal of ELIXIR is to upgrade Europe's bioinformatics infrastructure so that we can continue to provide life science researchers with seamless access to the many facets of biological information.

“WE WILL FOCUS ON RESEARCH THAT ENHANCES THE CARE OF CANCER PATIENTS AND THAT BENEFITS SOCIETY IN GENERAL. THESE ENDEAVOURS WILL INCLUDE CLINICAL TRIALS WITH NEW DRUGS, DEVELOPMENT OF DIAGNOSTIC AND PROGNOSTIC BIOMARKERS, AS WELL AS THE LAUNCH OF PERSONALISED CANCER TREATMENT STRATEGIES.”

MANUEL HIDALGO VICE-DIRECTOR OF TRANSLATIONAL RESEARCH

VICE-DIRECTION OF TRANSLATIONAL RESEARCH

MANUEL HIDALGO VICE-DIRECTOR

MOLECULAR PATHOLOGY PROGRAMME

MARÍA S. SOENGAS PROGRAMME DIRECTOR

All of us within the Molecular Pathology Programme share a common goal: a more rational approach to the prevention, diagnosis and treatment of aggressive cancers. A distinctive feature of our laboratories is their particular emphasis on clinical specimens. They provide a physiological platform for gene discovery and target validation, which we complement with a variety of cellular systems and genetically engineered mice.

The Programme is constituted by 2 Senior Groups and 1 Junior Group that pursue the identification of novel tumour genes from an organ-based perspective. The Epithelial Carcinogenesis Group, headed by Francisco X. Real, has made great strides in defining early events involved in the development of pancreatic cancer, as well as in discovering novel oncogenes and tumour suppressors in bladder carcinoma. The work of his Group on genetic mosaicism as a contributor to adult cancer, has also opened up new avenues of research. The Tumour Markers Junior Group, headed by Marta Sánchez-Carbayo, has also shed light on unanticipated drivers of bladder cancer. Various epigenetic changes are being validated for non-invasive diagnosis, and as prognostic and predictive biomarkers for patient stratification. My own laboratory focuses on melanoma. This tumour type keeps surprising us. This year, we discovered molecular switches that melanoma cells can tune up or down in a dynamic manner to favour proliferation or invasion. We are also proud of the first-in-class melanoma model that we generated to monitor metastatic events before the actual dissemination of the tumour cells, and of the progress made in the validation of potent anticancer nanoparticles.

The perspectives for the molecular pathology field, and in particular for our Programme, are exciting. Knowing the mutational profile of cancer cells is not sufficient for obtaining effective personalised medicine; understanding the plasticity of tumour cells is key to the design of compounds that provide long-term responses. The creation of a spin-off company (*Bioncotech Therapeutics*) and multiple ongoing collaborations with various biotechnology companies, illustrate the dynamic translational activities of our Programme. We also cherish our active participation in large national and international consortia, joining forces in order to achieve our goal of improving the management of cancer patients. ■

"OUR PROGRAMME STRIVES TO HELP THE CANCER COMMUNITY THROUGH THE IDENTIFICATION OF RISK FACTORS, TUMOUR BIOMARKERS, PROGNOSTIC INDICATORS AND NOVEL TARGETS FOR RATIONAL DRUG DESIGN. THE ROAD AHEAD IS WINDING, BUT WE ARE COMMITTED TO RIDING IT TOGETHER TOWARDS THE ULTIMATE GOAL OF TRANSLATING OUR KNOWLEDGE TO THE PATIENT'S BEDSIDE."

MELANOMA GROUP

María S. Soengas
Group Leader

Staff Scientists
Alicia González, David Olmeda, Erica Riveiro (until September)

Post-Doctoral Fellows
María García, Lisa Osterloh

Graduate Students
Direna Alonso, Daniela Cerezo (since September), Metehan Cifdaloz, Panagiotis Karras, Eva Pérez, Napala Ransom Pratini (since September)

Technicians
Tonantzin G. Calvo, Estela Cañón, Ángel Colmenar, Silvia Tamborero (from April to September)


María S. Soengas ESP


Alicia González ESP


David Olmeda ESP


Erica Riveiro BRA


María García ESP


Lisa Osterloh DEU


Direna Alonso ESP


Daniela Cerezo VEN


Metehan Cifdaloz TUR


Panagiotis Karras GRC


Eva Pérez ESP


Napala Ransom Pratini USA


Tonantzin G. Calvo ESP


Estela Cañón ESP


Ángel Colmenar ESP

OVERVIEW

The long term goal of our Group is to identify molecular mechanisms that are involved in the initiation, progression and resistance to therapy of malignant melanoma. In particular, we are interested in stress response programmes (involving apoptosis, autophagy, senescence and endosome mobilisation), which we have found to be deregulated in a melanoma-specific manner. Multimeric complexes controlling RNA stability, transcription and translation, are also central themes in our research. Our experimental settings include normal and tumour cells, as well as comprehensive collections of tissue specimens spanning early, intermediate and late stages of melanoma development. In addition, particular emphasis is placed on the generation of genetically modified mice that have the unique feature of allowing for non-invasive imaging of metastatic processes. These studies are performed in the context of multidisciplinary consortia of specialists in biology, chemistry, pharmacy, nanotechnology, molecular imaging, dermatopathology and clinical oncology. We also work in partnership with biotechnology companies to translate our discovery efforts to the bedside. As an example of these collaborative efforts, I would like to highlight the dsRNA-based nanocomplexes that are currently under development for clinical testing by *Bioncotech Therapeutics*.

"WE HAVE IDENTIFIED AN ONCOGENIC PATHWAY THAT IS UNIQUE TO MELANOMA, DISCOVERED TWO PUTATIVE PROGNOSTIC FACTORS, CONFIRMED A HIGHER ANTICANCER ACTIVITY OF NANOPARTICLES BASED ON dsRNA, AND GENERATED A SERIES OF ANIMAL MODELS THAT ALLOW US TO VISUALISE THE METASTATIC PROCESS BEFORE TUMOUR CELLS COLONISE DISTANT ORGANS."

RESEARCH HIGHLIGHTS

Molecular determinants of melanoma progression and cell plasticity

Comprehensive genome-wide analyses are revealing a striking inter-and intra-tumoural heterogeneity. Still, even highly unstable cancers retain traits that trace back to the organ or to the cell of origin. However, for many types, including melanoma, the identity of these lineage-specification oncogenes is not completely understood. To address this question, we have established a large collaborative group that is composed of researchers from the Bioinformatics Core Unit at the CNIO, the *Hospital 12 de Octubre* in Madrid, the Hospital of Zurich, and the Memorial Sloan-Kettering Cancer Center in New York.

These studies identified a cluster of lysosomal-associated pathways that distinguished melanoma from 35 other tumour types (FIGURE 1A and results not shown). One of the most enriched genes in this cluster was the RAB7 small GTPase (FIGURE 1B), which we demonstrated to be overexpressed also at the protein level. RAB7 was found to be a key modulator of the inherent plasticity of melanoma cells, favouring their ability to switch from proliferative to invasive phenotypes. Parallel studies also demonstrated an upregulation of the p62/sequestosome protein during melanoma progression. Importantly, overall survival (10-year follow up studies) strongly correlated with the levels of both p62 and RAB7. Further differences between normal melanocytes and melanoma cells in the context of MDM-2, autophagy modulators, RNA

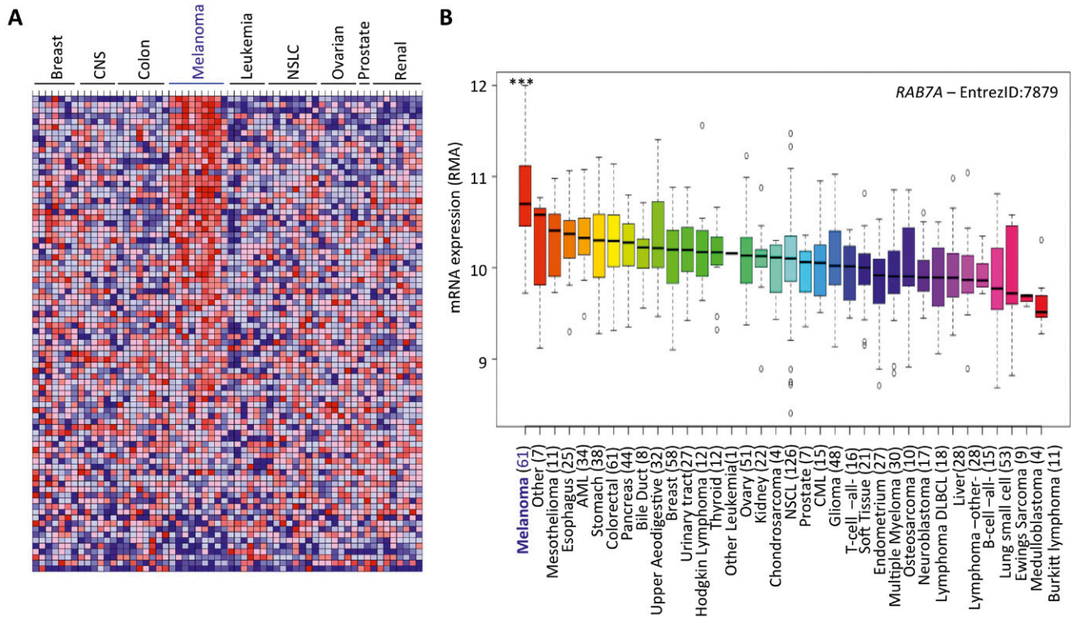

Figure 1 Identification of a melanoma lineage-specific lysosomal gene cluster. (**A**) Heat map showing a selective enrichment of the Lysosome Gene Ontology (GO) gene set in melanoma compared to the indicated tumour types. (**B**) Box plots showing the relative *RAB7* mRNA levels across 850 cell lines encompassing the indicated tumour types (datasets were obtained from the Cancer Cell Line Encyclopedia). The number of cell lines per tumour type are indicated in parenthesis.

splicing, transcription and translation are being analysed with collaborating laboratories at the University of Michigan, the Centre for Genomic Regulation (CRG) and the Institute for Research in Biomedicine (IRB) in Barcelona.

New mouse models for *in vivo* imaging of tumour metastasis: application to gene discovery and drug response in melanoma

Metastatic dissemination of cancer cells is a complex process invariably associated with neo-vascularisation. Intriguingly, while the presence of malignant cells in lymph nodes is a defining criterion in tumour staging, the specific contribution of lymphangiogenesis to tumour progression and drug response remains largely unknown. This is mainly due to the lack of markers and amenable models for the imaging and analysis of lymphangiogenesis by non invasive *in vivo* methods. In collaboration with the Transgenic Mice Core Unit of Sagrario Ortega, also at the CNIO, we have generated the first in-class 'lymphoreporter' mouse strains with melanoma. This mouse model is based on a knock-in of a GFP-luciferase fusion cassette at the 3' UTR region of *Flt4* (*Vegfr3*); a classical marker of lymphangiogenesis. These mice offer the unique opportunity of visualising activation of pro-metastatic signals 'in remote', before the tumour cells can actually be detected to colonise distant organs. The lymphoreporter mouse model has also proven to be a new cost-effective system for pharmacological testing of dsRNA-based nanoparticles (FIGURE 2) and other anticancer agents. ■

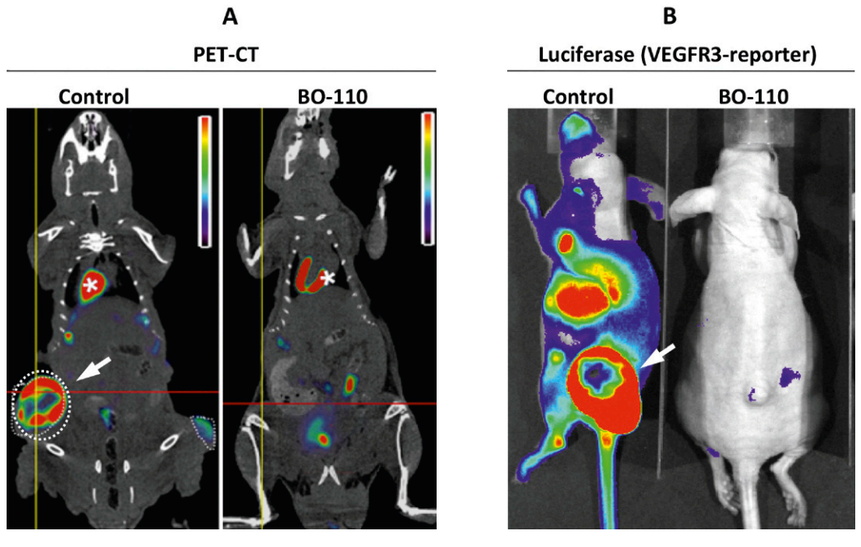

Figure 2 Imaging drug response in melanoma models. (**A**) Ability of the dsRNA-based nanocomplex BO-110 to block melanoma growth, measured by classical PET/CT (positron emission tomography/computerised tomography). Arrow points to cutaneous melanomas generated in a *Tyr:Cre; Ink4a/Arf*$^{-/-}$ background. (**B**) Drug response analysed in VEGFR3-luciferase reporter mice. Note the abrogation of VEGFR3 driven signals in the tumour area (arrow) and at distal sites by BO-110.

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AWARDS AND RECOGNITION

- Executive Editor, *Pigment Cell and Melanoma Research.*

EPITHELIAL CARCINOGENESIS GROUP

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OVERVIEW

Our main interest is to understand the molecular/cellular mechanisms involved in pancreatic and bladder cancer through a disease-oriented approach. Our strategy builds on a structure similar to that of a pyramid, having an equilateral triangle as a base. The 3 vertices correspond to the models used: patient samples, cell cultures, and genetically modified mice; the 3 models are all considered equally important in our work. The third dimension is represented by the projection of this knowledge onto the general population: we provide the biology to benefit the studies with patients. We are interested in the genetic susceptibility to cancer and in developing better molecular tools to predict patient outcome or response to therapy. Our primary observations can be made at either of these levels and they are then extended through additional work.

The focus on pancreatic ductal adenocarcinoma (PDAC) relates to the early events involved in tumour development; the control of cell differentiation as a critical tumour suppressor mechanism is a focal point. Using genetic mouse models, PDAC can originate both in pancreatic progenitors and acinar cells. The elucidation of the contribution of these cell types is crucial to design better strategies for early tumour detection and prevention in subjects at risk.

"OUR GROUP HAS IDENTIFIED NEW GENES THAT ARE INVOLVED IN PANCREATIC CELL DIFFERENTIATION AND THAT CAN ACT AS NOVEL TUMOUR SUPPRESSORS. WE HAVE SHOWN THAT VITAMIN D REGULATES FGFR3 AND CAN CONTRIBUTE TO THE RISK OF DEVELOPING SPECIFIC BLADDER CANCER SUBTYPES."

Regarding urothelial cell carcinoma (UCC), the focus is on identifying new genes, using them for improved tumour taxonomy, characterising the mechanisms through which they participate in tumourigenesis, and applying this knowledge for improved prediction of outcome.

RESEARCH HIGHLIGHTS

Pancreas cancer molecular pathophysiology

Cell differentiation as a tumour suppressor mechanism in the pancreas. *KRAS, p16*, *TP53*, and *SMAD4* are the major genes involved in PDAC. We have acquired strong evidence that novel genes that control acinar cell differentiation (i.e. *Gata6*) also play an important role. When *Gata6* is inactivated in pancreatic progenitors, the pancreas develops normally but acinar cells are unable to maintain their number and identity in the adult. This results in apoptosis, extensive acinar-ductal metaplasia and adipocyte transdifferentiation, as well as in impaired recovery upon induction of acute pancreatitis (FIGURE 1). Furthermore, Gata6 protein expression is lost in mutant *KRas*-driven PDAC as well as in a proportion of human tumours. *Gata6* inactivation also accelerates *KRas*-driven PDAC progression in mice. Our findings point to a role for the Gata6 protein as a novel tumour suppressor through the control of epithelial-mesenchymal transition, among others.

We are also studying other genes that play important roles in differentiation, pancreatitis and PDAC, including *Nr5a2, Hnf1a, Foxa1/2*, and *Myc*. RNA-Seq and ChIP-Seq studies have identified intricate relationships between them through regulatory networks controlling tumour suppressors, metabolic processes, and inflammatory cytokine cascades. Components of these networks can contribute to PDAC by modulating the risk of developing chronic pancreatitis and by controlling the expression of genes that can be targeted at the therapeutic level.

Our work on PDAC is enhanced through our close collaboration with the other CNIO Groups who are also working on this tumour (Mariano Barbacid, Christopher Heeschen, Manuel Hidalgo, and Núria Malats).

Urothelial cell carcinoma (UCC) genetics and biology

FGFR3 is the most commonly activated oncogene in UCC. *FGFR3*-activating mutations occur mainly in well differentiated, low grade, tumours, and its expression/activation is required for the maintenance of the epithelial phenotype. FGFR3 overexpression, as well as mutations, contributes

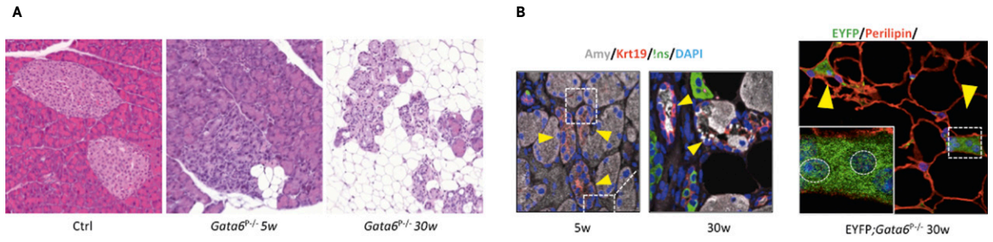

Figure 1 Gata6 is required for the normal development and maintenance of the exocrine pancreas. The pancreas of young (5 weeks) *Gata6*$^{P-/-}$ mice contains isolated metaplastic foci and focal areas of fat. At 30 weeks, massive atrophy, fat replacement, and metaplasia are present (**A**). Intermediate cells, co-expressing amylase and Krt19, are detected (**B**, left). EYFP expression in perilipin-expressing adipocytes in a *EYFP; Gata6*$^{P-/-}$ pancreas at 30 weeks, indicating that some of the adipocytes have an epithelial origin (**B**, right).

to the tumour phenotype and we have shown that vitamin D regulates FGFR3 expression at the transcriptional level. Using the EPICURO study resources, we have shown that insufficient vitamin D plasma levels are selectively associated with a higher risk of developing low FGFR3-expressing bladder tumours. These studies will lead to the design of clinical trials to assess the therapeutic potential of vitamin D.

Identifying new genes involved in UCC. We are using massive parallel exome sequencing and have identified new pathway genes contributing to UCC, including chromatin remodelling (i.e. *ARID1A*), DNA repair, and chromosome segregation (i.e. *STAG2*), among others. The relationship between these genes and the well established pathways of UCC progression are being analysed in the context of multi-centric collaborative studies (i.e. EPICURO and UROMOL). Our aim is to use this biological knowledge to improve UCC diagnosis and prognosis, and to identify novel therapeutic opportunities.

The weight of somatic postzygotic embryonic genetic alterations. Most current cancer models postulate that the genetic/genomic alterations associated with the common cancers of the aged, occur during adult life. Using both 'index case' and 'large scale' approaches, we have acquired strong evidence that somatic postzygotic embryonic genetic alterations (i.e. oncogene mutations and genomic aberrations) occur during embryonic development and contribute to adult cancer risk. Postzygotic mutations in *RAS* genes lead to mosaicism and increased risk of cancer, and are responsible for *Schimmelpenning* Syndrome and *Phacomatosis pigmentokeratotica*. We are also using genetic mouse models to analyse how embryonic mutations contribute to determine clonal tissue composition in the adult (FIGURE 2).

Figure 2 2D reconstruction of a pancreas at embryonic day 14.5 (E14.5). Assembly of a whole mount E14.5 *Ptf1a-Cre*$^{+/KI}$ *R26*$^{+/KI}$ pancreas stained for LacZ to assess clonal size and distribution.

This work is being conducted in collaboration with the groups of Núria Malats and Alfonso Valencia at the CNIO, C. Hafner (Univ. Regensburg), S. Chanock (NCI-NIH, Bethesda), I. Gut (CNAG, Barcelona), L. Pérez-Jurado (UPF, Barcelona), R. Pujol (*Hospital del Mar*-IMIM, Barcelona), and M. Torres (CNIC, Madrid). ■

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AWARDS AND RECOGNITION

- Member, Scientific Council, "Cancer Research for Personalized Medicine" (CARPEM) in Paris, France.
- Academic Editor, *PLoS ONE* Journal.

TUMOUR MARKERS JUNIOR GROUP

Marta Sánchez-Carbayo
Junior Group Leader

Technicians
Salvador Mena (until May),
Silvia Tamborero (until April)

Marta Sánchez-Carbayo ESP

OVERVIEW

The main goal of the Tumour Markers Junior Group is to contribute to the molecular characterisation of bladder cancer by integrating information from high-throughput approaches, with two aims: i) to understand how molecular alterations contribute to tumourigenesis and cancer progression; and ii) to translate this knowledge into improved clinical management of bladder cancer patients, providing multiplexed tools for the analysis of tissue biopsies (for disease stratification and outcome prediction) and body fluids (for early diagnosis and follow-up).

Our studies are designed to address 4 major clinical needs for biomarkers in bladder cancer: diagnosis, surveillance, progression into invasive and metastatic disease, and prediction of therapeutic response. We are using epigenetic and proteomic approaches to identify and validate individual and multiplexed biomarkers, as well as to dissect the molecular pathways through which genes of interest contribute to bladder cancer initiation and disease progression.

"OUR GROUP HAS CONTRIBUTED TO THE UNDERSTANDING OF KEY MECHANISMS INVOLVED IN BLADDER CANCER AND HAS IDENTIFIED BIOMARKERS THAT WILL IMPROVE NON-INVASIVE DIAGNOSTICS OF THESE TUMOURS. OUR ANALYSIS OF GENE METHYLATION AND PROTEIN EXPRESSION PATTERNS IN BLADDER AND COLON CANCER HAS YIELDED NOVEL BIOMARKERS THAT WILL FACILITATE PATIENT STRATIFICATION AND GUIDE THERAPEUTIC DECISIONS."

RESEARCH HIGHLIGHTS

We are addressing the role of epigenetic events in bladder cancer, using tumours and urinary samples, with the aim to use the resulting knowledge for disease stratification, prognosis and non-invasive diagnosis. More specifically, we have achieved: i) the detection of *miR-143*, *miR-222*, and *miR-452* in urine, and the validation of their targets as tools for the diagnosis, disease stratification and prognosis of bladder cancer; ii) the identification of the diagnostic and prognostic utility of myopodin gene methylation and protein expression patterns in colon cancer; iii) the discovery of the predictive value of *PMF1* methylation in high-risk non-muscle invasive T1G3 bladder tumours; iv) the identification of the diagnostic and prognostic utility of *KiSS-1* gene methylation and protein expression patterns in colon cancer; and v), the discovery of the functional and biomarker role of CUL3 in bladder cancer aggressiveness.

As part of collaborative studies involving groups at the CNIO and other centres, we have played a contributory role to: i) identify distinctive methylation patterns for different tumour types; ii) reveal the usefulness of Profilin protein expression as a biomarker for disease stratification and prognosis in bladder cancer; iii) establish the expression of the proteoglycan versican in bladder cancer as a marker to assess how RhoGDI2 suppresses metastasis by reducing macrophage infiltration; iv) validate in a multi-centric study the prognostic value of Cathepsin E, Maspin, Plk1, and Survivin protein expression for progression in non-muscle invasive bladder cancer; v) evaluate the expression patterns of Notch signalling components in human lung tumours in order to assess the therapeutic effect of gamma-secretase inhibition in non-small-cell lung carcinoma; vi) validate in a multi-centric study the prognostic value of Cyclin D1, MCM7, TRIM29, and UBE2C expression in bladder cancer; and vii), uncover the role of SPARC loss for carcinogenesis and progression in a murine model of bladder cancer. ■

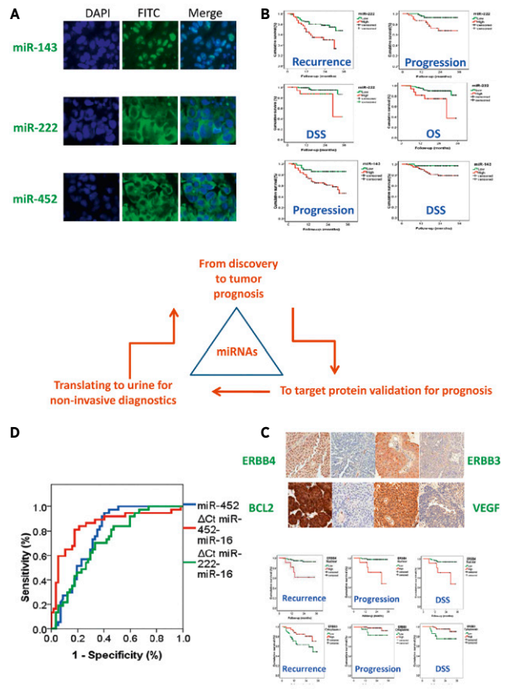

Figure Example of the translational approaches for biomarker discovery and validation in bladder cancer, from tumour to urinary non-invasive miRNAs. (**A**) Selected miRNAs are proven to be present in neoplastic cells in bladder tumours by *in situ* hybridisation. (**B**) Tumour miRNAs predict recurrence, progression, disease-specific (DSS) and overall survival (OS). (**C**) Immunohistochemistry of miRNAs targets predict clinical outcome in bladder tumours. (**D**) miRNAs can be detected in the urine for bladder cancer diagnosis.

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AWARDS AND RECOGNITION

- Toyota Merit Award and Best Scientific Poster Prize, 40th Anniversary Congress of the International Society of Oncology and BioMarkers (ISOBM), Israel.

HUMAN CANCER GENETICS PROGRAMME

JAVIER BENÍTEZ PROGRAMME DIRECTOR

The Human Cancer Genetics Programme conducts a wide range of translational research related to familial cancer in its four Research Groups, Genotyping Core Unit and Familial Cancer Consultancy. The principal goals of the Programme are the characterisation of genetic and cytogenetic tumours, the search for diagnostic and prognostic markers, as well as the discovery of novel cancer-related genes. These research activities are complemented by studies on the genetic and environmental factors that confer cancer susceptibility and modulate the tumour phenotype as well as its response to therapeutic interventions. The Programme also fosters cooperation with the clinical community through its Familial Cancer Consultancy at the *Hospital Universitario de Fuenlabrada* that, in close collaboration with the Medical Oncology Service of this Hospital, provides genetic counselling and clinical surveillance to patients and families at risk of suffering from hereditary cancer.

The Programme's education and training activities in 2012 comprised a total of 15 national and 11 international PhD research projects, including the successful defence of 6 doctoral theses, four 2-6 weeks short research stays of professionals from different international research centres, and trimestral training programmes in familial cancer and cytogenetics in which a total of 6 resident physicians participated. The Programme has entered into a total of 9 projects funded by European and American entities, signed 6 contracts with biotech companies, and sponsored 1 national and 4 international meetings.

Major milestones of the Programme in 2012 include:

- The Human Genotyping Unit-*CEGEN*, funded by the *Instituto de Salud Carlos III*, was reorganised into 2 nodes; one of them located in Santiago de Compostela and the other at the CNIO, providing a more operative structure than with the 3 previous nodes, without duplication of platforms and maintaining an optimal personnel level in both nodes.
- The Human Genotyping Centre-*CEGEN* was certified, as the first genotyping laboratory in Europe, according to the ISO15159 standard.
- The Familial Cancer Consultancy at the *Hospital Universitario de Fuenlabrada* has consolidated its position in the Autonomous Community of Madrid and is currently opening its service to other Autonomous Communities such as *Castilla la Mancha*. ■

"WE ARE PROUD OF THE TYPE OF RESEARCH THAT IS CARRIED OUT BY THE PROGRAMME, WHICH IS PURELY TRANSLATIONAL, FOCUSED ON THE STUDY OF PATIENTS AND DIRECTLY BENEFITING THEM. THIS IS OUR GOAL AND WE HOPE TO BE ABLE TO FULFILL IT."

HUMAN GENETICS GROUP

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"OUR STUDIES IMPROVE THE DIAGNOSES OF FAMILIES WITH CANCER, INCREASE THE IDENTIFICATION OF FAMILIES WITH A KNOWN GENETIC AETIOLOGY AND FACILITATE THEIR GENETIC COUNSELLING AND CLINICAL FOLLOW UP; THIS CONTRIBUTES TO EARLIER DIAGNOSIS, ALLOWING FOR PREVENTIVE MEASURES THAT MAY AVOID THE DEVELOPMENT OF CANCER OR FACILITATE ITS SUCCESSFUL TREATMENT."

OVERVIEW

The Human Genetics Group's interest lies in the understanding of the genetic bases of familial cancer; mainly of breast, ovarian and colorectal cancer, and to apply the resulting knowledge to clinical practice. The Group's main activities include the identification of new susceptibility genes for both sporadic and familial cases, the characterisation of genetic and environmental modifier factors that modulate cancer risk, and the discovery of diagnostic and prognostic biomarkers by using different 'omics' approaches that can help in the selection of candidate families for genetic studies.

Our approaches range from disease to gene, from individual to population level (genetic epidemiological studies), and from constitutional to somatic tissue. We use classical genetic approaches, such as case-control association studies, as well as new high-throughput technologies for whole exome genotyping and ultrasequencing. The development of novel bioinformatics tools for the analysis of complex data is also a priority. The Group further conducts functional studies for ultimate data interpretation purposes. Several national (through public and private funding) and international projects support our research activities.

The Group's strategic goals are: i) to gain insight into familial and sporadic breast and ovarian cancer by identifying new genes or modifier factors that can alter the final effect of the main gene, and the discovery of genetic markers associated with diagnosis and prognosis; ii) to better understand the genetic bases of familial colorectal cancer; and iii), to integrate high-throughput technologies, as is the case of massive exome sequencing, for the identification of high-susceptibility genes in families with rare cancer susceptibility syndromes or genetic heterogeneity.

RESEARCH HIGHLIGHTS

Breast cancer

Shorter telomeres have been linked to susceptibility to various types of cancers seeing as they predispose the genome to instability, and thus, to malignant transformation. We have demonstrated that telomere shortening in families with mutations in *BRCA1* and *BRCA2* genes, results from transmission of the mutation rather than from the disease itself, and that hereditary breast cancer shows genetic anticipation linked to shorter telomeres. In addition, we have confirmed that short telomeres are a risk factor in familial and sporadic ovarian cancer.

We participate in COGS, a large-scale project funded by the European Commission to reveal the genetics behind breast cancer; as a result many common genetic markers (SNPs) associated with cancer risk have been identified. We have completed the genotyping process and identified 41 new breast cancer susceptibility genes that explain around 5% of familial cancer risk. Furthermore, there are more than 1,000 genes that have been identified that confer each of them very low risk (OR<1.03). All together, these genes explain about 18% of familial cancer risk.

We have determined miRNA profiles associated with the different genetic subtypes in familial breast cancer, thereby yielding a set of 35 miRNAs that are differentially expressed in breast tumours with *BRCA1* and *BRCA2* mutations *versus* non-mutated tumours. We are currently validating these results in serum samples from patients. Similarly, hierarchical clustering of *BRCAX* tumours revealed high heterogeneity with 4 apparent subgroups, defined by specific miRNA signatures and different histological characteristics, which points to different routes of tumour evolution.

By using array-CGH we identified an amplification event at the 13q34 region. Further characterisation of the amplicon allowed us to define *CUL4A* as one of the most likely putative oncogenes. To elucidate the possible implication of *CUL4A* in the initial steps of the tumourigenic process, we have induced stable up-regulation of *CUL4A* in human mammary epithelial cells by lentiviral infection and stably silenced *CUL4A* in human breast cancer cell lines that present amplification or overexpression of *CUL4A*. *In vitro* data and *in vivo* results in athymic nude mice, support an oncogenic role for *CUL4A* in tumour cell proliferation and survival (FIGURE 1).

Ovarian tumours: genomic characterisation

We are interested in characterising the pattern of genomic alterations in the different subtypes of hereditary ovarian tumours. We have been able to define a chromosomal deletion at 6q24-26 that might be an independent prognosis factor associated with improved overall survival in epithelial ovarian cancer patients, especially in those with high FIGO stage tumours (FIGURE 2). The prognostic relevance of this deletion has been validated in two independent series, and we are currently pursuing the identification of candidate genes explaining such an association.

Familial colorectal cancer

In non-polyposis colorectal cancer patients, we have carried out a linkage analysis in a series of Type X families. Results support a very heterogeneous genetic basis in these families with an important role of low-moderate penetrance genes in cancer susceptibility. In Familial Adenomatous Polyposis Syndromes, more than 90% of classic forms are associated with germline mutations in *APC*. The contribution of the *MUTYH* gene is modest (4%). Other genes, like *AXIN2*, *GSK3b* or spindle assembly checkpoint genes, seem to play a role in several rare families with polyposis.

Diagnostic activity and genetic counselling in familial cancer

In 2012 we carried out over 750 studies involving more than 30 genes responsible for different types of familial cancer syndromes (breast, colorectal, endocrine, and other familial tumours). The Familial Cancer Consultancy in the *Hospital Universitario Fuenlabrada* was very active in 2012. Around 100 consultations have been conducted with families suspected of having hereditary cancer. Most of these patients come from the Autonomous Communities of *Castilla La Mancha* and Madrid. ■

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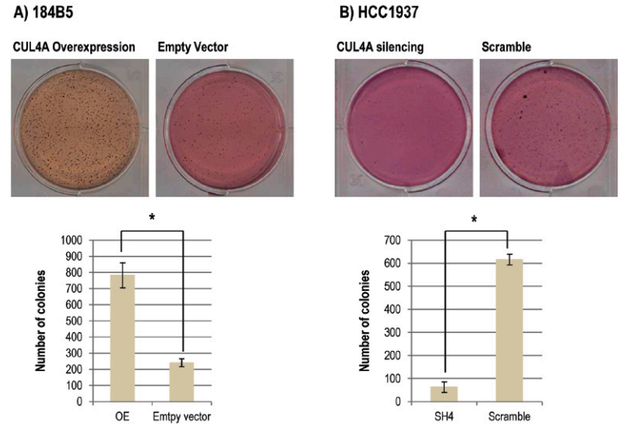

Figure 1 Modulation of *CUL4A* levels modifies colony formation ability in anchorage independent conditions. (**A**) Lentiviral overexpression of *CUL4A* induced an increased colony-forming ability in 184B5 human mammary epithelial cells. (**B**) Silencing of *CUL4A*, by using lentiviral shRNAs (*SH4*), resulted in the reduction of colony-forming ability in the HCC1937 breast cancer cell line. *$P \leq 0.05$ (2-sided paired Student's t-test). OE.- Overexpression.

Association of 6q24-26 deletion with clinical data

Region	Survival analysis	All carcinomas			High FIGO stage carcinomas		
		HR (95%CI)	*P_{adj}	*P*	HR (95%CI)	**P_{adj}	*P*
6q24.2-6q25.3 (without del vs. del)	**OS**	0.14 (0.04-0.49)	***0.002***	***0.03***	0.13 (0.04-0.48)	***0.002***	***0.002***
	5yrs-survival	0.12 (0.03-0.44)	***0.001***	***0.03***	0.12 (0.03-0.44)	***0.002***	***0.002***

*adjusted for: FIGO stage and residual tumor; ** adjusted for: residual tumor

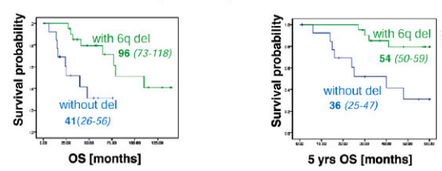

Figure 2 From all the alterations differentiating the clusters, deletion at 6q24-26 was found to be the most significantly associated with better survival.

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AWARDS AND RECOGNITION

- Editorial Board Member, *Methods in Next Generation Sequencing*.

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OVERVIEW

In almost all human cancers, next generation sequencing and other genomic approaches demonstrate that the chromosomes of tumour cells show structural rearrangements in the form of translocations, deletions, amplifications or numerical changes (aneuploidy). Among them, we are particularly interested in those rearrangements that generate chimaeric genes with new or altered biological activities, as well as in the molecular mechanisms by which these newly created fusion genes drive oncogenesis.

We are mainly working on myeloid leukaemia as a disease model. Myeloid leukaemia is the paradigm of a genetic neoplasia in which fusion genes collaborate with other genomic aberrations to induce abnormal cell proliferation and disrupt the differentiation programme of haematopoietic stem cells. Based on our ongoing collaborations with the clinical community, our research activity is being developed through: i) the characterisation of molecular cytogenetic and epigenetic markers; ii) the design of human stem cell models with defined chromosome rearrangements, rendering them unique tools to study the effects of these rearrangements on the biology of the tumour and the underlying molecular pathways of the observed effects; and iii) the translation of our findings to the clinical setting by providing molecular cytogenetic technology, such as spectral human and mouse karyotypes, as well as tailor-made FISH probes for both research purposes and their use as clinical reagents.

“WE ARE DESCRIBING HOW CHROMOSOME REARRANGEMENTS AND POINT MUTATIONS OF SPECIFIC GENES ARE THE MAJOR RESPONSIBLE AGENTS FOR THE PROFOUNDLY ABERRANT EPIGENOME AND SPLICEOSOME DISPLAYED BY MYELOID LEUKAEMIA CELLS. OUR FINDINGS OPEN NEW AVENUES FOR ALTERNATIVE AND SPECIFICALLY TARGETED CANCER THERAPIES.”

RESEARCH HIGHLIGHTS

Advances in the epigenomics of leukaemia

We have focused our research on the study of the AML1-ETO fusion protein that is present in 10% of cases of acute myeloid leukaemia as a consequence of a t(8;21)(q22;q22) translocation. This fusion protein is known to repress myeloid haematopoietic differentiation genes through the recruitment of chromatin-modifying proteins. We characterised the genomic distribution and epigenetic chromatin modifications induced by the AML1-ETO fusion protein, using an AML1-ETO-expressing CD34+ human haematopoietic stem/progenitor cell model. We identified 1.168 AML1-ETO target genes and among them – based on the analysis of ‘inactive’ heterochromatin marks – we selected 103 genes co-occupied by HDAC1 and with a

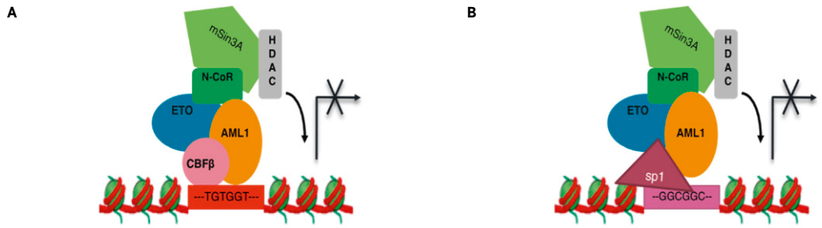

Figure 1 AML1-ETO DNA interaction models. (**A**) Classical AML1/ETO DNA interactions through AML1 TFBS. (**B**) Alternative AML1/ETO DNA interaction model through Sp1 TFBS. qChIP analysis and functional studies using an Sp1 inhibitor (mithramycin A) point to a crucial role of the Sp1 transcription factor as a key driver in AML1-ETO binding to some of its target genes.

loss of hyper-acetylation at histone H4, and 264 genes with an increase of the heterochromatin mark H3K9me3. These genes were involved in haematopoietic differentiation and in specific signalling pathways (i.e. TGF-β and Wnt/β-catenin). Furthermore, the presence of heterochromatin marks correlated with transcriptional repression in our stem/progenitor cell model and in patient samples, reinforcing the biological and clinical relevance of this setting in AML1-ETO leukaemias. Interestingly, a detailed sequence analyses identified an enrichment of Sp1 transcription factor binding sites among AML1-ETO target genes. This interaction with Sp1 was found to be a crucial element driving the AML1-ETO DNA-binding pattern and thus supports the relevance of Sp1-targeted therapeutic approaches for this leukaemia subtype (FIGURE 1).

We compared the micro RNA (miRNA) expression profiles of the two major groups of multiple myeloma (MM). MM patients are grouped into a non-hyperdiploid (nh-MM) (FIGURE 2A) group, highly enriched for IgH translocations, and into a hyperdiploid (h-MM) group that is characterised by trisomies of some odd-numbered chromosomes. We identified 16 miRNAs that were down-regulated in the h-MM group. Among them, we found that inhibition of *miR-425*, *miR-152* and *miR-24* correlated with the over-expression of CCND1, TACC3, MAFB, FGFR3 and MYC, a group of oncogenes that is up-regulated by the most frequent IgH translocations occurring in nh-MM (FIGURE 2B). Importantly, we showed that both the down-regulation of these specific miRNAs and the up-regulation of their targets also occur simultaneously in primary cases of h-MM. Together, our data provide further evidence for mis-regulated cyclin D signalling as the unifying key mechanism for both groups of MM.

Myeloid leukaemia: from genetic mutations to the generation of human stem cell models

We have identified and characterised 2 new leukaemic translocations, t(4;21)(q21;q22) and t(1;21)(p32;q22). Both types of rearrangements resulted in the absence of the transactivation function and inhibit the trans-activating activity of wild type AML1b, preventing its normal function, and most likely contribute to leukaemogenesis. Additionally, by integrating molecular cytogenetics, array-CGH and next-generation sequencing we have described new chromosome rearrangements and genetic mutations that result in the improper functioning of the spliceosome molecular machinery.

Since we are committed to transferring our research activities into potential clinical applications, we are generating biological models and tools to study the role of chromosome translocations in cancer. We have developed several human haematopoietic stem cell models, based on cell lines that have been genetically engineered to carry those novel chimaeras and mutated genes, such as *AML1-TMEM48*, *NUP98-HOXA*, *BCR-ABL*, and splicing knockouts.

Our group also provides state-of-the-art molecular cytogenetic services. In 2012, we carried out over 2.000 assays such as karyotyping of leukaemia and other tumours, design of FISH probes, spectral karyotyping, aneuploidy analysis for mouse models, and array-CGHs for experimental and clinically-oriented projects. As a reference laboratory in Molecular Cytogenetics, we are participating in several clinical assays, collaborative networks, and quantity performance studies both at the national and European level. ■

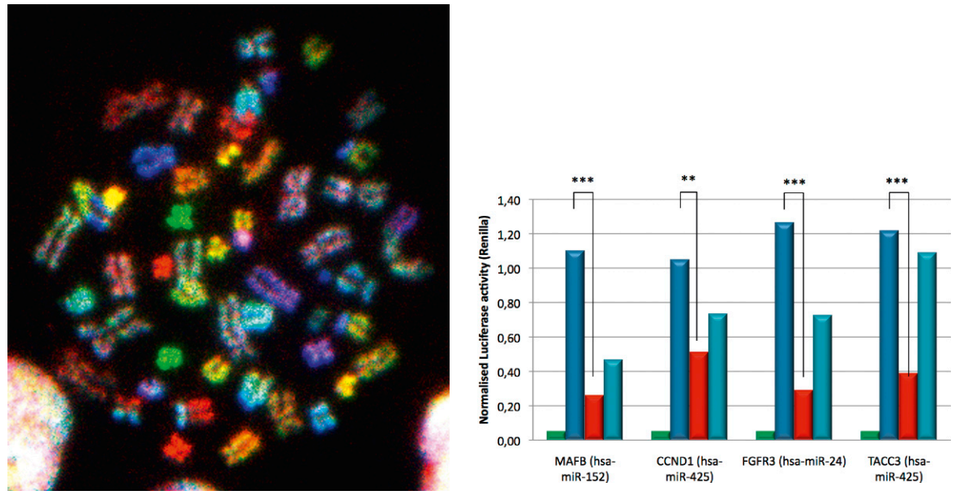

Figure 2 miRNA regulation in multiple myeloma. (**A**) Spectral karyotype of a multiple myeloma cell showing numerical and structural chromosome aberrations. (**B**) Luciferase assay of the 3'UTR promoter regions of each gene in the presence of specific miRNAs. Significant down-regulation of the expression of the most frequently affected genes by IgH translocations (*CCND1, TACC3, MAFB* and *FGFR3*) was observed (red columns), being this effect sequence-specific (light blue columns).

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AWARDS AND RECOGNITION

- President-Elect Nominee, International Society of Cellular Oncology.

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OVERVIEW

Our Group is interested in identifying high and low genetic risk factors involved in endocrine tumour susceptibility. To this end, we not only phenotypically classify patients according to features of their tumour genome, but we also apply a pioneer approach to detect epistatic effects among polymorphic variants that may explain part of the hidden heritability. We are interested in revealing differences between tumour transcriptomes, mirnomes, methylomes and chromosomal gains and losses according to the different individual genetic backgrounds. Such comprehensive characterisation allows us, not only to define diagnostic and prognostic markers associated with primary mutations, but also to specifically pinpoint altered pathways as potential therapeutic targets. For this purpose, we have obtained a large collection of endocrine tumours from patients with germline mutations in most of the known major susceptibility genes related to these diseases as well as sporadic cases.

We are also interested in defining genetic markers associated with differences in anticancer drug response and toxicity. In order to do so, we have compiled a large series of biological material and associated data regarding therapeutical 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 ADDED NOVEL INSIGHTS REGARDING THE ROLE OF *MAX* IN PHEOCHROMOCYTOMA AND OBTAINED PROGNOSTIC AND DIAGNOSTIC MARKERS RELATED TO THE INDIVIDUAL GENETIC BACKGROUND. THE IDENTIFICATION OF GENETIC FACTORS RESPONSIBLE FOR PACLITAXEL TOXICITIES BY OUR GROUP PROVIDES A NOVEL ROAD TO INDIVIDUALISED CHEMOTHERAPY."

RESEARCH HIGHLIGHTS

The specific transcriptional profile of *MAX*-related pheochromocytomas points to the mTOR pathway

The MYC-associated factor X *(MAX)* gene was identified by our Group in 2011 as the tenth susceptibility gene for pheochromocytoma (PCC). Its clinical relevance, however, was not addressed at this stage. Through an international collaboration that involved 17 referral centres from all over the world we were able to ascertain the prevalence of *MAX* mutations in PCC patients, extend the spectrum of *MAX*-related tumours to paraganglioma (PGL), uncover contributions of somatic *MAX* mutations to sporadic disease, and define an intermediate catecholamine phenotype that may guide testing of *MAX* gene in patients with PCC/PGLs. This study also confirms a preferential paternal mode of transmission with important consequences for genetic counselling. We have established that *MAX* germline mutations are responsible for the disease in 1.12% of cases, similarly to the recently described genes, *TMEM127, SDHAF2* or *SDHA*. In the light of our findings, *MAX* should now also be considered in the genetic work-up of affected patients.

It is noteworthy that the transcriptional profile of *MAX* tumours shows a significant enrichment of mTOR pathway components when compared to other mutated PCCs. This finding is particularly relevant, since deregulation of both the mTOR pathway, and the upstream PTEN/PIK3CA/AKT1 axis, seems to be essential for the development of many PCCs (i.e. with mutations in *RET*, *NF1*, *TMEM127* or *MAX*) (FIGURE 1). Considering the transcriptional profile of *MAX* tumours, and since a crosstalk between the PIK3CA/AKT1/mTOR and MYC/MAX/MXD1 pathways has been proposed, it is likely that mutations in *MAX* not only deregulate the MYC neoplastic switch but also lead to the impairment of the mTOR pathway and PCC development. It has been reported that mTOR inhibitors prevent the development of PCC in PTEN knock-out mice. In addition, it seems that sunitinib induces apoptosis in PC12 cells by inhibiting the VEGFR-2/AKT1/mTOR/S6K1 pathways. Since PC12 cells constitute a specific double-knockout model for *MAX*-related PCC, it seems plausible that targeting the mTOR pathway could be an effective therapy for malignant PCC patients with germline *MAX* mutations.

β-Tubulin polymorphisms as toxicity markers of the microtubule-binding drug paclitaxel

Paclitaxel is a microtubule-binding drug that stabilises the cellular microtubules through β-tubulin binding, leading to cell cycle arrest and apoptosis. This agent is an effective anticancer drug currently used to treat several solid tumours such as breast, lung, and ovary. One of its major clinical problems is the large and unpredictable inter-individual variability in the toxicities developed by the patients. Currently, dose-limiting paclitaxel toxicity is related to peripheral neuropathy. In this respect, we described a large inter-individual variability in the mRNA content of its neural target β-tubulin IIa. We found that 2 common polymorphisms in the proximal promoter of the gene conferred an increased transcription rate. The patients carrying these variants were protected from paclitaxel-induced peripheral neuropathy [HR, 0.62; 95% confidence interval, 0.42-0.93; P = 0.021, multivariate analysis], and an inverse correlation between β-tubulin IIa expression and paclitaxel-induced apoptosis was demonstrated *in vitro* (P = 0.001). With respect to paclitaxel-induced myelotoxicity, we found that the missense polymorphism T274M in the haematological β-tubulin VI isoform, significantly decreased sensitivity to paclitaxel-induced tubulin polymerisation *in vitro;* in patients this polymorphism was associated with lower thrombocytopenia as compared to homozygous wild-type patients (P = 0.031). Altogether, these studies define β-tubulins as key players for inter-patient variability in paclitaxel toxicities and provide novel markers for individualised paclitaxel chemotherapy. ■

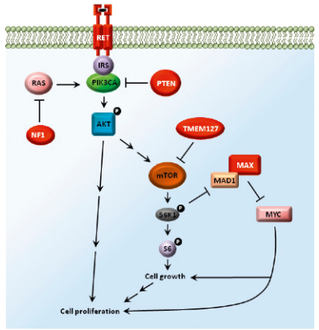

Figure 1 The PIK3CA/AKT1/mTOR pathway mediates downstream activation of genes involved in cellular processes such as regulation of growth, division, survival and, when disrupted, tumourigenesis.

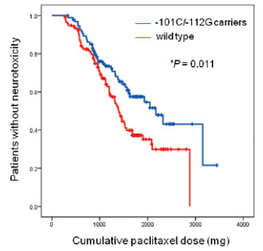

Figure 2 Kaplan–Meier analysis of cumulative doses of paclitaxel up to the development of grade 2 sensory peripheral neuropathy, according to -101C/-112G variants in *TUBB2A*. Patients treated with paclitaxel were grouped according to their *TUBB2A* genotype. Those carrying 1 or 2 variant alleles had a significantly lower risk of paclitaxel-induced neurotoxicity. The P value shown corresponds to univariate log-rank test.

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AWARDS AND RECOGNITION

- *Sergio Vidal* and *Fundación Mutua Madrileña* 2012 Awards for the publication in *Nature Genetics*, Spain.
- Scientific Co-Chair of the international *Pheochromocytoma and Paraganglioma Research Support Organization* (PRESSOR).

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OVERVIEW

The scope of our Group's research focuses on bladder, pancreatic, and breast cancer, and ranges from the identification of aetiological agents and mechanisms to the translation of these findings into the clinical and public health settings.

Today, epidemiology demands the alignment of scopes, data, and tools across the multiple disciplines involved. By applying such an integrative approach, we participate in large, international multidisciplinary studies and contribute to the development of methodological innovations in all aspects of epidemiology. We employ a wide range of biomarkers to better characterise risk exposures and cancer outcomes, as well as genetic patterns that predispose or protect against the disease, or determine the variability of its clinical course.

"THROUGH AN INTEGRATIVE APPROACH THAT COMBINES PATHOLOGICAL WITH MOLECULAR INFORMATION, OUR GROUP HAS MADE IMPORTANT CONTRIBUTIONS TO THE IDENTIFICATION OF ENVIRONMENTAL EXPOSURES (VITAMIN D, TRACE ELEMENTS) AND GENOME-WIDE VARIANTS, IN PARTICULAR OF INFLAMMATORY GENES THAT ARE INVOLVED IN CANCER DEVELOPMENT AND PROGRESSION."

The integration of 'omics' data in the context of epidemiologic studies may allow us to further dissect complex exposures, genetic susceptibility, and phenotypes.

The strategic goals of our Group are: i) identifying environmental exposures and genetic susceptibility factors, as well as gene-environment and gene-gene interactions involved in cancer development and progression; ii) studying the differential association of genetic germline variants and environmental exposures with cancer sub-phenotypes at the molecular/omics level; iii) developing and applying statistical/informatics tools to model risk and prognostics of patients with cancer, combining epidemiologic with 'omics' data; and iv), assessing clinical and public health strategies for cancer control using genomic tests and datasets.

RESEARCH HIGHLIGHTS

Urothelial bladder cancer (UBC)

During 2012, our Group found that low serum levels of 25-hydroxy-vitamin D_3 increase the overall risk of UBC and, in particular, of muscle invasive tumours lacking FGFR3 expression (FIGURE 1). We also explored the association of trace elements, potential modulators of epigenetic events, with UBC risk. We found that arsenic, iron, nickel, and selenium are associated with LINE-1/D4Z4 methylation levels, which in turn are associated with UBC risk.

Another potential risk factor for UBC is chronic inflammation. Variants in inflammatory genes have been shown to be associated with a UBC subtype characterised by cyclooxygenase-2 expression. Copy number variations (CNVs) may also explain

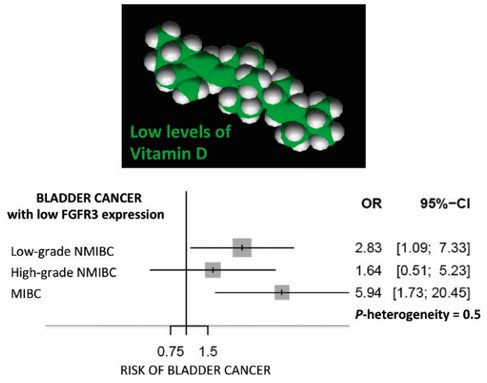

Figure 1 Risk (odds ratios and 95% confidence intervals) for the association between plasma 25-hydroxy-vitamin D_3 levels and risk of low-and high-grade non-muscle invasive (NMIBC) and muscle invasive (MIBC) bladder cancer tumours with low FGFR3 expression levels. Estimates are adjusted for age, gender, region, and smoking status.

part of the heritability of UBC. Through a genome-wide study on the allele and the number of copies for single nucleotide polymorphisms (SNPs) located in CNV regions, we identified CNV regions that are potentially associated with UBC risk, as well as SNPs for which both the allele and the number of copies may account for risk. Together with the MD Anderson Cancer Centre, and other international collaborators, we have performed one of the first genome-wide prognosis association studies and succeeded in identifying susceptibility loci that are independently associated with clinical outcomes of non muscle-invasive UBC (FIGURE 2). Ongoing efforts aim to integrate epidemiological with 'omics' data by developing and applying novel analytical strategies to facilitate disease modelling. Our Group also participates in several initiatives conducted within the International Consortium of Bladder Cancer (ICBC).

Pancreatic cancer

The Group reported on novel associations of lead, nickel, and selenium toenail concentrations with pancreatic cancer risk and confirmed previous associations with cadmium and arsenic. These findings point to the role of chronic inflammation in pancreas carcinogenesis. We have also shown that inflammatory polymorphisms, associated with pancreatic cancer risk, are also associated with chronic pancreatitis. Using the resources of the Breast Cancer Family Registry, we observed significant associations of *BRCA1*, *BRCA2* and *BRCAX* families with pancreatic cancer; thus, establishing genetic commonalities between these tumours and providing additional clues on the mode of inheritance of, as yet, unidentified genes in *BRCAX* families. In collaboration with the *Hospital Ramón y Cajal* in Madrid, we have created the Spanish Registry of Familial Pancreatic Cancer. This project also includes a screening programme for high-risk relatives and is now being extended to other centres in Spain.

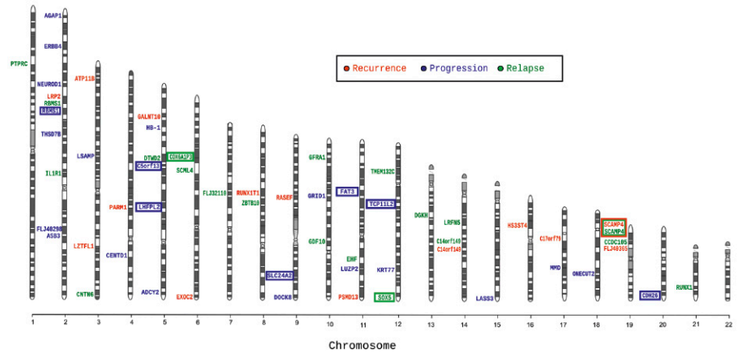

Figure 2 Germline variants associated with outcome in patients with non-muscle invasive bladder cancer (NMIBC). Chromosomal representation with the genomic location of the closest genes to the SNPs associated with NMIBC outcome (recurrence, red; progression, blue; relapse, green) in discovery analysis. Highlighted are those SNPs that were replicated in the validation phase. Results are from an international genome-wide prognosis study.

The European case-control study (PanGen-EU), conducted in 6 European countries, has included 2,216 cases and 813 controls; placing it in a unique position for post-genome-wide association studies. Our group also participates in the Pancreas Cancer Case-Control Consortium (PanC4) and collaborates with other similar international initiatives. Recently, we were selected by the European Commission to spearhead the COST Action "An integrated European platform for pancreatic cancer research: from basic science to clinical and public health interventions for a rare disease".

Breast cancer

Gene-gene interactions have been proposed to account for the 'missing heritability' in breast cancer. We have undertaken huge, computational resource-demanding analyses to explore these interactions with 72,611 candidate variants, genotyped in 46,450 breast cancer cases, and 42,461 controls of European origin from a European multi-consortia project (COGS). Surprisingly, no evidence of 2-way SNP interactions in breast cancer susceptibility was observed. The collaboration with International Consortia has resulted in the identification and replication of genetic variants in both sporadic and familial breast cancer patients.

Public Health and Genomics

We participate in the European Public Health and Genomics Network (PHGEN) with the aim to assess the implications of using genomic data and technology for large-scale population studies (genetic testing, biobanking, ethic and legal issues). ■

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OVERVIEW

Single nucleotide polymorphisms (SNPs) are the most abundant form of genetic variation in the human genome, although structural changes such as copy number variants (CNVs) are proving to be more common than expected. Many complex human phenotypes have a significant genetic component that is likely to be explained by such variants. Association studies involving large-scale analysis of both SNPs and CNVs can help to identify genes that underlie diverse human phenotypes, including complex diseases such cancer, as well as to predict drug response. Given the potential impact of such genetic variations on health, our Unit has implemented different high-throughput and cost-effective methods to measure thousands, and up to millions, of these variants. Complementarily, large-scale pharmacogenomic studies have been undertaken to identify predictive biomarkers for personalised cancer therapy.

"OUR GOAL IS TO IDENTIFY BIOMARKERS TO PREDICT RESPONSE TO CANCER TREATMENT THAT CAN BE USED TO DEVELOP MORE PERSONALISED THERAPIES AND THEREBY IMPROVE CANCER PATIENT CARE."

RESEARCH HIGHLIGHTS

Discovery of a new susceptibility gene for capecitabine-induced hand-foot syndrome

We carried out a genome-wide association study in patients treated with capecitabine, a drug used in breast and colorectal cancer to identify genetic susceptibility factors for severe capecitabine-related hand-foot syndrome (HFS); this adverse event is the most frequent reason for dose reduction or therapy cessation. We identified several cis-acting regulatory genetic variants of a cadherin gene that was associated with severe capecitabine-induced HFS and replicated the results in an independent patient series (FIGURE). Gene expression analysis suggested that these variants play a role in gene regulation. Thus, this study provides novel insights into the clinical response to capecitabine treatment that could be helpful in taking precise preventive measures for patients at high risk of severe HFS appearance.

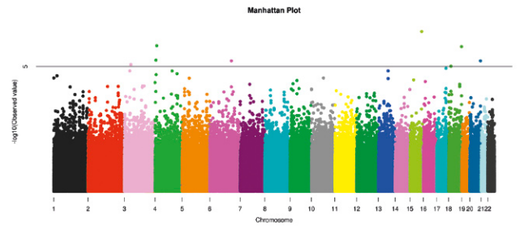

Figure Manhattan plot of –log10 P-values for SNPs from Cox regression – adjusted for sex, tumour type and treatment regimen – by chromosomal position 10 markers with $P < 1 \times 10^{-5}$ were selected for replication in an independent patient series.

Discovery of genetic variants related to Ewing's sarcoma prognosis after treatment

Ewing's sarcoma (ES) is the second most common bone malignancy in children and adolescents. Multidisciplinary management, incorporating advances in diagnosis, surgery, radiation and, in particular, chemotherapy, has substantially improved the survival for ES patients to almost 70%. Despite these advances, many individuals still relapse or suffer from adverse drug reactions; this has motivated the search for predictive factors. We examined 224 SNPs in 17 genes involved in the transport/metabolism of 6 widely-used ES chemotherapeutic agents in 512 patients from 5 European countries. The data from this large-scale study are currently being analysed.

Translational research

This year, the Unit has received accreditation by the National Accreditation Entity (ENAC) in recognition of both its technical competence and quality management. The Unit has also been accredited, as the first of its kind in Europe, as a clinical laboratory complying with UNE EN ISO 15189:2007 standards for large-scale SNP genotyping, using high-throughput technologies (No. 984/LE 1873). ■

PUBLICATIONS

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AWARDS AND RECOGNITION

- National Representative and Board Member, European Association for Predictive, Preventive and Personalised Medicine (EPMA).

CLINICAL RESEARCH PROGRAMME

MANUEL HIDALGO PROGRAMME DIRECTOR

The Clinical Research Programme (CRP) aims to translate advances in cancer research into the diagnosis and care of patients with cancer. The major goals of the CRP include the design, conduction and analysis of early clinical trials with novel drugs; the implementation of a programme for personalised cancer treatment; and the expansion of these activities to clinical centres across the country by establishing associate clinical units and by launching a training programme in drug development.

The CRP is composed of one Research Group, 3 Clinical Research Units (CRUs), and 3 support Units. Chistopher Heeschen leads the Stem Cell and Cancer Research Group that works on the molecular identification and targeted elimination of cancer stem cells. The Gastrointestinal Cancer CRU, led by Manuel Hidalgo, studies novel therapeutics in pancreatic cancer. Miguel Quintela-Fandino leads the Breast Cancer Junior CRU that centres its efforts on the development of kinase and angiogenesis inhibitors in breast cancer. In 2012, we recruited David Olmos, from the Royal Marsden Hospital in London, to head the *CRIS* Foundation-CNIO Prostate Cancer and Genitourinary Tumours Junior CRU that focuses on novel therapeutics and biomarkers in prostate cancer. In 2012, we also recruited Fátima Al-Shahrour to lead the Translational Bioinformatics Unit that aims to provide genomic analysis for personalised cancer treatment. The Molecular Diagnostics CRU headed by Luis Lombardía focuses on the implementation of molecular markers in clinical trials, and the Clinical Trials Management Unit coordinates our clinical trials activities.

In 2012, our main activities were based on the expansion of our clinical trials activities through collaborations with several hospitals in Spain. At the *Hospital de Madrid*, we consolidated our phase I clinical trials activity by launching over 20 phase I studies; more than 100 patients are projected to be treated this year. We have also implemented collaborative agreements with other hospitals in Madrid and in other Auotonomous Communities of Spain in order to create an early clinical trials network. Furthermore, we have established multi-centric clinical trials in breast cancer that involve several Spanish hospitals. Finally, we initiated the CNIO's personalised cancer treatment initiative based on the 'Avatar' mouse model; this innovative approach has gained significant acceptance in the scientific community. ■

"THROUGH OUR PROGRAMME, CNIO SPEARHEADS A PIONEERING PHASE I CLINICAL TRIALS NETWORK IN SPAIN THAT BRINGS NEW CANCER TREATMENTS FROM THE LABORATORY BENCH TO THE PATIENT'S BEDSIDE."

GASTROINTESTINAL CANCER CLINICAL RESEARCH UNIT

Manuel Hidalgo
Clinical Research Unit Head

Staff Scientist
Pedro P. López

Post-Doctoral Fellows
Antonio Calles, Monica A. Musteanu

Graduate Students
Spas Dimitrov (since December), Raquel Martínez, Jorge Oliver, Natalia Sherina

Technicians
Natalia Baños, Camino Menéndez, Manuel Muñoz

Clinical Investigator
Carlos Gómez

Clinical Research Fellow
Elena Garralda


Manuel Hidalgo ESP


Pedro P. López ESP


Monica A. Musteanu ROU


Spas Dimitrov BGR


Raquel Martínez ESP


Natalia Sherina RUS


Natalia Baños ESP


Camino Menéndez ESP


Manuel Muñoz ESP


Carlos Gómez ESP


Elena Garralda ESP

OVERVIEW

The Gastrointestinal (GI) Cancer Clinical Research Unit focuses on the clinical development and personalised application of novel therapeutic approaches for patients with cancers of the alimentary tract. Our principal activity is the design, conduction, and analysis of clinical trials with novel anticancer 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.

Key to our work, is the development and characterisation of 'Avatar' mouse models for drug screening, biomarker development, and personalised medicine. Over the last few years we have developed and characterised the largest collection of these models in pancreatic cancer, both from primary and metastatic tumours, and have also developed a growing collection of colon cancer models. We are using the Avatar models in two critical applications. On one hand, we are conducting co-clinical trials, in which 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. On the other hand, we are using the Avatar models for personalised cancer treatment by integrating data from next-generation sequencing approaches.

"IN 2012 WE HAVE INITIATED MORE THAN 20 NEW CLINICAL TRIALS – INCLUDING FIRST-IN-CLASS/FIRST-IN-HUMAN TRIALS WITH NEW ANTICANCER AGENTS – AND HAVE ENROLLED OVER 300 PATIENTS. *NAB*-PACLITAXEL – A DRUG THAT WE HELPED TO DEVELOP – IMPROVES THE SURVIVAL OF PATIENTS WITH PANCREATIC DUCTAL ADENOCARCINOMA."

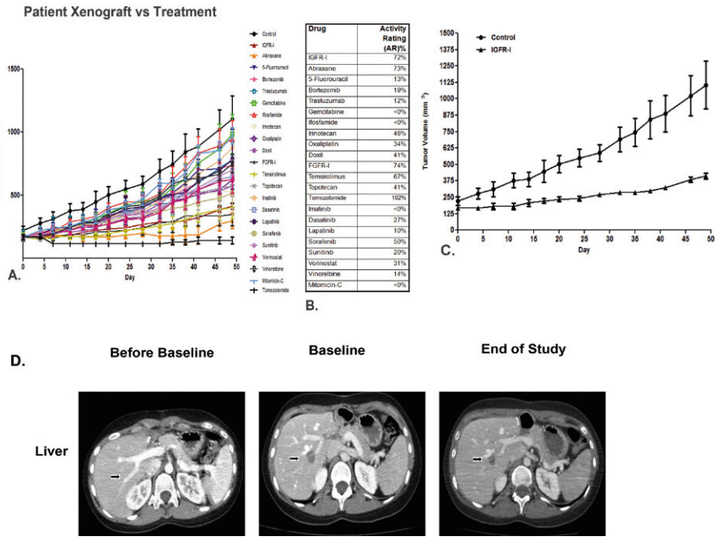
RESEARCH HIGHLIGHTS

During 2012, we have continued lines of work that were initiated in the previous year, including preclinical studies with novel anticancer agents, conduction of clinical trials in our associated hospitals, expansion of our network of collaborative centres, and the launch of several personalised medicine studies.

Avatar mouse model development and characterisation

Our group has continued its efforts to develop and characterise Avatar mouse models based on xenografts 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 pancreatic ductal adenocarcinoma xenografts from pancreatic ductal adenocarcinomas (PDA) is the largest and best characterised collection available so far, and represents an important resource for academic and industry investigators.

We have collaborated in several published studies (listed below) that use our collection for drug development and for biological studies. We have also continued to exploit our Avatar mouse models for personalised oncology. Figure 1 summarises the application of an Avatar model to select phase I treatments for a patient with salivary gland carcinoma. This study identified an IGFR1 inhibitor as the most effective agent that was administered to the patient with clinical improvement. Based on this observation, we initiated parallel clinical and preclinical studies in patients with this disease as well as in Avatar models derived from patients with this cancer type; these studies have been completed very recently with encouraging results.



Drug	Activity Rating (AR)%
IGFR-I	72%
Abraxane	73%
5-Fluorouracil	13%
Bortezomib	19%
Trastuzumab	12%
Gemcitabine	<0%
Rosfamide	<0%
Irinotecan	48%
Oxaliplatin	34%
Dosil	41%
FGFR-I	74%
Temsirolimus	67%
Topotecan	41%
Temozolomide	162%
Imatinib	<0%
Dasatinib	27%
Lapatinib	10%
Sorafenib	59%
Sunitinib	20%
Vorinostat	31%
Vinorelbine	14%
Mitomicin-C	<0%

Figure 1 Personalised cancer treatment approach. (**A**, **B**) Treatment response of a patient's Avatar mouse model. (**C**) Activity of the IGF1R inhibitor figitumumab in the patient's Avatar model. (**D**) CT-scan response of a liver metastasis.

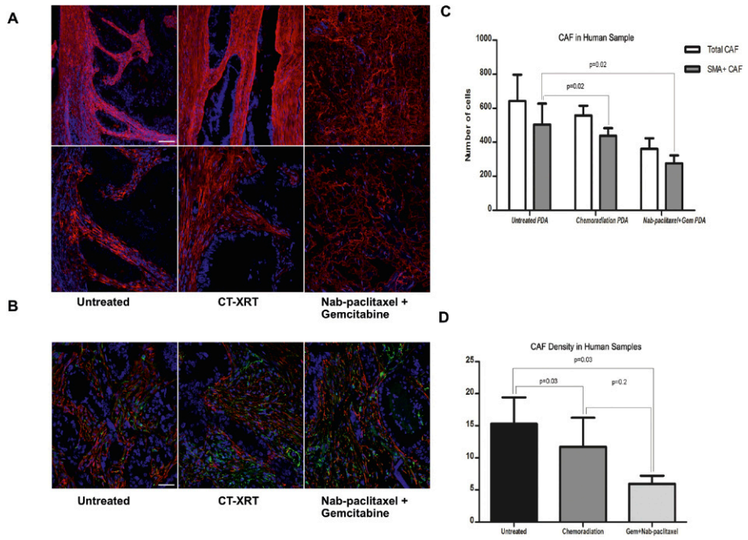


Figure 2 Effects of *nab*-paclitaxel in PDA. (**A**) Effects of *nab*-paclitaxel in the stroma of PDA; a group of untreated patients and patients treated with conventional therapy are shown for comparison. (**B**) Presence of activated cancer-associated fibroblasts in tumour tissues. (**C**, **D**) Quantitation of cancer-associated fibroblasts in tumour tissues.

Development of novel anticancer agents

We have significantly expanded our portfolio of early clinical trials in patients with GI and other malignancies. At present, the GI Cancer Clinical Research 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. These studies include first-in-class/first-in-human clinical trials and studies with relevant biomarker analysis, as well as co-clinical studies in mouse models. An example of our contributions to drug development is the work conducted with *nab*-paclitaxel in patients and mouse models of PDA. In initial phase I-II clinical and co-clinical studies we observed that *nab*-paclitaxel was very effective in patients with advanced PDA and that one of the biological effects was a profound remodelling of the PDA stroma. To gain further understanding of this observation, we performed a preoperative study of *nab*-paclitaxel in patients with operable PDA. Analysis of tumours resected at the time of surgery showed massive stromal disorganisation with a decreased concentration of cancer associated fibroblasts (FIGURE 2). A subsequent preclinical trial in mouse models of PDA suggested that these effects are specific for *nab*-paclitaxel and not for gemcitabine. *Nab*-paclitaxel has now completed phase III testing in PDA patients with positive results and is likely to become approved for this indication.

Personalised treatment of pancreatic cancer

Our goal is to implement a stepwise protocol for personalised cancer treatment, spanning from the selection of first line treatment, to the selection of the most effective experimental treatments and the investigation of new targets and drugs. In keeping with this global aim, we have implemented a protocol to guide chemotherapy selection in patients with advanced pancreatic cancer; we have enrolled the first patients in this trial. We have also implemented a programme to integrate next-generation sequencing of Avatar mouse models for personalised cancer treatment. So far we have completed over 20 individual patient genomes and have generated mouse models from 8 of these patients. Preliminary results of this innovative work provide compelling evidence that it is possible to find drug targets for many of these patients and that the Avatar mouse models are instrumental in the making of therapeutic decisions. ■

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- Calvo-Plaza I, Ugidos L, Miró C, Quevedo P, Parras M, Márquez C, de la Cruz JJ, Suárez-Gauthier A, Pérez FJ, Herrero M, Marcos M, García-Aranda M, Hidalgo M, Estévez LG. Retrospective study assessing the role of MRI in the diagnostic procedures for early breast carcinoma: a correlation of new foci in the MRI with tumor pathological features. *Clin Transl Oncol* (in press).

AWARDS AND RECOGNITION

- Scientific Advisory Board Member, Department of Oncology at the University of Leuven, Belgium.
- External Advisory Board Member, *Instituto Oncológico de kutxa (Onkologikoa),* Spain.

STEM CELLS AND CANCER GROUP

Christopher Heeschen
Group Leader

Staff Scientists
Susana García, Bruno Sainz, Patricia Sancho (since June)

Post-Doctoral Fellows
Maria Azevedo (since September), Anamaria Balic, Jenifer Clausell, Jorge Dorado, Patrick C. Hermann, Enza Lonardo (until May), Marina Roy (since September), Sara Trabulo, Yolanda Sánchez (since March)

Graduate Students
Michele Cioffi, Javier Frias (until Dcember), Irene Miranda, Sladjana Zagorac

Technicians
Sonia Alcalá, M. Mercedes Alonso (until September), Mario Bautista (until January), Ildiko Meny, Catarina L. Reis, Iker Rodríguez (until October)

Visiting Scientist
Alexandra Aicher


Christopher Heeschen DEU


Susana García ESP


Bruno Sainz ESP


Patricia Sancho ESP


Maria Azevedo PRT


Anamaria Balic HRV


Jenifer Clausell ESP


Jorge Dorado ESP


Patrick C. Hermann DEU


Marina Roy ESP


Sara Trabulo PRT


Yolanda Sánchez ESP


Michele Cioffi ITA


Javier Frias ESP


Irene Miranda ESP


Sladjana Zagorac SRB


Sonia Alcalá ESP


Ildiko Meny DEU


Catarina L. Reis PRT


Iker Rodríguez ESP


Alexandra Aicher DEU

OVERVIEW

Accumulating evidence from several laboratories, including our own, supports the existence of cancer stem cells (CSCs) in pancreatic cancer. CSCs represent a subpopulation of cells distinguishable from the bulk of the tumour by their exclusive ability to drive tumourigenesis and metastasis (FIGURE 1). They play a crucial role in disease relapse due to their inherent resistance to current therapies.

"RESULTS FROM OUR STUDIES SHOULD ULTIMATELY ALLOW US TO DEVELOP NOVEL TARGETED THERAPIES TO SPECIFICALLY ELIMINATE CSCS AS THE ROOT OF PANCREATIC CANCER. 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."

Therefore, in order to enhance our understanding of the regulatory machinery of these CSCs, we are running a combined basic and translation research programme, which synergistically combines studies on the biology of mouse and human cancer stem cells, including their *in vivo* microenvironment. First, we are aiming for a comprehensive understanding of the cellular origin of pancreatic cancer stem cells, including their relation to different environmental conditions and how this alters the function of the arising cancer stem cells. Secondly, we are studying their consecutive genetic and epigenetic evolution during tumour progression, including acquisition of invasive and metastatic phenotypes.

In particular, research in our group focuses on: i) the identification of novel biomarkers for CSCs; ii) tracking, isolation and characterisation of circulating CSCs; iii) *in vivo* studies of CSC biology in mouse models; iv) the development of novel CSC-targeting therapies; v) the generation of nanoparticles for targeted drug delivery; and vi), the clinical translation of novel treatment modalities.

Pancreatic stellate cells form a niche for cancer stem cells

Emerging data indicate that the elimination of CSCs as the root of the cancer is of pivotal importance for efficient treatment of pancreatic cancer. Recently, we demonstrated – in a highly relevant preclinical mouse model for primary pancreatic cancers – that the combination of CSC-targeting strategies in combination with a stroma-targeting agent, such as a hedgehog pathway inhibitor and conventional chemotherapy, resulted in significantly enhanced long-term and progression-free survival. This year, we were able to show, mechanistically, that Nodal-expressing pancreatic stellate cells (PSCs) are an important component of the tumour stroma due to their capability to create a paracrine niche for pancreatic cancer stem cells. Secretion of the embryonic morphogens, Nodal/Activin by PSCs, accounts for PSC invasiveness in an Activin Receptor-Like Kinase 4 (Alk4)-dependent manner (FIGURE 2). These findings imply that the pancreatic CSC phenotype is promoted by paracrine Nodal/Activin signalling at the tumour-stroma interface. Therefore, targeting the tumour microenvironment does not only improve drug delivery but, even more importantly, also destroys the cancer stem cell niche and, therefore, should be an integral part of cancer stem cell-based treatment strategies.

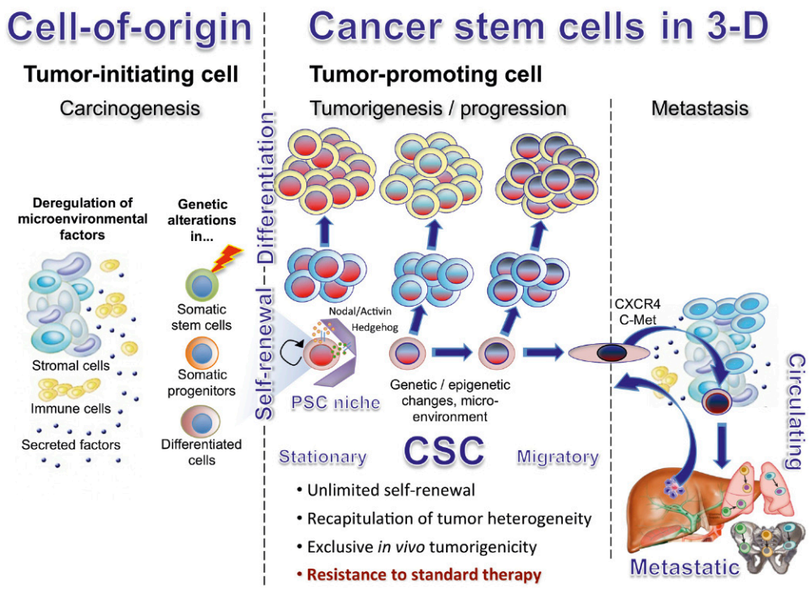

Figure 1 Cancer stem cell concept. Although the origin of CSCs still remains illusive for most cancer entities, distinct populations of CSCs have already been identified. For example, tumour resident CD133+ pancreatic CSCs bearing a self-renewal capacity, are able to repopulate the entire tumour population and are highly resistant to chemo-and radiotherapy. A distinct subpopulation of migrating CSCs, identified by additional expression of CXCR4 and/or c-Met, can be detected in the invasive front as well as in the circulating blood. Detection of these circulating CSCs could serve as prognostic and therapeutic biomarkers and, even more importantly, their prospective isolation as a liquid biopsy serves – in our laboratory – as a valuable source of metastatic CSCs from individual patients.

Metformin targets pancreatic cancer stem cells

As opposed to the cancer cells that make up the bulk of the tumour, CSCs are a small subset of cells that are resistant to conventional therapy for pancreatic cancer. Recent data from our group now indicate that metformin, a widely used and well-tolerated drug for the treatment of diabetes, is capable of efficiently eliminating CSCs. In particular, we found that metformin-pretreated CSCs were especially sensitive to alterations to their metabolism through the activation of 5' adenosine monophosphate-activated protein kinase (AMPK). In fact, metformin treatment resulted in the death of CSCs. In contrast, treatment of more differentiated cancer cells with metformin only arrested the cells' growth. As the cancer stem cells represent the root of pancreatic cancer, their extinction by reprogramming their metabolism with metformin, in combination with the stalling of the proliferation of more differentiated cells, should result in tumour regression and long-term, progression-free survival. In support of this hypothesis, we found that treatment of immunocompromised mice, implanted with a diverse set of patient-derived tumours, with a combination of the anti-diabetic drug metformin and gemcitabine – the standard chemotherapeutic treatment for pancreatic cancer – resulted in reduced tumour burden and the prevention of relapse as compared with treatment based on either drug alone. ■

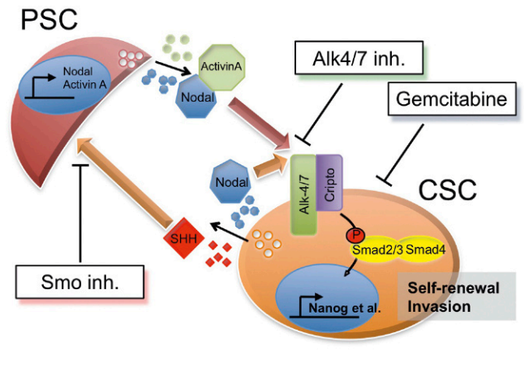

Figure 2 Paracrine Nodal signalling, combined treatment targeting stroma and CSCs. Autocrine (from CSCs) and paracrine (from PSCs) Nodal and Activin A signalling promote stemness and invasiveness of CSCs. In turn, sonic hedgehog (SHH) is released from CSCs and activates paracrine hedgehog signalling in PSCs, resulting in their subsequent activation. This vicious circle can be interrupted by combined inhibition of Alk4/7 in CSCs and smoothened (Smo) in PSCs.

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PATENT

- Heeschen C, Hermann P C, Lonardo E (2012). Triple combination for the treatment of cancer. *PCT/EP2012/071851.*

AWARDS AND RECOGNITION

- Patrick C. Hermann has been awarded the *Hector-Forschungspreis Onkologie* for his outstanding work on the role of cancer stem cells in colon and pancreas cancer.

BREAST CANCER JUNIOR CLINICAL RESEARCH UNIT

Miguel Quintela-Fandino
Junior Clinical Research Unit Head

Staff Scientist
Paloma Navarro

Post-Doctoral Fellow
M. José Bueno (since July)

Clinical Research Fellow
Elena Hernández

Graduate Student
Ivana Zagorac

Technicians
Tamara Mondejar, Jesús Sánchez

Research Associate
Ramón Colomer (from March to September)


Miguel Quintela-Fandino ESP


Paloma Navarro ESP


M. José Bueno ESP


Elena Hernández ESP


Ivana Zagorac SRB


Tamara Mondejar ESP


Jesús Sánchez ESP


Ramón Colomer ESP

OVERVIEW

Our Clinical Research Unit is devoted to the implementation of the findings of CNIO's basic research units into the clinical setting. Generally, we focus on three areas of activity with the aim of creating rational steps towards the improvement of breast cancer management; all the questions driving forth our projects, regardless of whether they concern animal model studies or clinical trials sponsorship, are geared towards their eventual clinical applicability.

- → Preclinical level: determination of tumour reprogramming mechanisms that explain their escape from targeted therapies.
- → Translational level: large collections of breast cancer samples are interrogated with different high-throughput platforms to define clinically significant patient subgroups (i.e. response or resistance to specific agents).
- → Clinical level: concept-driven clinical trials in biomarker-defined breast cancer patient populations.

"THE UNIT HAS EXTENDED ITS CLINICAL NETWORK THIS YEAR, LAUNCHING TRIALS IN 16 HOSPITALS OF THE SPANISH HEALTH SYSTEM AND INTERTWINING ITS ACTIVITIES WITH THE 2 LARGEST SPANISH BREAST CANCER COLLABORATIVE GROUPS, *SOLTI* AND *GEICAM*. TWO NOVEL CONCEPT-DRIVEN TRIALS HAVE BEEN LAUNCHED IN 2012."

RESEARCH HIGHLIGHTS

Hormone receptor-positive breast cancer represents more than two thirds of the total breast cancer cases. All the patients within this subtype are managed with the same treatment strategies (chemotherapy, hormonal blockade), however, great heterogeneity is observed in terms of development of distant metastasis, survival, and response to standard treatments. Recently, different studies published by consortiums involved in the sequencing of large number of tumours have reported a very low incidence of driving mutations in breast cancer (100-fold lower as compared to other malignancies such as melanoma); thus, these findings do not provide an explanation for the observed heterogeneity. Therefore, we hypothesised that other gains-and loss-of-function mechanisms might account for this heterogeneity.

To this end, we interrogated – using comparative genomic hybridisation arrays – a training set of patients characterised by extreme behaviour. Paired by all the known prognostic factors, 2 subgroups constituted the training set: a set of patients with distant metastatic relapse in less than 4 years, and a second set with no evidence of disease after more than 12 years following diagnosis. This setting aims to determine amplifications and deletions (likely to drive the phenotype) – enriched in the aggressive or indolent cases – that help segregate the clinical behaviour of incident cases.

Through this approach, we identified 16 genomic regions with aberrant copy number that occurred only in the bad-prognosis subgroup and another 15 that were only found in the good-prognosis group. These regions are small and contain few genes, which allowed us to determine the gene of interest in each amplicon. A signature composed by these regions helped us to discriminate the clinical outcome of a validation-set series with higher power than conventional prognosis factors. Moreover, as shown (FIGURE), the identified alterations have therapeutic significance since some of these regions contain genes encoding proteins that can be therapeutically targeted by currently available drugs. ■

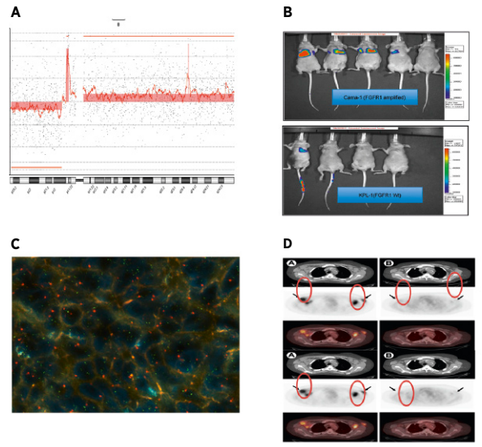

Figure (**A**) shows an amplicon, detected in 25% of aggressive breast cancer cases, that contains coding sequence for the Fibroblast Growth Factor Receptor 1 (FGFR1). (**B**) depicts mice with tamoxifen-treated lung metastasis originated from FGFR1-amplified and non-amplified hormone-positive breast cancer cell lines. Targeting FGFR1 in a patient with refractory breast cancer and FGFR1 amplification (**C**), led to an improved response to therapy (**D**).

PUBLICATIONS

- Arpaia E, Blaser H, Quintela-Fandino M, Duncan G, Leong HS, Ablack A, Nambiar SC, Lind EF, Silvester J, Fleming CK, Rufini A, Tusche MW, Brüstle A, Ohashi PS, Lewis JD, Mak TW (2012). The interaction between caveolin-1 and Rho-GTPases promotes metastasis by controlling the expression of alpha5-integrin and the activation of Src, Ras and Erk. *Oncogene* 31, 884-896.
- Turrado C, Puig T, García-Cárceles J, Artola M, Benhamú B, Ortega-Gutiérrez S, Relat J, Oliveras G, Blancafort A, Haro D, Marrero PF, Colomer R, López-Rodríguez ML (2012). New synthetic inhibitors of fatty acid synthase with anticancer activity. *J Med Chem* 55, 5013-5023.

AWARDS AND RECOGNITION

- Ramón Colomer has been appointed Head of Oncology, *Hospital La Princesa in* Madrid, Spain.

CRIS FOUNDATION-CNIO PROSTATE CANCER AND GENITOURINARY TUMOURS JUNIOR CLINICAL RESEARCH UNIT

David Olmos (since September)
Junior Clinical Research
Unit Head

Research Associate
Jesús García-Donas
(since September)

Visiting Scientist
Nuria Romero (since December)

David Olmos ESP

OVERVIEW

Prostate cancer is the second most common form of cancer in males, with approximately 900,000 cases every year worldwide. Despite radical treatment, up to a third of all these patients may succumb to advanced, castration resistant disease.

Our Unit plans to focus on translating basic cancer research into clinical practice, especially in the following areas:

- → Basic research: to characterise the role of DNA repair deficiency and chromosomal instability in prostate cancer progression and castration-resistance.
- → Translational research: to individualise patients' management by implementing the molecular characterisation of circulating tumour cells and other novel blood-borne circulating biomarkers as less invasive and more reproducible approaches.
- → Clinical research: to conduct early biomarker-driven clinical trials with novel agents in patients with this disease.

"WE AIM TO TRANSLATE ADVANCES IN PROSTATE CANCER RESEARCH INTO IMPROVEMENTS IN PATIENT CARE BY DESIGNING AND CONDUCTING BIOMARKER-DRIVEN EARLY CLINICAL TRIALS WITH NOVEL DRUGS, FOLLOWING THE PRINCIPLES OF SOLID BIOLOGICAL RATIONALE, EXHAUSTIVE PRECLINICAL TESTING AND PERSONALISED MEDICINE."

PUBLICATIONS

- Olmos D, Brewer D, Clark J, Danila DC, Parker C, Attard G, Fleisher M, Reid AH, Castro E, Sandhu SK, Barwell L, Oommen NB, Carreira S, Drake CG, Jones R, Cooper CS, Scher HI, de Bono JS (2012). Prognostic value of blood mRNA expression signatures in castration-resistant prostate cancer: A prospective, two-stage study. *Lancet Oncol* 13, 1114-1124.

Article in press

- Castro E *et al.* (incl. Olmos D). Germline BRCA mutations are associated with higher risk of nodal involvement, distant metastasis and poor survival outcomes in prostate cancer. *J Clin Oncol* (in press).

Publications at other institutions

- Olmos D, A'hern RP, Marsoni S, Morales R, Gomez-Roca C, Verweij J, Voest EE, Schöffski P, Ang JE, Penel N, Schellens JH, del Conte G, Brunetto AT, Evans TR, Wilson R, Gallerani E, Plummer R, Tabernero J, Soria JC, Kaye SB (2012). Patient selection for oncology phase I trials: a multi-institutional study of prognostic factors. *J Clin Oncol* 30, 996-1004.
- Arkenau HT, Plummer R, Molife LR, Olmos D, Yap TA, Squires M, Lewis S, Lock V, Yule M, Lyons J, Calvert H, Judson I (2012). A phase I dose escalation study of AT9283, a small molecule inhibitor of aurora kinases, in patients with advanced solid malignancies. *Ann Oncol* 23, 1307-1313.
- Mezynski J, Pezaro C, Bianchini D, Zivi A, Sandhu S, Thompson E, Hunt J, Sheridan E, Baikady B, Sarvadikar A, Maier G, Reid AH, Mulick Cassidy A, Olmos D, Attard G, de Bono J (2012). Antitumour activity of docetaxel following treatment with the CYP17A1 inhibitor abiraterone: clinical evidence for cross-resistance? *Ann Oncol* 3, 2943-2947.
- Molife LR, Alam S, Olmos D, Puglisi M, Shah K, Fehrmann R, Trani L, Tjokrowidjaja A, de Bono JS, Banerji U, Kaye SB (2012). Defining the risk of toxicity in phase I oncology trials of novel molecularly targeted agents: a single centre experience. *Ann Oncol* 23, 1968-1973.
- Olmos D, Ang JE, Gomez-Roca C, Morales-Barrera R, Vulink AJ, Massard C, Kaye S (2012). Pitfalls and limitations of a single-centre, retrospectively derived prognostic score for phase I oncology trial participants-reply to Fussenich *et al.*: a new, simple and objective prognostic score for phase I cancer patients. *Eur J Cancer* 48, 594-596.
- Naing A, Aghajanian C, Raymond E, Olmos D, Schwartz G, Oelmann E, Grinsted L, Burke W, Taylor R, Kaye S, Kurzrock R, Banerji U (2012). Safety, tolerability, pharmacokinetics and pharmacodynamics of AZD8055 in advanced solid tumours and lymphoma. *Br J Cancer* 107, 1093-1099.
- Ploquin A, Olmos D, Lacombe D, A'Hern R, Duhamel A, Twelves C, Marsoni S, Morales-Barrera R, Soria JC, Verweij J, Voest EE, Schöffski P, Schellens JH, Kramar A, Kristeleit RS, Arkenau HT, Kaye SB, Penel N (2012). Prediction of early death among patients enrolled in phase I trials: development and validation of a new model based on platelet count and albumin. *Br J Cancer* 107, 1025-1030.
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RESEARCH HIGHLIGHTS

Whole blood RNA expression profiles in prostate cancer as an example for novel circulating biomarkers

Our recent work – the major part of which was conducted when our group was still located at the Institute of Cancer Research and The Royal Marsden NHS Foundation Trust in the United Kingdom – showed that it is possible to learn about prostate cancer from its footprint in blood. Such signatures of prostate cancer in the blood allow us to develop a test that is potentially more accurate than the presently available ones and to further the benefits for patients by circumventing the need for a biopsy.

We analysed the whole transcriptome of high-quality RNA obtained from whole blood collected from 100 patients with prostate cancer (69 patients with advanced cancer and 31 control patients with low-risk, early-stage cancer and managed by active surveillance) by using the Affymetrix HGU133 plus 2 genechip. By means of statistical modelling, with an unsupervised Bayesian method called Latent Process decomposition (LPD), we divided the patients into 4 groups according to gene expression patterns. One of these patterns, LPD1, was associated with high-risk baseline characteristics such as elevated LDH or ALP, higher PSA levels or augmented circulating tumour cell counts. Differentially expressed genes in this pattern were functionally associated with depression of B-and T-cell function and mobilisation of early red cell precursors from bone marrow, probably as markers of early bone-marrow failure and immune depression. After a follow-up of 2.5 years we found that patients in LPD1 had survived for significantly less time than patients in the other groups. The prognostic value of this gene expression signature from whole blood was confirmed in 70 patients with advanced prostate cancer from Memorial Sloan-Kettering Cancer Centre in New York, demonstrating that patients in LPD1 survived 9.2 months – more than 1 year less (21.6 months) – than patients who did not exhibit this gene expression signature. ■

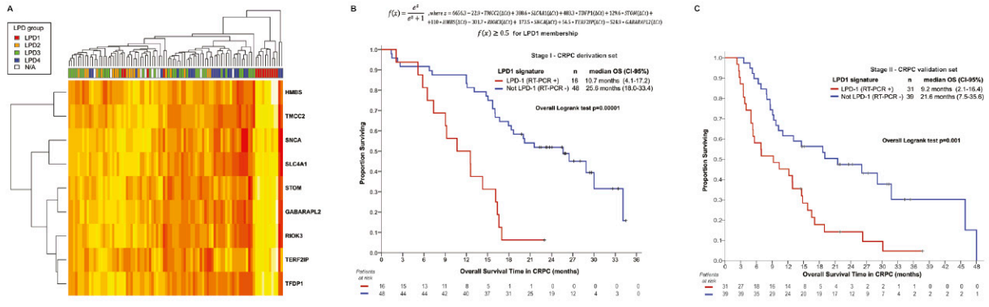

Figure A reduced 9-gene signature was developed to classify LPD1 patients. (**A**) Heat-map of the 9-gene classifier. Kaplan-Meier overall survival curves, according to the LPD1 status in (**B**) 64 patients with advanced, castration resistant prostate cancer (CRPC) included in the derivation set and (**C**) 70 patients with CRPC included in the validation set.

MOLECULAR DIAGNOSTICS CLINICAL RESEARCH UNIT

Luis Lombardía
Clinical Research Unit Head

Clinical Investigator
Elena García

Technician
Diana Romero

Luis Lombardia ESP

Elena García ESP

Diana Romero ESP

OVERVIEW

The activities of the Molecular Diagnostics Clinical Research Unit focus on developing, implementing and making available a wide variety of highly sensitive and specific molecular diagnostics tools and services that are scarcely available in the Hospitals of the Spanish National Health System. Our portfolio of assays allows the determination of alterations in the sequence or expression levels of key genes in cancer crucial for the early diagnosis of neoplasias before their clinical manifestation, the detection of Minimal Residual Disease in patients showing clinical remission, and the follow-up of the response of the patients to therapeutic interventions. Our Unit also collaborates closely with CNIO's Clinical Research Programme in the design and implementation of pharmacodiagnostic approaches by developing new tests based on novel biomarkers and state-of-the-art technologies.

"DESPITE BUDGET CUTS IN THE NATIONAL HEALTH SYSTEM, THE UNIT HAS MAINTAINED THE VOLUME OF DIAGNOSTICS TESTS OFFERED TO SPANISH HOSPITALS AS COMPARED TO 2011, CONTRIBUTING TO BETTER-INFORMED CLINICAL DECISIONS THAT MAXIMISE DRUG EFFICACY, AND, IN SOME CASES, ADD VALUE TO DRUG DEVELOPMENT PIPELINES."

PUBLICATIONS

- Langerak AW *et al.* (incl. Lombardía L) (2012). EuroClonality/BIOMED-2 guidelines for interpretation and reporting of Ig/TCR clonality testing in suspected lymphoproliferations. *Leukemia* 26, 2159-2171.
- Alvarez-Díaz S, Valle N, Ferrer-Mayorga G, Lombardía L, Herrera M, Domínguez O, Segura MF, Bonilla F, Hernando E, Muñoz A (2012). MicroRNA-22 is induced by vitamin D and contributes to its antiproliferative, antimigratory and gene regulatory effects in colon cancer cells. *Hum Mol Genet* 21, 2157-2165.
- Kerguelén Fuentes AE, Hernández-Maraver D, Lombardía L, Canales Albendea MA, Rodríguez de la Rúa A (2012). Clinical significance of the quantification of JAK2V617F allele burden in classical Ph-negative myeloproliferative neoplasms. *Med Clin (Barc)* 139, 373-378.

Article in press

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AWARDS AND RECOGNITION

- Editorial Board Member, *Dataset Papers in Biology.*

RESEARCH HIGHLIGHTS

During 2012, we increased our routine capability with new molecular diagnostic tests allowing the detection of:

- Mutations in exon 10 of the *MPL* gene, improving our competence in the identification of patients with myeloproliferative disorders previously diagnosed negative for *JAK2* mutations.
- Mutations in exon 3 of the *NRAS* gene that are found in solid tumours, including melanoma, colorectal and thyroid cancers; the detection of these mutations may be useful to classify subtypes of the disease and to guide personalised therapies.
- Genomic amplifications of the *c-MET* gene that are associated with poor prognosis in various types of tumours and predict the therapeutic response to MET inhibitors.

We have also completed the experimental phase of a collaboration in a research project linked to a clinical trial (ENEST First) coordinated by Novartis Pharmaceuticals SA. We are now in the analytical stage; our goal is to take advantage of the clinical trial's endpoint results for the identification of biomarkers that are predictive of a complete molecular response in patients with chronic myeloid leukaemia treated first-line with the drug nilotinib.

Among the multiple challenges facing the molecular diagnostics of cancer, a key issue is to detect the maximum number of patients affected by a malignancy. With this aim in mind, our Unit has designed a diagnostic platform to detect – at a sustainable cost and time allocation– the gene translocations observed in more than 95% of patients with synovial or Ewing Sarcomas (FIGURE). In collaboration with the *Hospital 12 de Octubre* in Madrid, we will soon start validation experiments for its definitive implementation.

Our Unit has also maintained a strong policy regarding QC/QA procedures – by participating in international and Spanish groups devoted to External Quality Assessment programmes – as well as by providing training and educational programmes; during 2012 our Unit trained 2 pre-doctoral students, 1 laboratory technician and 4 medical residents. ■

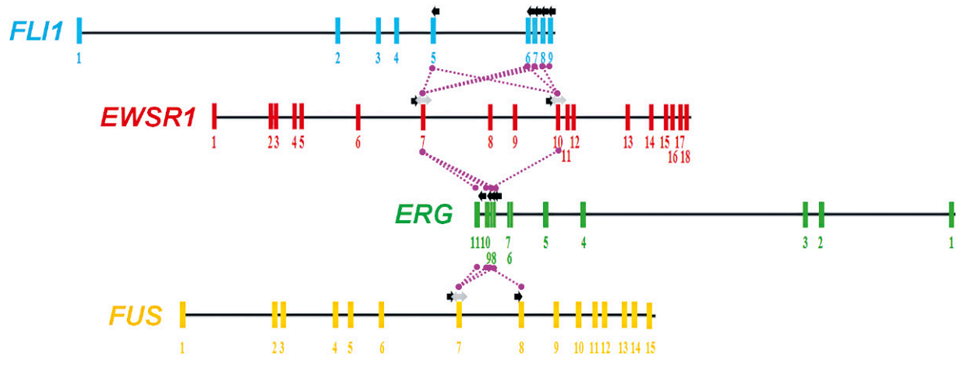

Figure Localisation of primers (black arrows) and probes (grey arrows) for the implementation of a diagnostic platform that has been designed to detect, by means of qRT-PCR, the 18 most frequent translocations (dotted purple lines) between the genes *FLI1*, *EWSR1*, *ERG* and *FUS*, commonly observed in patients with Ewing Sarcomas.

TRANSLATIONAL BIOINFORMATICS UNIT

Fátima Al-Shahrour (since February)
Unit Head

Fátima Al-Shahrour ESP

OVERVIEW

Translational Bioinformatics has rapidly evolved from an emerging research field into a complex multidisciplinary area that intersects biomedical research, informatics and clinical research.

The official definition of Translational Bioinformatics is the development of storage, analytic, and interpretive methods to optimise the transformation of increasingly voluminous biomolecular data into proactive, predictive, preventive and participatory health.

The main focus of the Translational Bioinformatics Unit at the CNIO includes research on the development of novel computational techniques for the integration of cancer genomic multidimensional data with clinical and pathological features, and to apply these new methodologies to detect therapeutic targets and biomarkers of patient response. Our final goal is to translate the knowledge from cancer genome studies to effective treatment for cancer patients.

"DURING 2012 WE HAVE BEEN WORKING ON THE IMPLEMENTATION OF A NOVEL PIPELINE MODULE FOR THE ANALYSIS OF NEXT-GENERATION SEQUENCING DATA FROM PATIENTS' TUMOURS. THIS MODULE WILL ALLOW US TO PRIORITISE GENETIC VARIANTS THAT ARE POTENTIAL THERAPEUTIC TARGETS."

RESEARCH HIGHLIGHTS

The Translational Bioinformatics Unit was established in February 2012. During this first year, we have focused on the development of computational genomic-based clinical tests to help categorise patients' tumours and match them to effective drugs or treatments based on their genomic alterations. These alterations include mutations, copy number variations, genomic rearrangements and activation/deregulation profiles obtained from high-throughput technologies, such as next-generation sequencing and gene expression microarrays.

Integrative genomics

Diversity and complexity are both hallmarks of cancer genomes. Large-scale international cancer genome projects (ICGC, TCGA) have emerged to comprehensively characterise the molecular alterations of diverse cancer types. Thus, the analysis of human cancer includes the integration of these large sets of data and information.

In this context, our 3 main goals are: i) to develop bioinformatics approaches – that integrate genomic multidimensional data with clinical and pathological risk factors – into useful biomarkers that can classify patients' tumours by prognosis and response to therapeutic modalities; ii) to discover novel cancer-associated molecular markers or pathways that are useful targets for therapy; and iii), to generate an efficient predictor methodology, based on previously analysed genomic data, that may help to further refine and guide future treatment decisions (FIGURE).

Personalised cancer medicine

Personalised medicine has the potential to fulfil the promise of delivering the right drug dosage, for the right indication, to the right patient by specifically targeting the genomic aberrations that drive an individual patient's tumour behaviour.

The Clinical Research Programme at the CNIO is pioneering personalised cancer treatment and, in collaboration with associated hospitals, aims to implement individualised and targeted therapies that are based on tumour genome profiling and on the Avatar mouse response to candidate drugs.

The Translational Bioinformatics Unit plays a key role in CNIO's personalised cancer medicine platform by providing the framework and expertise for the systematic interpretation of individual cancer genomes. ■

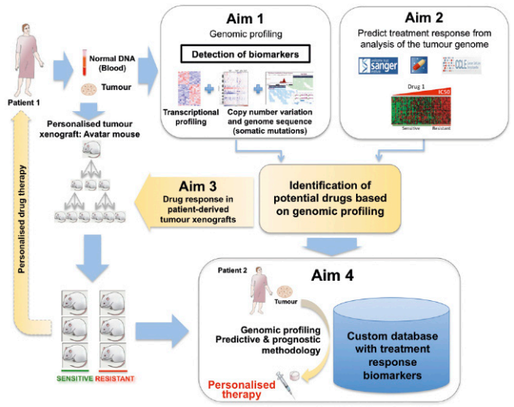

Figure The 3 main aims of the Translational Bioinformatics Unit for personalised cancer medicine: 1) genome profiling, 2) discovery of novel druggable biomarkers, and 3), development of a drug response predictor based on known response biomarkers.

PUBLICATIONS

Publications at other institutions

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- Weaver Z, Difilippantonio S, Carretero J, Martin PL, El Meskini R, Iacovelli AJ, Gumprecht M, Kulaga A, Guerin T, Schlomer J, Baran M, Kozlov S, McCann T, Mena S, Al-Shahrour F, Alexander D, Wong KK, Van Dyke T (2012). Temporal molecular and biological assessment of an erlotinib-resistant lung adenocarcinoma model reveals markers of tumor progression and treatment response. *Cancer Res* 72, 5921-5933.

BIOBANK

Manuel M. Morente
Biobank Director

Associate Director
Elena García

Technical Coordinator
Lydia Sánchez

Technicians
M. Jesús Artiga, Francisco de Luna,
M. Cruz Marín (since February)


Manuel M. Morente ESP


Lydia Sánchez ESP


M. Jesús Artiga ESP


Francisco de Luna ESP


M. Cruz Marín ESP

OVERVIEW

Access to human biological samples and their associated data is considered as a major bottleneck in biomedical research. Between 2000 and 2012, the former CNIO Tumour Bank Core Unit provided samples and associated data, as well as related ethical documentation support, to CNIO researchers through the Spanish National Tumour Bank and Biobank networks; this network involves 63 institutions, is promoted by the *Instituto de Salud Carlos III* (ISCIII), and is coordinated by the CNIO (www.redbiobancos.es).

Upon the entering into force of the new legal framework for biobanking – based among others on RD1716/2011 – the former Tumour Bank Unit ceased its activity and was transformed into today's Biobank under the umbrella of CNIO's Vice-Direction for Translational Research. The main objectives of the newly created Biobank include: i) supplying tumour samples and associated data to intramural and extramural research groups; ii) offering sample management services to CNIO researchers who work with human sample collections; iii) offering ethical and technical advice to intramural and extramural research groups, cooperative networks and clinical trials; and iv), promoting a harmonised approach to biobanking through the coordination of the Spanish National Biobanks Network and the European Biobank Network (BBMRI-ERIC).

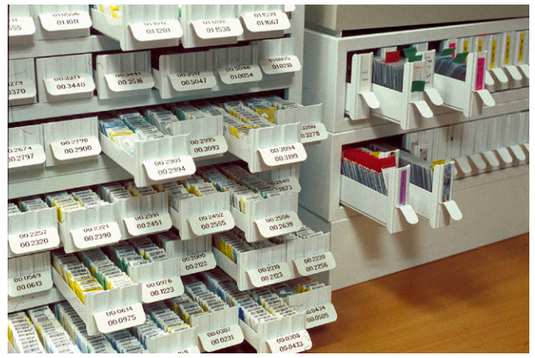
"THE LAST TWO DECADES HAVE BEEN MARKED BY A HUGE TECHNICAL DEVELOPMENT IN GENETICS THAT HAS TRANSFORMED ACCESS PROCEDURES TO HUMAN BIOLOGICAL MATERIAL; THEREBY CREATING A NEW ETHICAL PARADIGM AND, CONSEQUENTLY, AN EXTREMELY STRICT LEGISLATION REGARDING PERSONAL DATA PROTECTION THAT INCLUDES GENETIC INFORMATION OBTAINED FROM HUMAN SAMPLES."

RESEARCH HIGHLIGHTS

Tumour Bank Core Unit (until September)

As a biological resource centre, we have supported 25 research projects throughout 2012, most of which belong to multicentre cooperative groups. The mean impact factor of the 28 publications supported by our Unit in 2012 was 6.7. We also provided sample and/or documental support for the CNIO's Molecular Diagnostic Core Unit (279 cases) and the familial cancer activities of the CNIO's Human Cancer Genetics Programme (23 cases).

Furthermore, our Unit actively collaborated with the ISCIII in the coordination of the *Red Temática de Biobancos Hospitalarios*, as well as in activities related to the ESFRI Pan-European infrastructure on biobanking BBMRI-ERIC. 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 International Society for Biological and Environmental Repositories (ISBER), the European, Middle East and African Society of Biopreservation and Biobanking (ESBB), international think tanks such as the Marble Arch International Working Group on Clinical Biobanking, European (ESFRI) Platforms such as BBMRI-ERIC and the Spanish Society of Pathology (SEAP).



CNIO Biobank (since October)

The CNIO-Biobank has been set up as a transversal service platform for all CNIO researchers and the general research community. Its purpose is to obtain, process, store and share human biological samples for research excellence in cancer, in line with the best technical, ethical and quality standards. The institutional commitment of the CNIO is apparent through the creation and support of this Biobank that complies fully with the present legal framework. In keeping with its objectives, the Biobank will further increase its capacities to host specimens collected for specific research projects at the CNIO as well as for projects that fall within the framework of collaborative networks involving Spanish hospitals. ■

PUBLICATIONS

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- Molina-Privado I, Jiménez-P R, Montes-Moreno S, Chiodo Y, Rodríguez-Martínez M, Sánchez-Verde L, Iglesias T, Piris MA, Campanero MR (2012). E2F4 plays a key role in Burkitt lymphoma tumorigenesis. *Leukemia* 26, 2277-2285.
- Czachorowski MJ, Amaral AF, Montes-Moreno S, Lloreta J, Carrato A, Tardón A, Morente MM, Kogevinas M, Real FX, Malats N; for the SBC/EPICURO investigators (2012). Cyclooxygenase-2 expression in bladder cancer and patient prognosis: results from a large clinical cohort and meta-analysis. *PLoS One* 7, e45025.
- Guisado-Vasco P *et al.* (incl. Morente M) (2012). Stage IV and age over 45 years are the only prognostic factors of the International Prognostic Score for the outcome of advanced Hodgkin lymphoma in the Spanish Hodgkin Lymphoma Study Group series. *Leuk Lymphoma* 53, 812-819.
- Álava E, Arias J, Ariza A, Cuatrecasas M, Fernández PL, Morente M, Nicolas P (2012). Preguntas y recomendaciones sobre integración de los archivos diagnósticos de los servicios de Anatomía Patológica en biobancos. *Rev Esp Patol* 45, 215-217.

Article in press

- Moya P, Esteban S, Fernández-Suárez A, Maestro M, Morente M, Sánchez-Carbayo M. KiSS-1 methylation and protein expression patterns contribute to diagnostic and prognostic assessments in tissue specimens for colorectal cancer. *Tumor Biol* (in press).

AWARDS AND RECOGNITION

- President-Elect of the European, Middle East & African Society of Biopreservation and Biobanking (ESBB).

“THE DIRECTION OF INNOVATION ENHANCES THE VALUE GENERATED BY CNIO’S SCIENTIFIC ACTIVITIES, EXPANDING THEIR SCOPE TOWARDS PRODUCT AND SERVICE DEVELOPMENT, AND GENERATING POSITIVE SOCIAL AND ECONOMIC IMPACTS.”

MARISOL QUINTERO DIRECTOR OF INNOVATION

DIRECTION OF INNOVATION

MARISOL QUINTERO DIRECTOR

"THROUGH THE ESTABLISHMENT OF COLLABORATIONS WITH PRIVATE PARTNERS, WE LEVERAGE OUR EXISTING RESOURCES AND CAPABILITIES, BRINGING ADDITIONAL EXPERTISE AND INVESTMENT TO IMPROVE OUR COMPETITIVENESS."

MARISOL QUINTERO DIRECTOR OF INNOVATION

Since its establishment in October 2011, the Direction of Innovation aims to fulfil one of the major strategic goals of the CNIO, namely, the translation of discoveries into practical solutions. The Biotechnology and Experimental Therapeutics Programmes, together with the Technology Transfer and Valorisation Office, constitute the Direction of Innovation. The Biotechnology Programme, composed of different units that act together as a technology service provider, is engaged in the development of novel technologies, such as antibodies, research tools, or the improvement of existing technological platforms. The Experimental Therapeutics Programme is dedicated to identifying novel drug targets and to validating them as potential therapeutic opportunities for the treatment of cancer. The Technology Transfer and Valorisation Office has been very active in attracting partners to commercialise or further develop CNIO's technologies.

The achievements of the Direction of Innovation were threefold:

- During 2012 we were able to strengthen our collaborations with existing partner companies. One example is the agreement signed with Roche. This alliance is unique in Spain and is the third one of its kind in Europe; it is framed within Roche's Extending Innovation Network. Our partnership with another pharmaceutical company, Eli Lilly & Company, has also been expanded and now the CNIO is part of their Open Innovation Drug Discovery platform. This initiative enables us to share some of our proprietary compounds, with the goal of identifying potential new medicines that would act through novel mechanisms.
- A second key component is the generation of new ventures to promote technology transfer. At the CNIO, we believe that young researchers are instrumental to the generation and diffusion of new technologies, and for that reason we launched a new initiative, in collaboration with *Instituto de Empresa* Business School, *CNInnOtrain*. Thanks to the support of *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.
- Finally, the Direction of Innovation aims to assist the CNIO in achieving an important goal: attract additional funding from private sponsors. Ongoing fundraising activities will enable the accomplishment of future strategic endeavours. ■

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 that are widely used in biomedical research, as well as to implement and develop state-of-the-art biotechnological tools and reagents for cancer research. The Programme is currently composed of 9 Core Units that cover major areas in biotechnology; namely, Genomics, Proteomics, Monoclonal Antibodies, Histopathology, Flow Cytometry, Confocal Microscopy, Molecular Imaging and Transgenic Mice, as well as an Animal Facility. Although the Core Units are mainly focused on meeting the internal demand from the CNIO Research Groups, they also provide services and collaborate with groups from other institutions in both the public and private domains.

During 2012, the Programme underwent two significant organisational changes. The search for a new Head of the Proteomics Core Unit culminated with the recruitment of Javier Muñoz; a brilliant young proteomics specialist coming from the University of Utrecht who joined the CNIO in October. We wish him lots of success in leading the Unit over the forthcoming years.

Also during this past year, the two previous units that provided support in histology and immunohistochemistry technologies for human and mouse tissues, were reorganised and merged into one single Histopathology Core Unit; under the leadership of Marta Cañamero who previously headed the Comparative Pathology Core Unit. Lydia Sánchez, the former Head of the Histology and Immunohistochemistry Core Unit, has moved to the CNIO Biobank as its Technical Coordinator. We wish them good luck with their new responsibilities.

Last but not least, 2012 has been a very productive year for the Programme from a scientific point of view. The Transgenic Mice Core Unit, led by Sagrario Ortega, published their transgenic 'lymphoreporter' mouse model that allows tracking the development of lymphatic vessels by optical imaging. Likewise, Jorge L. Martínez (Proteomics Core Unit), published the characterisation of human antibody fragments (scFv) against ephrinB2, with anti-angiogenic properties *in vitro* and *in vivo*, as potential candidates for the development of novel therapies. These molecules have been licensed to an external company for their eventual commercial exploitation. These publications, together with others also released this year, reflect the excellence of the Programme as a generator of biotechnological tools and methods. ■

"THE BIOTECHNOLOGY PROGRAMME HAS DEMONSTRATED ITS COMPETITIVE EDGE IN 2012: WE HAVE SUCCEEDED IN DYNAMICALLY ADAPTING OUR STRUCTURE AND PORTFOLIO OF SERVICES TO THE EVOLVING DEMANDS OF CNIO RESEARCHERS, AND MADE ESSENTIAL CONTRIBUTIONS TO SEVERAL LANDMARK PUBLICATIONS, INCLUDING PAPERS REPORTING NEW ADVANCED BIOTECHNOLOGICAL TOOLS AND APPLICATIONS LED BY THE UNITS."

GENOMICS CORE UNIT

Orlando Domínguez
Core Unit Head

Technicians
Purificación Arribas, Martha L. Campo, Guadalupe Luengo, Jorge Monsech, David B. Rodríguez, Ángeles Rubio


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Jorge Monsech ESP


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OVERVIEW

Genomics deals with the study of all the genes in an individual, of their interactions and orchestrated activities, and with the study of functional elements – genes or not – in its genome sequence. It deals with both the structure and dynamics of the genome, with the interactions of those genes with one another, and with their environment. The genome is a conglomerate of functional and non-functional elements, deserts, fossils or mobile genetic pieces, each with a different tempo and capabilities. The genome is chemically made of DNA, but packed, maintained and interpreted by a myriad of protein complexes. The genome is a display of all the genes, active or dormant, within a living cell or tissue. Not immutable, the genome changes and adapts to events such as damage or a changed environment; source of either individual miseries or chance for a species' evolution.

"AS A SERVICE-PROVIDING FACILITY, THE GENOMICS CORE UNIT CONFIGURES A TOOLBOX FOR DNA AND RNA ANALYSES DEDICATED TO AN ARRAY OF APPLICATIONS, EITHER AT THE SINGLE LOCUS OR AT A MORE GLOBAL GENOMIC LEVEL. THE UNIT ACTIVELY EMPLOYS ITS RESOURCES TO HELP CNIO SCIENTISTS TO UNDERSTAND THE MOLECULAR PROCESSES UNDERLYING CANCER."

RESEARCH HIGHLIGHTS

Cancer is eminently a genetically based condition whose complexity can only be appreciated with the broadness of genomics approaches. Genomics' insights reveal basic clues for the understanding of the disease, its evolution and diversity, within a tumour and within a patient. Genomics 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 variants and differences between samples or conditions at a structural level; mutations, location of protein factors or complexes, structural variations in chromatin, prognostic biomarkers or even therapeutic targets. Others are suitable to examine functional choreographies; the dynamic gene life/activity with its varied response to stimuli or treatments, or as affected by mutations.

The Genomics Unit provides services at 2 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, or genome-wide location of interacting protein factors on chromosomal DNA by ChIPseq. These applications are based on the use of a Genome Analyser IIx from Illumina. In addition, gene expression or transcriptome and detection of chromosomal copy number aberrations can also be dissected with DNA microarrays. The latter are based on a DNA microarray scanner G2505C from Agilent. At the single locus level different services 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. The Unit also provides a service for the identification of transgene insertion sites in genetically engineered mouse models, and a locus methylation analysis through bisulfite sequencing technology. ■

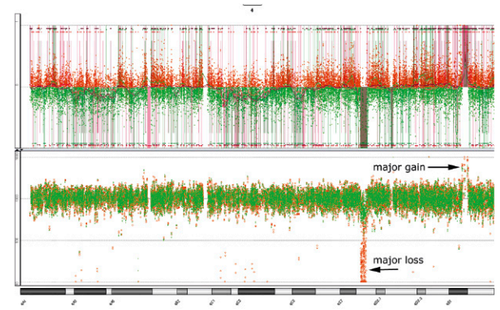

Figure Sooner or later, cancer develops unbalanced chromosomal abnormalities in which gains or losses of genetic material occur. Array Comparative Genomic Hybridisation (aCGH) provides a genome-wide screening for such copy-number variations. Two genomes, test and control, are analysed in a microarray that contains probes evenly spaced at little genomic distance. The figure represents a linear representation of the probes along a chromosome showing segment losses and gains in a tumour.

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TRANSGENIC MICE CORE UNIT

Sagrario Ortega
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Graduate Student
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OVERVIEW

Genetically engineered mouse models (GEMMs) have become an essential tool for studies of gene function and disease mechanisms, as well as for drug discovery and target validation. The Transgenic Mice Core Unit offers state-of-the-art technology for the manipulation of the mouse genome and for the cryopreservation of genetically modified mouse strains. In collaboration with the CNIO Animal Facility, the Unit is also in charge of the re-derivation of mouse strains and manages a collection of tool strains (Cre/Flp transgenes, reporter lines, etc.), which have either been developed in-house or acquired from external sources. The Unit gives support to CNIO researchers and collaborates with them on many aspects related to research with embryonic stem (ES) and induced pluripotent stem (iPS) cells, as well as any type of embryo-and mouse model-based research. Finally, the Unit leads its own research projects that are aimed at the generation of GEMMS for studies of tumour vascularisation and its contribution to metastasis.

"THIS YEAR WE HAVE GENERATED 15 GEMMS WITH CANCER-RELATED MUTATIONS AND HAVE DEVELOPED A NOVEL MOUSE MODEL TO STUDY TUMOUR METASTASIS BY *IN VIVO* IMAGING OF LYMPHATIC VESSELS AND LYMPHANGIOGENESIS. THESE ANIMAL MODELS WILL ENHANCE OUR UNDERSTANDING OF TUMOUR BIOLOGY AND FACILITATE THE DEVELOPMENT OF CANCER THERAPIES."

RESEARCH HIGHLIGHTS

Core activities

Throughout 2012, 15 new GEMMs have been generated by gene targeting in ES cells or by BAC transgenesis. Moreover, 30 new lines have been imported to the CNIO Animal Facility by re-derivation, and close to 100 strains have been cryopreserved by sperm and embryo freezing.

In March 2012, a collaboration agreement between the CNIO and the Spanish node of the European Mouse Mutant Archive (EMMA), at the *Centro Nacional de Biotecnología* (CNB) in Madrid, was signed regarding the transfer of genetically modified mouse strains – generated and cryopreserved at our Unit – to the European archive. Through this agreement, the CNIO facilitates the distribution of mouse strains that are of extraordinary interest for the international cancer research community. The transfer of these strains is entirely coordinated by our Unit in collaboration with the Spanish node.

Together with CNIO's IT Department, we are creating a database for the management of cryopreserved strains. During 2012, the database for sperm cryopreservation was completed and has in the mean time been made available to CNIO researchers via our Unit's intranet.

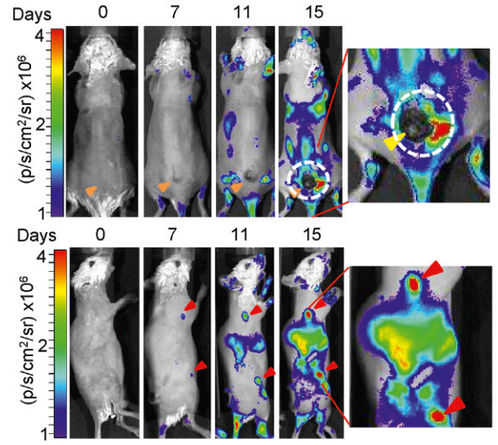

Figure *In vivo* dorsal (top) and lateral (bottom) luciferase signal in *Vegfr3*^{EGFPLuc} mice measured at different times after subcutaneous injection of B16-V5 melanoma cells. Orange arrowheads: site of injection and tumour. Yellow arrowheads: luciferase emission from the periphery of the tumour (white circle). Red arrowheads: lymph node luciferase signal (Martínez Corral *et al*., *Proc Natl Acad Sci USA*, 2012).

Research activities

We have created a mouse model in which, for the first time, lymphatic vessel growth or lymphangiogenesis can be monitored *in vivo* with non-invasive imaging techniques. In this model, the fluorescent/luminescent reporter EGFP-Luciferase is expressed under the endogenous transcriptional control of the *Vegfr3* gene, a classical marker for the lymphatic endothelium. This genetic modification enables the tracking and quantifying of lymphangiogenesis that is associated to inflammation and tumour growth. Moreover, the response of the lymph nodes to metastatic tumours, which spread mostly through the lymphatic network, can also be monitored *in vivo* with this strain. The model represents a powerful tool for studying lymphatic vessels in physiological and pathological processes, as well as for the discovery and validation of anti-angiogenic drugs. The publication of this technical advance was commented in the 'Research Highlights' section of *Nature* ("Follow the lymph vessels", *Nature,* 2012). ■

PUBLICATIONS

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MONOCLONAL ANTIBODIES CORE UNIT

Giovanna Roncador
Core Unit Head

Technicians
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M. Mar López ESP


Lorena Maestre ESP


Ana I. Reyes ESP

OVERVIEW

Hybridoma technology for the production of monoclonal antibodies (mAbs) represents one of the most relevant methodological advances in biomedicine. The availability of mAbs has significantly improved our knowledge about cell biology and has opened up new possibilities for basic and applied research. Their use in medicine has also improved the diagnostics, prevention and treatment of a large number of diseases including cancer.

Our Unit provides Research Groups at the CNIO with *à la carte* generated mAbs that are indispensable tools for the characterisation of novel pathways and targets relevant to cancer diagnostics and treatment. We are highly specialised in mouse and rat mAb production. Our services include mAb production in genetically engineered mouse models, mAb characterisation and validation, medium-scale mAb production, and also *Mycoplasma* testing for the Cell Culture Facility.

"THE UNIT PRODUCES NOVEL AND HIGH-QUALITY MONOCLONAL ANTIBODIES THAT ARE USED IN BASIC RESEARCH TO GAIN NOVEL INSIGHTS INTO CANCER BIOLOGY. BEING HIGHLY SPECIALISED IN MONOCLONAL ANTIBODY CHARACTERISATION, WE PROVIDE CNIO RESEARCHERS WITH RELIABLE AND WELL-VALIDATED REAGENTS THAT ADD VALUE TO THEIR RESEARCH PROJECTS."

RESEARCH HIGHLIGHTS

During the last 12 years, the Unit has generated and characterised a large number of mAbs, mostly targeting molecules for which mAbs are not commercially available. The mAbs produced to date are directed against the following proteins: AID, Annexin-IV, ARK5, a,b,cMYB BCL2, BCL6, BCL7a, β-galactosidase, BMP4, BTLA, CD5, CD8, CD15, CD30, CD43, CD1D, CDK6, CEMP-C1, cMYB, CSF1, CSF1R, E4F1, EED, EstrogenR, EphrinB2, Fc, FOXP3, FRA2, GAPDH, Gasdermin, Gastrin, GCET1, GFP, GST, HLA-DP-DQ-DR, HP1alpha, HJURP, KLHL-6, Lipocalin-2, LMO2, Luciferase, LKB1, MASTL, MALT-1, MAP17, MASTL, MBP, MNDA, MSP58, MYBL1, NANOG, NIK, NSE2, p15, p16, p21, p27, PIM2, PDGFB, Pol-mu, PRDM1/Blimp-1, PSF1, PSF2, PSF3, PSGL-1, SOX4, SPIB, SUZ12, TdT, TIMP3, VPREB3, WRN, XBP-1(s), XBub1, XMCAK and XSGO.

In 2012, our Unit has produced and characterised 7 different mAbs in collaboration with CNIO researchers. Furthermore, in collaboration with J. F. García-García, Head of the Pathology Department at the MD Anderson Cancer Centre, we have produced and characterised a new mAb against the 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 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 single cell level in lymphoid tissue, showing that CSF1R mAb is a specific marker of the normal monocytes/macrophages lineage. We are currently establishing the predictive value of CSF1R protein expression as a diagnostic biomarker for lymphoma patients by evaluating the correlation of its expression with patient outcome, in routine paraffin tissue sections from a large set of lymphomas. ■

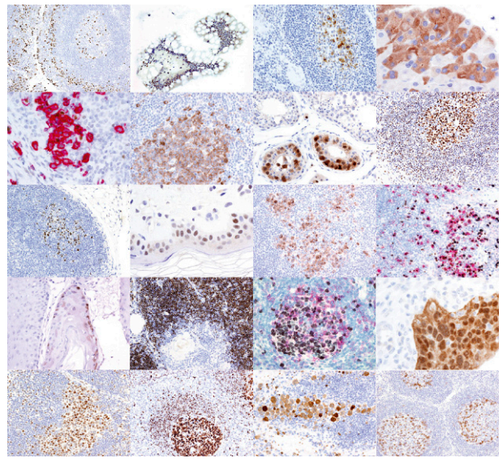
Figure Immunohistochemistry with mAbs produced by the Monoclonal Antibodies Core Unit.

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PATENT

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HISTOPATHOLOGY CORE UNIT

Marta Cañamero
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Technicians
Virginia Álvarez, M. Carmen Arriba (until March), Nuria Cabrera (since September), Ana Díez, Elvira Gil, María Gómez, Patricia González, María Lozano, Raquel Pajares, Zaira Vega


Marta Cañamero ESP


Virginia Álvarez ESP


Nuria Cabrera ESP


Ana Díez ESP


María Gómez ESP


Patricia González ESP


María Lozano ESP


Raquel Pajares ESP


Zaira Vega ESP

"THE WORK DEVELOPED AT THE UNIT IS THE BASIS FOR THE ROUTINE DIAGNOSIS, PROGNOSIS AND TREATMENT OF CANCER. WE COLLABORATE WITH RESEARCHERS AT CNIO IN THE PHENOTYPING AND HISTOPATHOLOGICAL CHARACTERISATION OF DIVERSE MOUSE MODELS FOR CANCER RESEARCH. FURTHERMORE, WE PARTICIPATE IN EUROPEAN PROJECTS SUCH AS THE CANCEROPÔLE NETWORK."

OVERVIEW

Histopathology is the branch of pathology dealing with the tissue diagnosis of disease. The Histopathology Core Unit at the CNIO offers a full range of services for both human and mouse tissue samples, including processing, embedding, and cutting of paraffin tissue specimens. We also provide most standard histological stains, as well as research/diagnostic immunohistochemistry, antibody workup, *in situ* hybridisation (including microRNAs) and TUNEL, tissue microarray, digital slides & analysis, and laser capture microdissection services; these are all provided to CNIO's researchers as well as to the surrounding hospitals and the academic community in general.

Our Unit is equipped with all the instruments required to automate most of the processes included in our service package; this allows us to obtain highly reproducible results. The Unit also offers specialised pathology consulting to the CNIO Research Groups and is heavily involved in training activities, thus providing students at the CNIO with the skills and expertise in histopathology that they require for the successful execution of their research projects.

RESEARCH HIGHLIGHTS

The Histopathology Core Unit is a new Unit that has been formed this year after an internal reorganisation that led to the merge of the former Comparative Pathology and Histology/Immunohistochemistry Core Units. This reorganisation has allowed us to optimise our resources and generate synergies by combining the complementary knowledge and expertise from both units regarding mouse and human tissue studies.

We have increased our panel of anti-human and anti-mouse antibodies to more than 525 and 270 available antibodies, respectively. It is worth mentioning the development of an anti-beta-Gal monoclonal antibody for mouse tissues. Likewise, we have developed new *in situ* hybridisation (ISH) probes, including one for ALU sequences that allows to discriminate between mouse and human cells in the same slide; this is very useful for working with tissues derived from mouse xenograft models. Furthermore, we have developed new ISH probes against diverse miRNAs (*miRNA-21*, *-203*, *-494*) in order to study their expression profiles in both human and murine tumour paraffin sections. The Histopathology Core Unit has created its own catalogue of new rat monoclonal antibodies (mAbs) against mouse antigens, and is currently working in collaboration with CNIO's Monoclonal Antibodies Core Unit to develop new mAbs against proteins that are the subject of study in several CNIO Research Groups (e.g., Hes-1 or Nanog).

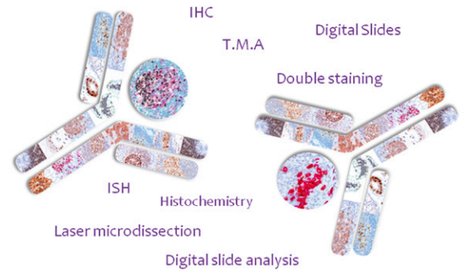

Figure State-of-the-art services offered by the Histopathology Core Unit.

Our Unit participates in several External Quality Assessment Services/Programmes (EQAS), such as NordiQC, UK NEQAS and SEAP GCP. Equally, it has obtained the accreditation as 'Testing Laboratory' in accordance with the requirements – specified by the regulation UNE EN ISO/IEC 17025 for assays of histology, immunohistochemistry and chromogenic *in situ* hybridisation – as indicated in the scope of the accreditation (No. 984/LE1911-Human samples). It is the first laboratory of its kind in Spain, and the second in Europe, to fulfil these criteria. This accreditation ensures the quality and reliability of the results obtained in the laboratory. ■

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MOLECULAR IMAGING CORE UNIT

Francisca Mulero
Core Unit Head

Graduate Student
Monika Musko (since May)

Technicians
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Francisca Mulero ESP


Monika Musko POL


Elena Andrés ESP


Juan A. Cámara ESP


Silvia Sánchez ESP


Coral Velasco ESP

OVERVIEW

Molecular imaging is a discipline that enables the visualisation of cellular functions and the tracking of the molecular processes in living organisms without perturbing them. The multiple and numerous potential benefits offered by this field are applicable to the study and diagnosis of a variety of diseases including cancer. Imaging techniques can contribute to improve the treatment of cancer by optimising the preclinical and clinical testing of new drugs.

Molecular imaging differs from traditional imaging in that known biomarkers are used as probes to facilitate the visualisation of particular targets or pathways. Biomarkers interact chemically with their environment and, in turn, alter the image according to molecular changes occurring within the area of interest. Furthermore, molecular imaging allows for quantitative analysis, providing a higher degree of objectivity.

"BY LENDING OUR WIDE-RANGING SUPPORT TO CNIO RESEARCHERS THROUGH AN ASSORTMENT OF STATE-OF-THE-ART TECHNICAL EQUIPMENT AND HIGH-QUALITY TECHNIQUES FOR IMAGING *IN VIVO*, WE HAVE PROVIDED A POWERFUL TOOL FOR MONITORING TREATMENT RESPONSE."

RESEARCH HIGHLIGHTS

Tumour hypoxia is a key factor in predicting tumour response to treatment. Drugs that modulate vascularisation offer extraordinary therapeutic opportunities for the treatment of many cancer types. Positron emission tomography-computed tomography (PET-CT), using [^{18}F]-labelled fluorodeoxyglucose as a probe, has proven its usefulness in the assessment of cancer treatment responses regarding aggressiveness and metabolic aspects. [^{18}F]-fluoromisonidazole, a biomarker of hypoxic tissues, is more specific in tracing the effect of anti-angiogenic drugs. The binding of this probe to the cell requires reduction of a nitro group in its imidazole ring, which depends on the activity of the enzyme nitroreductase. In the presence of less than 10 mmHg pO_2, [^{18}F]-fluoromisonidazole gets trapped in the hypoxic cell and the PET scanner can image it. We have used this hypoxia marker in mouse models of breast and pancreatic cancer for the assessment of the tumour's response to therapy and the optimisation of treatment protocols (FIGURE).

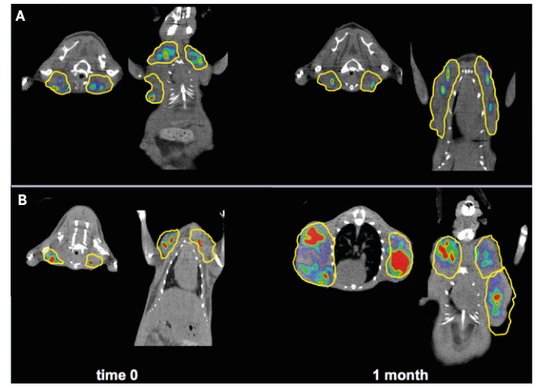

Figure Imaging hypoxia. [^{18}F]-fluoromisonidazole PET-CT images of a mouse bearing breast tumours, treated with either an anti-angiogenic drug (**A**) or vehicle (**B**) at the indicated times. Treatment decreases [^{18}F]-fluoromisonidazole uptake (yellow perimeter) reflecting a reduction of hypoxic areas, while in vehicle-treated mice hypoxia increased.

In collaboration with the *CIEMAT* and the CNIO Melanoma Research Group we have developed and tested ^{68}Ga-NAPamidea; a new PET radio-labelled probe that specifically targets the α-melanocyte-stimulating hormone (MSH) *in vivo*. MSH peptide analogues bind specifically to melanocortin receptors that are overexpressed in human and mouse melanoma cells.

We continued to develop novel protocols for ultrasound imaging, using a high-resolution ultrasound system Vevo770, directed towards the early diagnosis of tumours and the monitoring of therapeutic interventions. We also continued the provision of support and the testing of new probes for optical imaging, as well as the standardisation of protocols for densitometry analysis. We have also actively participated in the setup of a clinical PET-CT (64 slices) and a 3-Tesla MRI system at the *Hospital Universitario de Fuenlabrada*, dedicated to imaging support in clinical trials conducted by CNIO's Clinical Research Programme. Furthermore, we continued our participation in both national (CDTI-funded Advanced Molecular Imaging Techniques (AMIT) Consortium) and international (Madrid-MIT M+Vision Consortium) research and technology development networks. ■

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AWARDS AND RECOGNITION

- Scientific Advisory Board Member and Faculty, M+Vision Consortium, Spain-USA.

FLOW CYTOMETRY CORE UNIT

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Core Unit Head

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

Lola Martínez ESP

Ultan P. Cronin IRL

Miguel Ángel Sánchez ESP

OVERVIEW

Flow cytometry is a fast, multiparametric technique that allows for the identification, quantification and isolation of defined subpopulations of cells, based on the expression levels of fluorescent markers and their relation to each other. Nowadays, flow cytometry is an indispensable tool in cancer research. We provide CNIO Research Groups with technical and scientific advice regarding flow cytometry technologies and assays, and collaborate with them in data analysis and interpretation. Our Core Unit is equipped with 4 analysers and 2 high-speed cell sorters, with different configurations of lasers and detectors. Analysers are available to users, after appropriate training, so that they can operate them independently, whereas sorters are exclusively handled by the Core Unit staff. Our sorters can separate up to 4 defined populations at a time, as well as perform single cell cloning. One of the sorters is installed in a biosafety cabinet, allowing us to sort human samples according to biosafety regulations.

"WE ACTIVELY DEVELOP TAILOR-MADE ASSAYS IN COLLABORATION WITH CNIO RESEARCH GROUPS, HELPING THEM TO ANSWER BIOLOGICAL QUESTIONS THAT ARE RELEVANT TO THEIR PROJECTS. AS AN EXAMPLE, WE HAVE BEEN ISOLATING CELLS FROM MURINE TRF-1-GFP IPS CELLS, BASED ON THEIR LEVEL OF EXPRESSION AND CELL CYCLE STATUS, WHICH ALLOWED RESEARCHERS TO ASSESS THE ROLE OF TRF-1 IN PLURIPOTENCY."

RESEARCH HIGHLIGHTS

We provide state-of-the-art equipment and data analysis 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. Flow cytometry applications developed and validated in our Unit include:

- → Cell proliferation studies (CFSE, BrdU or EdU incorporation, DNA content, etc.).
- → Apoptosis studies (Annexin V, TMRE, Caspase 3, etc.).
- → Multicolor Immunophenotyping.
- → Functional Assays (side population detection, Ca^{2+} flux, intracellular pH, etc.).
- → Cytometric Bead Arrays for the simultaneous determination of different cytokines in cell extracts and plasma.

During 2012, we developed several new multicolour panels for the detection of progenitors and inflammatory cells from different sample types, such as foetal liver and skin, and combined these panels with the detection of proliferation, cell death and cytokine production. We further assessed new fluorochromes and incorporated them in certain panels with the aim of improving resolution of subpopulations of interest. We have quantified several cytokines simultaneously in serum and cell extracts, wich provides valuable information for the description of inflammatory and pathogenic processes. Furthermore, by means of our 561 nm excitation line, we have significantly improved the detection of red fluorescent proteins, which allows us to combine red fluorescent cells with multicolour immunophenotyping panels for further characterisation.

The Core Unit has developed comprehensive training courses for different applications of flow cytometry techniques. This year, training courses benefited from the collaboration of Kylie Price (Flow Cytometry Manager, Malaghan Institute, Wellington, NZ) who visited our Core Unit for a 3-month period; this seminar series was a great success in terms of attendance by internal and external users, and has resulted in the successful implementation of novel protocols developed by our Core Unit. This type of training course will be offered again in a similar format next year. ■

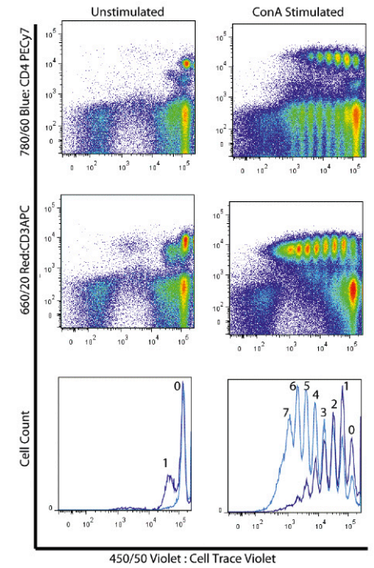

Figure Representative dot plots show the number of divisions of CD3 and CD4 T cell subsets obtained from murine splenocytes. Cells were labelled with Cell Trace Violet and stimulated with 1.5 micrograms /ml Concavalin A for 96 hours. Bottom panels show overlays of non-stimulated CD4 and CD3 T cells *versus* stimulated cells.

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CONFOCAL MICROSCOPY CORE UNIT

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Core Unit Head

Graduate Student
Aleksandra Amelian (since June)

Technicians
Manuel Pérez, Joaquim Soriano


Diego Megías ESP


Aleksandra Amelian POL


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OVERVIEW

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

The Confocal Microscopy Core Unit provides the CNIO Research Groups with a wide range of 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 are of interest to Research Groups at the CNIO. Training activities are also an essential component of our mission.

"THE CONFOCAL MICROSCOPY CORE 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. The Unit is also equipped with 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-Throughput Content Screening (HTS) system that allows running HTS 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 high content feeding of the instrument, not only in multi-well plates but also in tissue sections.

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

During 2012, the Confocal Microscopy Core Unit has continued to establish numerous scientific collaborations with CNIO researchers, covering several aspects of cancer studies such as the Intelligent Screening of single cell behaviour in cellular arrays *in vivo* and in tissues. In addition, the Unit is developing new methods for automated high resolution acquisition and analysis of cell invasion studies, and is assisting researchers in the measurement of protein activity reporters using Fluorescence Resonance Energy Transfer (FRET) technology. Moreover, the Unit is dedicating a significant effort towards the development and implementation of HTS technology at CNIO; for example, during this last year we helped to run screenings aimed at testing compounds that may eventually modify key aspects of tumourigenesis such as mitotic checkpoint regulation, integrity of nucleoli, DNA damage, cell survival and proliferation. ■

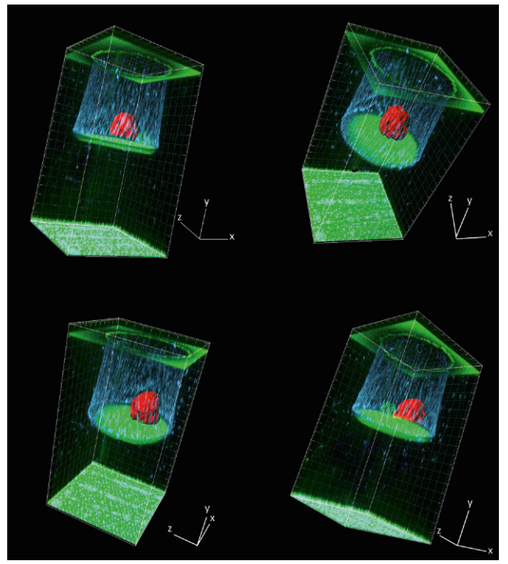

Figure 3D-reconstruction of a micro-well – 80 microns high and 80 microns wide – that contains a single living cell on a microarray composed of 4,000 poly-dimethyl-siloxane wells.

PUBLICATIONS

- Maraver A, Fernández-Marcos PJ, Herranz D, Cañamero M, Muñoz-Martin M, Gómez-López G, Mulero F, Megías D, Sánchez-Carbayo M, Shen J, Sánchez-Cespedes M, Palomero T, Ferrando A, Serrano M (2012). Therapeutic Effect of γ-Secretase Inhibition in *Kras*^{G12V}-Driven Non-Small Cell Lung Carcinoma by Derepression of DUSP1 and Inhibition of ERK. *Cancer Cell* 22, 222-234.
- Abéngozar MA, de Frutos S, Ferreiro S, Soriano J, Pérez-Martínez M, Olmeda D, Marenchino M, Cañamero M, Ortega S, Megíaas D, Rodríguez A, Martínez-Torrecuadrada JL (2012). Blocking ephrin-B2 with highly specific antibodies inhibits angiogenesis, lymphangiogenesis, and tumor growth. *Blood* 119, 4565-4576.
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PROTEOMICS CORE UNIT

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Core Unit Head

Staff Scientist
Jorge L. Martínez

Graduate Student
Natalia Miekus (since June)

Technicians
Fernando García, Rut González, Nuria Ibarz, Encarna Pucheta (since April), M. Isabel Ruppen, Pilar Ximénez de Embún


Javier Muñoz ESP


Jorge L. Martínez ESP


Natalia Miekus POL


Fernando García ESP


Rut González ESP


Nuria Ibarz ESP


Encarna Pucheta ESP


M. Isabel Ruppen ESP


Pilar Ximénez de Embún ESP

OVERVIEW

The global analysis of proteins (i.e. proteomics) provides, arguably, the principal and ultimate level of information required to understand how cells function. Proteomic analyses, however, are challenging due to the highly dynamic range and diversity of protein modifications. A further challenge is the interconnectivity of proteins into complexes and signalling networks that are highly divergent in time and space. Nowadays, large-scale proteome analysis has become within reach and heavily relies on mass spectrometry (MS)-based protein sequencing. MS-based proteomics is starting to mature and provide answers to important biological questions thanks to a combination of developments regarding instrumentation, sample preparation, and computational analysis.

"THE CNIO PROTEOMICS CORE UNIT IMPLEMENTS AND DEVELOPS TOP-NOTCH MS-BASED PROTEOMICS METHODOLOGIES IN ORDER TO BETTER UNDERSTAND, AT THE PROTEOME LEVEL, THE UNDERLYING MOLECULAR BASIS OF CANCER. THE UNIT ALSO PROVIDES RECOMBINANT PROTEIN EXPRESSION METHODOLOGIES AND QUANTITATIVE PROTEIN ASSAYS."

RESEARCH HIGHLIGHTS

Throughout 2012, the Unit has established robust pipelines for shotgun protein identification/quantification. The use of iTRAQ labels allows the comparison of up-to 8 samples in one analysis with high precision. Alternatively, label-free approaches are being used for the identification of protein interactions. Moreover, a collaboration with Agilent has recently been established with the goal of implementing the 'Bravo Platform' for automated and robust processing of a high number of biological samples. The large potential and applicability of proteomics in cancer research is illustrated by the Unit's high level of interaction with all the CNIO Programmes. Through a tight collaboration with these groups we have conducted several studies, including proteome expression profiling, analysis of post-translational modifications (e.g. phosphoproteomics) and identification of protein-protein interactions, altogether, providing new insights into the pathogenesis of cancer.

Regarding the recombinant protein expression activities, the Unit has continued to participate in projects aimed at recombinant cancer-related protein production in prokaryotic and eukaryotic expression systems for multiple purposes. Among these projects, it is worth mentioning the production of recombinant CSFR1 for the diagnosis/prognosis of lymphomas (in collaboration with MD Anderson and the CNIO Monoclonal Antibodies Core Unit), and the experimental validation to understand the biophysical reasons that underlie the different sensitivities of some tyrosine kinases (c-Src, c-Abl) to type II inhibitors such as the anticancer drug imatinib (in collaboration with the CNIO Computational Biophysics Junior Group). The Unit is also involved in the development and validation of recombinant antibody fragments (scFv) against proteins related to angiogenesis. We are currently carrying out a characterisation of ephrinB2 antibody fragments with potent anti-angiogenic and anti-tumoural activities, with the aim of exploring a putative therapeutic application in cancer. ■

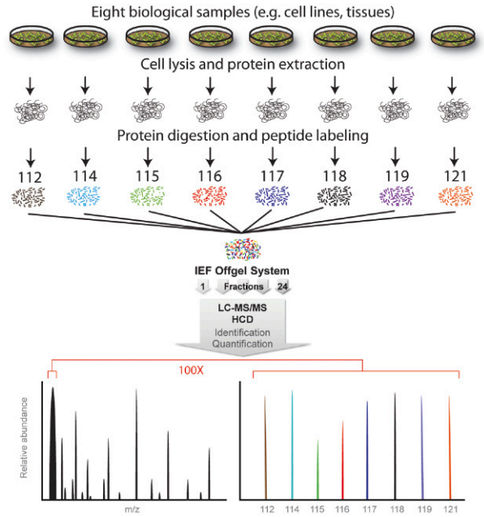

Figure Schematic workflow illustrating the quantitative proteomic comparison of 8 samples by using iTRAQ labelling.

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ANIMAL FACILITY

Isabel Blanco
Core Unit Head

Charles River Laboratories International, Inc.
Management

The picture, “A star has been born”, won the *Third Prize of Photography* at the 11th Meeting of the Spanish Society of Laboratory Animals (*SECAL*).

Photo by D. Sanguino.

Photo by D. Sanguino

The CNIO has a state-of-the-art Animal Facility that is managed by Charles River Laboratories. The Animal Facility’s primary responsibility is the supply, husbandry and quality control of laboratory animals used by CNIO’s Research Programmes in their experimental protocols. The strict compliance to national, European 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 (Molecular Pathology, Clinical Research), as well as with some Sections and Units from the Experimental Therapeutics and Biotechnology Programmes.

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 re-derivation.

“THE ANIMAL FACILITY PROVIDES THE CNIO RESEARCHERS WITH ALL THE SUPPORT REQUIRED TO WORK WITH MOUSE MODELS, WHICH, IN TURN, IS CRITICAL TO UNDERSTAND THE PROCESSES INVOLVED IN TUMOUR GENERATION AND DEVELOPMENT, AS WELL AS INDISPENSABLE FOR THE VALIDATION OF NOVEL DRUG TARGETS AND THERAPEUTIC INTERVENTIONS.”

We also have an additional area with a capacity for 1,800 type II cages dedicated for the use of non-replicative strains of adenovirus, lentivirus and retrovirus, as well as for housing xenograft models. To maintain clean air in the premises, mice are housed in ventilated racks with integration of Individually Ventilated Caging (IVC) units in the building’s 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 the potentially hazardous tasks such as the processing of dirty bedding, the washing/filling of cages and bottles, etc. These automated systems generate the highest productivity possible and ensure the quality standards in our washing and sterilising areas. All records concerning breeding protocols and animal inventory are computerised and stored in a web-based application accessible via the CNIO intranet.

The Animal Facility currently harbours more than 1,500 genetically modified mouse lines, either as live animals or as cryopreserved embryos or sperm, carrying more than 300 gene targeted alleles and close to 200 transgenic integrations. More than 100 gene targeted alleles and 50 transgenic mouse strains of cancer-related genes have been generated by the Research Groups at the CNIO, plus around 200 genetically modified lines 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, exposure to UV light and volatile carcinogenic agents, as well as surgery procedures. In addition, the monitoring of the mouse models on-site, through non-invasive imaging technologies, is provided by the Molecular Imaging Core Unit that has integrated all their image acquisition instrumentation into the Animal Facility. Likewise, the work of the Transgenic Mice Core Unit is performed in a laboratory inside the SPF barrier. Finally, a necropsy laboratory, equipped with instruments for the haematological and biochemical analysis of blood and urine, complements the pathology and clinical diagnostics capabilities.

All the work carried out by the Animal Facility complies with both national and European legislation – Spanish Royal Decree RD1201/2005 and EU Directive 86/609/CEE as modified by 2003/65/CE, respectively – 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) that has been set up to comply with the new European Directive 2010/63/UE.

The Royal Decree RD1201/2005 stipulates that all animal procedures are to be carried out by qualified people in possession of the corresponding accreditation as issued by the competent authority. To abide with this requirement, the Animal Facility offers CNIO staff an official ‘Category C’ qualification annual training course focused 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 EXPERIMENTAL THERAPEUTICS PROGRAMME PLAYS A KEY ROLE IN CNIO'S EFFORTS TO FILL THE GAP BETWEEN EARLY DISCOVERIES AND THE GENERATION OF DRUG DISCOVERY-ORIENTED RESULTS; THUS, PAVING THE WAY TOWARDS THE DEVELOPMENT OF NEW ANTI-CANCER THERAPIES. PATIENTS ARE WAITING FOR US."

The Experimental Therapeutics Programme (ETP) underwent a profound reorganisation during 2012. The Programme, as it stands today, is truly nested and integrated into CNIO's Basic and Translational Research Groups. The main focus of the ETP is to support drug discovery projects derived from CNIO research by delivering balanced and optimised lead compounds (activity, off-target selectivity and Absorption-Distribution-Metabolism-Excretion, Toxicity (ADME-Tox) properties) with demonstrated *in vivo* proof-of-concept (PoC) after oral administration (mechanism of action and efficacy in tumour models).

The Programme's capabilities range from High-Throughput Screening (HTS) assay development, medicinal chemistry for hit generation, hit-to-lead and lead optimisation, to *in vivo* pharmacokinetics and PoC studies in animal models of human cancer. Currently, the main focus is on the discovery of novel inhibitors for kinases that, by becoming mutated or deregulated, act as key drivers in tumour development and progression; this includes the proto-oncogene serine/threonine-protein kinase Pim (PIM), dual PIM/Phosphatidylinositol 3-kinase (PI3K) inhibition, ataxia telangiectasia and Rad3 related (ATR), and microtubule associated serine/threonine kinase-like (MASTL).

In addition to full-blown drug discovery projects, ETP offers its capabilities and skills to the CNIO Research Groups, including:

- → HTS Platform: transfer of manually developed assays (biochemical and cellular) by CNIO researches into a high-quality HTS environment.
- → Chemical Library and Tool Compounds: library of 50K chemically diverse compounds with a focus on cancer targets, and a collection of FDA approved drugs for their 'repositioning' in cancer therapy. Assistance in the identification of chemical tools and synthesis of non-available compounds.
- → Expertise covering *in vivo* pharmacokinetics, pharmacokinetics/pharmacodynamics (PK/PD) and efficacy studies.

Major milestones achieved in 2012:

- → PIM: delivery of optimised leads from 2 distinct chemical series meeting 'Targeted Product Profile' with demonstrated *in vivo* PoC.
- → ATR: lead ETP-142 has demonstrated *in vivo* PoC in several tumour models; a novel, patentable, series of selective ATR inhibitors with oral bioavailability has been prepared.
- → Dual PIM-PI3K inhibition: a novel series of orally available dual inhibitors has been prepared; simultaneous down-regulation of both pathways and strong anti-proliferative activity in the MV4:11 cell line has been demonstrated.
- → MASTL: limited screening of ETP-collection has yielded several hits. ■

MEDICINAL CHEMISTRY SECTION

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Section Head

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Post-Doctoral Fellow
Francisco Javier Ramos (until October)

Technicians
María Elena Cendón, Virginia Rivero


Sonia Martínez ESP


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Carmen Varela ESP


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Virginia Rivero ESP


Esther González ESP


Ana Isabel Hernández ESP


María del Rosario Rico ESP


Rosario C. Riesco ESP

OVERVIEW

The aim of the Medicinal Chemistry Section is to discover novel anti-cancer compounds with demonstrated *in vivo* mechanism of action (MoA) and efficacy in mouse models mimicking human cancer. This process covers different phases, from hit generation and hit identification (selection of screening sets) to hit-to-lead phase (HtL) and Lead Optimisation (LO). Our Section designs and identifies hits, and introduces structural modifications in selected molecules that confer not only pharmacological activity and selectivity, but also 'drug-like' properties that are a requisite for successful drug development; this includes optimisation of the molecules' biological activities against their main targets and selectivity against off-targets, as well as optimisation of *in vitro/ in vivo* Absorption-Distribution-Metabolism-Excretion, Toxicity (ADME-Tox) properties. To this end, different molecules are designed, taking into account information regarding the target or other known ligands, as well as medicinal chemistry expertise. Based on biological and structural data, we establish Structure Activity Relationships (SAR) and Structure Properties Relationships (SPR) that guide the subsequent steps of the molecules' optimisation. Multifactorial optimisation of the pharmacological and 'drug-like' properties is achieved through an iterative process in close collaboration with ETP's Biology Section.

"WE HAVE DESIGNED AND SYNTHESISED 4 SELECTIVE PIM AND ATR INHIBITORS WITH EXCELLENT PHARMACOLOGICAL AND 'DRUG-LIKE' PROPERTIES, AS WELL AS *IN VIVO* MOA AND EFFICACY IN MOUSE MODELS MIMICKING HUMAN CANCER; THUS, HIGHLIGHTING THEIR EXTRAORDINARY POTENTIAL TO YIELD NOVEL AND EFFICIENT DRUGS FOR CANCER TREATMENT."

In 2012, our Section has been involved in 4 different projects: i) inhibitors of proto-oncogene serine/threonine-protein kinases (PIM) (LO); ii) Ataxia telangiectasia and Rad3-related (ATR) inhibitors (LO); iii) dual PIM/Phosphatidylinositol 3-kinase (PI3K) inhibitors (HtL); and iv) microtubule associated serine/threonine kinase-like (MASTL) inhibitors (hit finding). All ETP leads have been protected by their corresponding patent applications.

RESEARCH HIGHLIGHTS

PIM inhibitors

The diverse biological roles of PIM in cancer and over-expression in various haematopoietic malignancies and solid tumours render PIM an attractive therapeutic target for cancer treatment. We generated 3 chemical series that contain molecules with different PIM isoform-specific inhibitor profiles. *In vivo* pharmacokinetic characterisation yielded 2 chemical series with oral bioavaibility that were taken forward to late LO. Taking into account aspects of cardiovascular safety – a known issue in the development of PIM inhibitors – and following chemical exploration and structural modifications of these series, we identified 2 lead compounds with an optimal profile in terms of potency, selectivity and oral bioavailability. Structural information about these lead compounds has been generated in collaboration with CNIO's Macromolecular

Crystallography Group in order to interpret their binding mode in the ATP-binding pocket of the PIM protein and, thus, confirm the rationale behind their design (FIGURE 1). Both lead compounds showed high selectivity against PIM 1,2 and 3 isoforms (FIGURE 2); *in vivo* studies further demonstrated good MoA and efficacy in different cancer mouse models (more details in ETP's Biology Section).

ATR inhibitors

Discovery of novel ATR inhibitors is relevant to cancer treatment due to their potential to increase chemotherapy/ radiotherapy efficacy and to specifically target tumours exposed to oncogenic replicative stress. ETP-142 was discovered as a lead compound for *in vivo* characterisation. This lead has demonstrated high selectivity in the 456 kinomescan assay. Furthermore, we have been working on the optimisation of ETP-142, and several analogues have progressed to *in vivo* pharmacokinetic studies. As a complementary strategy, we have generated a back-up chemical series containing potent ATR inhibitors that are selective against PI3K, mTOR, DNA-PK and ATM. *In vivo* studies with selected compounds demonstrated MoA in allo-Eμ-myc models.

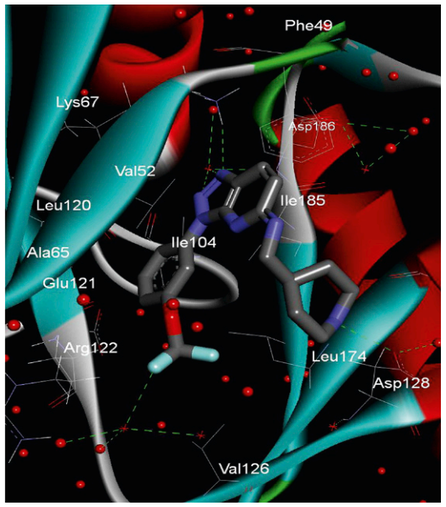
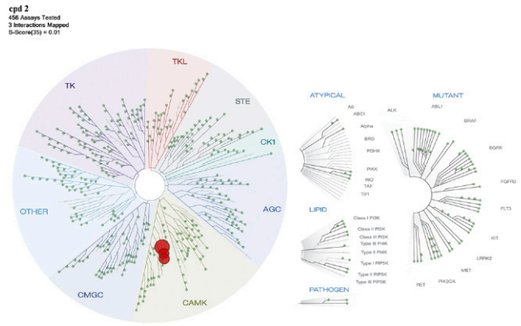


Figure 1 Compound from one of our advanced chemical series that binds to the ATP binding site of PIM-1 in its typical inactive conformation (i.e., Phe49 pointing to the inner side of the ATP binding site). Coordinates for the PIM-1-inhibitor complex have been deposited in the protein databank (access code 4A7C).



ASSAY MAPPED INTERACTIOS FOR CP2

KINOMEscan	ASSAY LABEL	ASSAY GROUP	%CTRL
PIM1	PIM1	CAMK	1.3
PIM2	PIM2	CAMK	0.7
PIM3	PIM3	CAMK	1.8

Figure 2 Selected lead PIM inhibitor with high selectivity when tested in a 456 kinase panel (Kinomescan); only PIM1, PIM2 and PIM3 inhibition is observed (red circles).

Dual PIM-PI3K inhibitors

High synergism between PIM and PI3K inhibitors has been reported by our Group. The design of compounds that combine these activities in a single molecule would be of high interest. We anticipate that such novel profiles will improve cancer treatment by providing advantages in their clinical development over the combination of two independent inhibitors. Through the exploration of our chemical platform and by introducing structural modifications, we were able to obtain selective PIM and PI3K inhibitors, as well as combined inhibitors of PIM-PI3K and PIM-PI3K-mTOR. Dual PIM-PI3K inhibitors demonstrated efficiency in downregulating both pathways in the MV4-11 cell line and more potent anti-proliferative properties when compared to single PIM and PI3K inhibitors. Some of the obtained compounds had oral bioavailability and were selected for further *in vivo* studies.

MASTL Kinase inhibitors

Screening of a selected subset of 200 compounds from our library by CNIO's Cell Division and Cancer Group yielded some promising hits. ■

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OVERVIEW

Our Section is devoted to the biochemical, cellular and *in vitro*/*in vivo* pharmacological characterisation of compounds synthesised by the Experimental Therapeutics Programme's Medicinal Chemistry Section. During the past year we have been working on inhibitors of proto-oncogene serine/threonine-protein kinases (PIM) and Ataxia telangiectasia and Rad3-related (ATR), and developed dual PIM/Phosphatidylinositol 3-kinase (PI3K) inhibitors.

PIM proteins belong to a family of serine/threonine kinases that are upregulated in many haematological malignancies and solid tumours, contributing to the survival, cell cycle progression and migration/homing of tumour cells, as well as to the development of drug resistance. Biological data suggest that PIM inhibitors may be particularly effective in combination with classical chemotherapy or targeted drugs, such as PI3K inhibitors. For this reason, we have been pursuing the identification and characterisation of selective inhibitors of PIM and dual PIM/PI3K inhibitors.

"WE HAVE PROVIDED *IN VIVO* PROOF-OF-CONCEPT IN DIFFERENT TUMOUR MOUSE MODELS WITH PIM AND ATR LEAD COMPOUNDS. WE ARE THEREFORE VALIDATING BOTH TARGETS FOR FURTHER CLINICAL DEVELOPMENT TO TREAT CANCER PATIENTS."

ATR is activated in response to ultraviolet light, certain chemotherapeutic drugs, and replication stress, which renders ATR a potential target to increase the efficacy of chemotherapy and radiotherapy, or to fight tumours with a high level of oncogene-induced replicative stress. For this reason we have been pursuing the discovery and characterisation of selective ATR inhibitors. This project has been carried out in collaboration with CNIO's Genomic Instability Group.

RESEARCH HIGHLIGHTS

During the past year, the Section has been involved in 3 major projects: the development of PIM, dual PIM-PI3K and ATR inhibitors. PIM is the more advanced project of the Programme, reaching the stage of analysis of the best suited compounds for the different series. In addition to High-Throughput Screening (HTS) evaluation of PIM inhibitor activities at the biochemical and cellular levels, we have performed Absorption-Distribution-Metabolism-Excretion, Toxicity (ADME-Tox) assays, including a cardiotoxicity hERG (human Ether-à-go-go-Related Gene) assay, as well as a panel of Cytochrome P450 (CYP) enzymatic and Microsomal Stability (MS) assays. All these assays were performed in-house in HTS format. Out of 400 tested compounds, 118 were selected for hERG and MS assays and finally, 38 compounds were characterised at the level of CYP inhibition.

Selected compounds were successively evaluated for their pharmacokinetics, after which they were further profiled in tolerance and pharmacokinetic/pharmacodynamic (PK/PD) studies and, finally, in efficacy models. This process yielded 4 lead candidates from 2 different chemical series that were extensively characterised *in vivo*. Candidates were profiled in a 456 kinase panel (see Medicinal-Chemistry section for details). The screening cascade for the Lead Optimisation phase – to evaluate the mechanism of action and efficacy of the PIM inhibitors – was performed with two different xenograft models for non-small-cell lung cancer (NSCLC) and acute myeloid leukaemia (AML) (A549 and MV4:11 cells, respectively), 1 allograft model (Eμ-myc), and a mouse model for NSCLC carrying a K-Ras(G12V) oncogenic mutation. Of note, several PIM inhibitors demonstrated potent anti-tumoural activity (FIGURE 1).

Since PI3K and PIM share common signalling pathways, it is conceivable that these pathways may also overlap to some extent in tumours. In fact, we observed significant synergy between selective PIM and PI3K inhibitors in a variety of tumour cell lines (FIGURE 1C). In order to exploit this synergy,

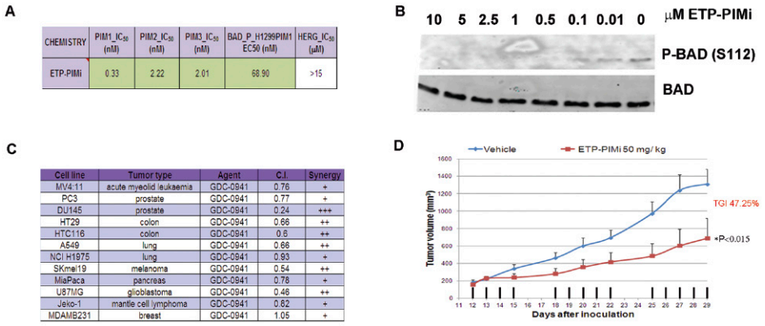

Figure 1 Characterisation of an advanced PIM inhibitor. (**A**) Biochemical and cellular inhibition of PIM. (**B**) PIM biomarkers inhibition in Jeko-1 cells. (**C**) Synergistic anti-proliferative activity with a PI3K inhibitor. (**D**) Anti-tumoural activity of the inhibitor in MV4-11 xenografts. Black lines represent days of treatment.

we generated dual PIM/PI3K and tri-functional PIM/PI3K/mTOR inhibitors and characterised them at the biochemical and cellular level. In MV4:11 cells, dual inhibitors potently impaired the PIM and PI3K pathways; they had stronger anti-proliferative activities than single PIM or PI3K inhibitors. Compounds with oral bioavailability were selected for further *in vivo* studies.

Finally, we are progressing in the development of ATR inhibitors by using a cellular assay that measures the induction of checkpoint kinase-1 phosphorylation under replicative stress. Out of 645 evaluated compounds, 66 were found to be active; upon evaluation of MS, 19 of them were tested *in vivo,* and 3 were selected for mechanistic and efficacy studies. We also performed selectivity profiling for the complete set of 66 active compounds. Since ATR is a member of the PIKK family, we evaluated the biochemical activity of these compounds against PI3K, mTOR, DNA-protein kinase, as well as their cellular activity against Ataxia Telangiectasia Mutated (ATM). Compounds for two different chemical series were mechanistically characterised in two different models: i) as a single agent in the allo-Eμ-myc model that has a high level of replicative stress, and ii) in pancreatic and colon cancer xenografts in combination with standard treatments. In both models, an increase in replicative stress or DNA damage was observed. Treatment of allo-Eμ-myc mice with the compound ETP-142 induced 50% tumour growth inhibition (FIGURE 2); similarly, we have observed potentiation of standard-of-care efficacy in the xenograft studies. These results confirm the therapeutic potential of this class of compounds. ■

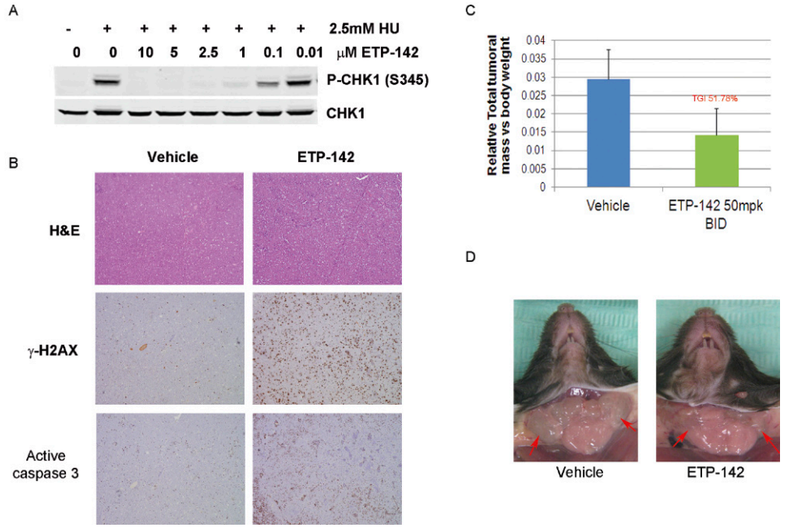

Figure 2 Characterisation of ETP-142. (**A**) Inhibition of ATR in HT29 cells. (**B**) Haematoxylin-eosin staining, γ-H2AX and active caspase-3 immunohistochemistry in mammary lymph nodes of allografted Eμ-myc mice. (**C**) Anti-tumoural activity of ETP-142 in Eμ-myc allografts. (**D**) Representative pictures of a salivary lymph-node.

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LILLY-CNIO CELL SIGNALLING THERAPIES SECTION

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SCOPE OF THE ELI LILLY-CNIO PARTNERSHIP

Eli Lilly and CNIO are collaborating on the identification and validation of novel targets in cancer metabolism. The Lilly-CNIO Cell Signalling Therapies Section – funded through a research contract with Eli Lilly – focuses on the identification of small and low 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. For this purpose, the Section is developing a series of biochemical and cell-based assays, exploiting advanced techniques such as NMR and metabolomics. Each drug target goes through an *in vivo* validation process, taking advantage of the availability of a wide range of mouse models at the CNIO that mimic different types of human cancer, as well as benefiting from the Centre's expertise in their characterisation with non-invasive *in vivo* tumour imaging technologies.

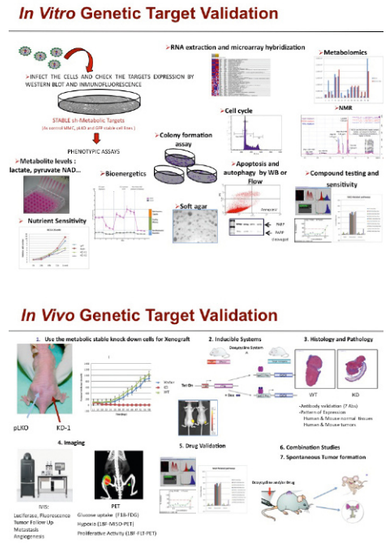
"THE MECHANISTIC UNDERSTANDING OF CANCER METABOLISM HAS REVIVED CONSIDERABLE INTEREST IN DEVELOPING THERAPEUTIC APPROACHES THAT TARGET KEY DRIVERS OF TUMOUR METABOLISM. WE ARE USING A COMBINATION OF STATE-OF-THE-ART *IN VITRO* AND *IN VIVO* APPROACHES TO OBTAIN A COMPLETE PICTURE OF THE METABOLIC STATUS OF TUMOURS."

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, who was awarded the Nobel Prize in 1931, argued that this altered metabolic state was the underlying cause for cancer. The preferential use of glucose by cancer cells has been clinically exploited to image tumours through the utilisation of ^{18}F-fluoro-deoxyglucose-positron emission tomography (^{18}F-FDG-PET). The molecular mechanisms driving the glycolytic phenotype have only recently begun to be understood in light of the results from large-scale genome and metabolomic profiling studies. Recent publications demonstrate that targeting key enzymes in the glutamine metabolic pathway can limit tumour growth in preclinical tumour xenograft models. The abnormal consumption of both glucose and glutamine in tumour cells further indicates that an altered metabolic programme may be at the root of the malignant transformation process.

A number of oncogenes and tumour suppressors have now been identified as key effectors in modulating cell metabolism. Genome sequencing studies have uncovered somatic mutations in metabolic enzymes, including succinate dehydrogenase (SDH), fumarate hydratase (FH), isocitrate dehydrogenase (IDH 1 and 2) in gliomas and leukemias, and gene amplification of phosphoglycerate dehydrogenase (PHGDH) in breast tumours. The loss of function mutations in SDH and FH impart a glycolytic phenotype on tumour cells, possibly through inhibition of the hypoxia-induced factor (HIF)-directed prolyl hydroxylases that is required for HIF degradation in the presence of oxygen. The end product of mutant IDH-1 and 2, 2-hydroxyglutarate (2-HG), functions as an onco-metabolite, altering the regulation of a broad spectrum of cellular pathways ranging from cell differentiation to gene regulation, DNA repair, lipid metabolism and HIF-1 activity.

In addition to mutations in metabolic enzymes, recent studies have shown that many oncogenes, including Myc and Ras, confer a glycolytic phenotype to cancer cells by up-regulating



the expression of genes involved in glycolysis (e.g. GLUT-1, PDHK, LDH5 etc). Von Hippau Lindal (VHL), an E3 ligase required for HIF-1 degradation, is a tumour suppressor that is deleted in almost 50% of kidney cancers. The loss of VHL results in the stabilisation of HIF-1α, even in the presence of oxygen, and imparts a glycolytic phenotype with enhanced glucose uptake and high levels of lactate production. Another important tumour suppressor, TP53, has also been shown to control the expression of key regulatory enzymes of metabolic pathways, including the pentose-phosphate pathway, glutamine metabolism and oxidative phosphorylation.

Together, these recent insights into the mechanisms of metabolic pathways relevant to cancer, open up novel avenues to the development of therapeutics that target key enzymes in tumour metabolism. ■

TECHNOLOGY TRANSFER AND VALORISATION OFFICE

MARISOL QUINTERO HEAD OF OFFICE

Anabel Sanz ESP

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 relationship between the researchers and companies as well as 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 CNIO.

In 2012, CNIO-TTO has actively collaborated with 18 CNIO Research Groups and Units. The Office has evaluated 5 invention disclosures, out of which 3 new patent applications have been filed. It has concluded 5 license agreements for the commercial exploitation of CNIO's assets. Furthermore, the Office has been successful in establishing new partnerships with industry, including framework research collaboration agreements with leading companies in the healthcare sector such as F. Hoffmann-La Roche Ltd.; CNIO and Roche will collaborate for a 5-year period in order to launch innovative projects aimed at providing novel and cutting-edge approaches to fight cancer. ■

"THE TECHNOLOGY TRANSFER AND VALORISATION OFFICE AIMS TO FOSTER SCIENTIFIC PROGRESS FOR THE BENEFIT OF SOCIETY BY TRANSLATING THE RESULTS OF CNIO RESEARCHERS INTO USEFUL APPLICATIONS."

“IN 2012, THE CNIO HAS A STRONG RECORD OF HIGH-QUALITY PUBLICATIONS AND HAS INCREASED EXTERNAL FUNDING THROUGH COMPETITIVE PUBLIC AND PRIVATE GRANTS, AS WELL AS THROUGH SPONSORSHIP.”

MARIA A. BLASCO DIRECTOR

FACTS AND FIGURES

SCIENTIFIC PRODUCTIVITY

CNIO ARTICLES 2012

BELOW IS THE LIST OF 96 SCIENTIFIC ARTICLES PUBLISHED BY CNIO SCIENTISTS AS SENIOR/CORRESPONDING AUTHOR, RANKED BY THEIR CORRESPONDING IMPACT FACTOR; THE AVERAGE IMPACT FACTOR (IF)* OF THESE ARTICLES WAS 8.23. FURTHERMORE, 10 ARTICLES WERE PUBLISHED IN JOURNALS WHICH DO NOT HAVE AN IMPACT FACTOR ALLOCATED, AND AN ADDITIONAL 13 ARTICLES WERE IN PRESS.

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COLLABORATIONS

IN 2012, CNIO SCIENTISTS CO-AUTHORED 133 ARTICLES FROM COLLABORATIVE WORK CARRIED OUT WITH OTHER INSTITUTIONS (PRINCIPAL OR SENIOR/CORRESPONDING AUTHOR NOT FROM CNIO). OF THOSE, 76 ARTICLES WERE IN COLLABORATION WITH FOREIGN INSTITUTIONS; WITH AN AVERAGE IF OF 9.91. THE OTHER 57 ARTICLES WERE COLLABORATIONS WITH SPANISH INSTITUTIONS; THE AVERAGE IF OF THESE ARTICLES WAS 6.44. FURTHERMORE, 8 ARTICLES WERE PUBLISHED IN PEER-REVIEWED JOURNALS WHICH DO NOT HAVE AN IF ALLOCATED, AND AN ADDITIONAL 11 ARTICLES WERE IN PRESS.

COLLABORATIONS WITH FOREIGN INSTITUTIONS

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AWARDS AND RECOGNITION

IN 2012 THE WORK OF SEVERAL SCIENTISTS AT THE CNIO HAS BEEN RECOGNISED THROUGH SCIENTIFIC AWARDS, APPOINTMENTS AND RECOGNITIONS AS DETAILED BELOW.

AWARDS

→ **Mariano Barbacid**
- *V de Vida A*ward, *Asociacion Española Contra el Cáncer* (*AECC*), Spain
- *I Premio Internacional de Investigación Oncológica Ramiro Carregal,* Spain

→ **Óscar Fernández-Capetillo**
- Howard Hughes Medical Institute (HHMI) International Early Career Award, USA

→ **Patrick C. Hermann**
- *Hector-Forschungspreis Onkologie*, Germany

→ **Mercedes Robledo**
- *Sergio Vidal* and *Fundación Mutua Madrileña* 2012 Awards, Spain

EDITORIAL BOARDS

→ **Javier Benítez**
- Editorial Board Member, *Methods in Next Generation Sequencing*

→ **Luis Lombardía**
- Editorial Board Member, *Dataset Papers in Biology*

→ **Francisco X. Real**
- Academic Editor, *PLoS ONE* Journal

→ **María S. Soengas**
- Executive Editor, *Pigment Cell and Melanoma Research*

OTHER RECOGNITIONS

→ **Mariano Barbacid**
- Member (Foreign), National Academy of Sciences of the USA

→ **Maria A. Blasco**
- Honorary Ambassador of the Spain Brand (*Embajadora Honoraria de la Marca España*), Leading Brands of Spain Forum *(FMRE)*
- Member of the Board of Trustees and President of the External Advisory Board of the Spanish National Centre for Research on Ageing (*Centro Nacional de Investigación en Envejecimiento, CNIE*)
- Member, *Faculty of 1000* (Ageing Section)

→ **Juan C. Cigudosa**
- President-Elect Nominee, International Society of Cellular Oncology

→ **Anna González-Neira**
- National Representative and Board Member, European Association for Predictive, Preventive and Personalised Medicine (EPMA)

→ **Manuel Hidalgo**
- Scientific Advisory Board Member, Department of Oncology at the University of Leuven, Belgium
- External Advisory Board Member, *Instituto Oncológico de kutxa (Onkologikoa)*, Spain

→ **Manuel M. Morente**
- President-Elect of the European, Middle East & African Society of Biopreservation and Biobanking (ESBB)

→ **Francisca Mulero**
- Scientific Advisory Board Member and Faculty, M+Vision Consortium, Spain-USA

→ **Francisco X. Real**
- Member, Scientific Council, "Cancer Research for Personalized Medicine" (CARPEM) in Paris, France

→ **Mercedes Robledo**
- Scientific Co-Chair of the international *Pheochromocytoma and Paraganglioma Research Support Organization* (PRESSOR)

COMPETITIVE FUNDING

The CNIO finances a substantial proportion of its research through competitive grants from Spanish, European and international public funding agencies, as well as from private entities. In 2012, researchers at the CNIO were involved in 139 projects that received extramural funding. Of these, 28 were international collaborative projects – 2 of which are coordinated by the CNIO – and 29 were collaborative projects with other groups in Spain. In addition to these collaborative projects, researchers at the CNIO attracted funding for projects that are executed by individual groups. In 2012, 21 of these projects received international funding and 61 received national funding.

The many grants and awards attributed to individual research projects demonstrate the excellence and international competitiveness of CNIO's scientists. These include: 6 Advanced and Starting Grants of the European Research Council (ERC), 1 Howard Hughes Medical Institute (HHMI) International Early Career Award, and several grants from the European Commission (EC), the Association for International Cancer Research (AICR), the US National Institutes of Health (NIH), the European Science Foundation (ESF), and the European Foundation for the Study of Diabetes (EFSD).

INTERNATIONAL GRANTS COLLABORATIVE PROJECTS

AXA FOUNDATION

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

EUROPEAN COMMISSION FRAMEWORK PROGRAMMES

INNOVATIVE MEDICINES INITIATIVE JOINT UNDERTAKING

PRINCIPAL INVESTIGATORS	PROJECT TITLES
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 PROJECTS

PRINCIPAL INVESTIGATORS	PROJECT TITLES
Barbacid, Mariano	CHEMORES: Molecular mechanisms underlying chemotherapy resistance, therapeutic escape, efficacy and toxicity
Barbacid, Mariano	EUMODIC: The European mouse disease clinic – a distributed phenotyping resource for studying human disease
Blasco, Maria A.	MARK-AGE: European study to establish biomarkers of human ageing
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	A BLUEPRINT of haematopoietic epigenomes
Valencia, Alfonso	ASSET: Analysing and striking the sensitivities of embryonal tumours
Valencia, Alfonso	MICROME: A knowledge-based bioinformatics framework for microbial pathways genomics
Valencia, Alfonso	RD-CONNECT: An integrated platform connecting registries, biobanks and clinical bioinformatics for rare disease research

MARIE CURIE ACTIONS

PRINCIPAL INVESTIGATORS	PROJECT TITLES
Fernández-Capetillo, Óscar	ITN aDDRess: Joint training and research network on chromatin dynamics and the DNA damage response
Losada, Ana	ITN Nucleosome4D: Nucleosome structure & function across biological scales and biological function

NETWORKS OF EXCELLENCE

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Barbacid, Mariano	EUROCAN PLATFORM: A European platform for translational cancer research

RESEARCH INFRASTRUCTURES

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Valencia, Alfonso	ELIXIR: European life-science infrastructure for biological information

SMALL OR MEDIUM-SCALE FOCUSED RESEARCH PROJECTS

PRINCIPAL INVESTIGATORS	PROJECT TITLES
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
Malats, Núria	DECanBio: Novel MS-based strategies to discover and evaluate cancer biomarkers in urine: application to diagnosis of bladder cancer
Malats, Núria	UROMOL: Prediction of bladder cancer disease course using risk scores that combine molecular and clinical risk factors
Robledo, Mercedes	ENS@T-CANCER: European network for the study of adrenal tumours – structuring clinical research on adrenal cancers in adults
Serrano, Manuel	RISK-IR: Risk, stem cells and tissue kinetics – ionising radiation

EXECUTIVE AGENCY FOR HEALTH AND CONSUMERS (EAHC)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Malats, Núria	PHGEN II: European best practice guidelines for quality assurance, provision and use of genome-based information and technologies

US NATIONAL INSTITUTES OF HEALTH (NIH)

PRINCIPAL INVESTIGATORS	PROJECT TITLES
Malats, Núria	Bladder cancer risk and genomic alterations
Valencia, Alfonso	GENCODE: Integrated human genome annotation: generation of a reference gene set

ASSOCIATION FOR INTERNATIONAL CANCER RESEARCH (AICR)

PRINCIPAL INVESTIGATORS	PROJECT TITLES
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
Malats, Núria	Bladder cancer risk: The role of trace metals and oxidative stress
Pérez-Moreno, Mirna	Role of p120 catenin in inflammatory skin cancer development
Soengas, María S.	Biosensor and response indicators in dsRNA-based anti-melanoma therapy

EUROPEAN COMMISSION FRAMEWORK PROGRAMMES

EUROPEAN RESEARCH COUNCIL (ERC)

PRINCIPAL INVESTIGATORS	PROJECT TITLES
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 Starting grant CHROMOREPAIR: Genome maintenance in the context of chromatin
Heeschen, Christopher	ERC Advanced Grant Pa-CSC: Molecular characterisation 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

MARIE CURIE ACTIONS

PRINCIPAL INVESTIGATORS	PROJECT TITLES
Fernández-Capetillo, Óscar	HISTONEDDR: Role of histone modifications in DNA damage response in mammals
Gervasio, Francesco L.	DmoNickaseDesign: Safer gene repair and targeting based on the monomeric meganuclease I-DmoI by design of homologous-recombination-inducing nickase activity
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
Real, Francisco X.	PANCLON: Clonal analysis in pancreatic development, differentiation and carcinogenesis

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 INVESTIGATORS	PROJECT TITLES
Hidalgo, Manuel	Tailoring new drugs in pancreatic cancer
Soengas, María S.	The unfolded protein response in melanoma progression and chemoresistance

EUROPEAN SCIENCE FOUNDATION (ESF)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Robledo, Mercedes	11th Scientific meeting of the European network for the study of adrenal tumours (ENS@T)

NATIONAL GRANTS COLLABORATIVE PROJECTS

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

PRINCIPAL INVESTIGATORS	PROJECT TITLES
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 Gervasio, Francesco L. 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-Torrecuadrada, Jorge L.	*Programa ANGIOBODIES 2: Desarrollo de anticuerpos recombinantes para uso terapéutico y diagnóstico en angiogénesis patológica y para la identificación de nuevos marcadores angiogénicos*
Montoya, Guillermo	*Programa INTERACTOMICS: Interactomica 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*

HEALTH RESEARCH FUND / *FONDO DE INVESTIGACIONES SANITARIAS (FIS)*

PRINCIPAL INVESTIGATORS	PROJECT TITLES
Benítez, Javier	*Red Temática de Investigación Cooperativa en Cáncer (RTICC)*
Malats, Núria	*Red Temática de Investigación Cooperativa en Cáncer (RTICC)*
Morente, Manuel M. (coordinator)	*Red Temática de Investigación Cooperativa en Salud (RETICS)-* *Red Temática de Biobancos Hospitalarios (Red Nacional de Biobancos)*
Valencia, Alfonso	*Red Temática de Investigación Cooperativa en Biomedicina Computacional (COMBIOMED)*

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

PRINCIPAL INVESTIGATORS	PROJECT TITLES
Gómez, Carlos Jesús	Chemosensitivity profiles for the personalised therapy of advanced colorectal cancer
Hidalgo, Manuel	Personalised treatment for pancreatic cancer patients

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

PRINCIPAL INVESTIGATORS	PROJECT TITLE
Barbacid, Mariano (coordinator) Blasco, Maria A. Fernández-Capetillo, Óscar Malumbres, Marcos Real, Francisco X. Serrano, Manuel	*Programa CONSOLIDER ONCOBIO: Biología del cáncer*
Benítez, Javier	*Proyecto INNPRONTA LIFE: Desafío integral al cáncer de mama*
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: 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, Lola	*Programa EUROCIENCIA: Plan estratégico de participación en el 7º Programa Marco*
Losada, Ana Méndez, Juan	*Programa CONSOLIDER: Inestabilidad genómica*
Montoya, Guillermo	*Programa CONSOLIDER CENTROSOMA-3D*
Soengas, María S.	*Programa CONSOLIDER RNAREG: Una aproximación integrada a la regulación post-transcripcional de la expresión génica y su papel en enfermedad"*
Soengas, María S.	*Proyecto INNPACTO: Nanocomplejos de ARN sintético como nueva terapia contra cánceres agresivos para los que no se dispone de tratamiento efectivo*
Valencia, Alfonso	*Programa CONSOLIDER SYEC: Supercomputación y eCiencia*
Valencia, Alfonso	*Proyecto ACI-PROMOCIONA INB-ELIXIR: Ampliación del INB como nodo español en la infraestructura Europea de Bioinformática*

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

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Cigudosa, Juan C.	*Neoplasias hematológicas: Terapia apoyada en el diagnóstico molecular*

CENTRE FOR INDUSTRIAL TECHNOLOGICAL DEVELOPMENT / *CENTRO PARA EL DESARROLLO TECNOLÓGICO INDUSTRIAL (CDTI)*

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Mulero, Francisca	*Programa Cenit AMIT: Tecnologías de imagen molecular avanzada*

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

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Fernández-Capetillo, Óscar	*Premio Miguel Catalán 2008 de la Comunidad de Madrid. Modalidad: "Jóvenes investigadores menores de 40 años"*

HEALTH RESEARCH FUND / *FONDO DE INVESTIGACIONES SANITARIAS (FIS)*

PRINCIPAL INVESTIGATORS	PROJECT TITLES
Álvarez, Sara	Identification of biomarkers that predict the clinical response to DNA hypomethylating therapies on myelodisplastic syndromes
Benítez, Javier	Identification and analysis of risk factors in breast and ovarian cancer families (INTRASALUD)
Cascón, Alberto	Ultrasequencing of recurrent regions of loss of heterozygousity in patients with familial pheochromocytoma not associated to mutations in the known susceptibility genes
Cigudosa, Juan C.	Human stem cell models with inducible chromosome translocations: Integrated genomic and biological study of the t(7;11)(p15;p15), its fusion gene *NUP98-HOXA9* and its leukeamic effects (INTRASALUD)
Colomer, Ramón	Structural disruption of oncogene addiction in breast cancer by blocking fatty acid synthase
García, María José	Definition of pathogenic pathways involved in familial and sporadic epithelial ovarian cancer
Guerra, Carmen	Preventative and therapeutic strategies in noonan, costello and cardio facio cutaneous syndromes
Heeschen, Christopher	Molecular characterisation of cancer (stem) cells for the development of novel targeted treatments modalities
Hidalgo, Manuel	Targeting Pancreatic Cancer Stroma
Malats, Núria	Ductal adenocarcinoma of the pancreas and chronic inflammation: An integrative approach in an international study
Martínez, Beatriz	Role of miRNAs as biomarkers for early diagnosis in hereditary breast cancer. Expression profiling of tumours, blood and serum
Milne, Roger L.	FGF Receptors and breast cancer susceptibility: An analysis of main effects, gene-gene and gene-environment interactions at an international level
Milne, Roger L.	Common variation in genes in the FGF and inflammation pathways and breast cancer risk
Quintela-Fandino, Miguel	Development of an integrated translational platform for the study of predictive factors and resistance mechanism for antiangiogenic drugs in early breast cancer
Robledo, Mercedes	Use of massive analysis platforms on endocrine tumours studies: From OMICS to patients
Sánchez-Beato, Margarita	BCR in lymphomas: Activation markers; identification of genetic and epigenetic alterations
Urioste, Miguel	Research of the tumourigenic pathways involved in familial colorectal cancer type X. Analysis of: 1) specific genes; 2) methylation patterns; 3) telomeric dysfunction; and 4), expression of proteins with a relevant role in colorectal tumourigenesis

LILLY FOUNDATION / *FUNDACIÓN LILLY*

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Blasco, Maria A.	Lilly Foundation Award for Preclinical Research

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

NATIONAL PROGRAMME FOR BASIC RESEARCH PROJECTS / *PROGRAMA NACIONAL DE PROYECTOS DE INVESTIGACIÓN FUNDAMENTAL*

PRINCIPAL INVESTIGATORS	PROJECT TITLES
Barbacid, Mariano	Inhibition of oncogenic K-Ras signalling in cancer
Blasco, Maria A.	Mammalian telomeres and telomerase: From chromatin structure to stem cell biology
Djouder, Nabil	Decoding the URI role in the hepatocellular carcinoma (HCC) development
Fernández-Capetillo, Óscar	Exploring the role of replication stress in cancer and ageing
Gervasio, Francesco L.	An experimentally validated computational approach to study protein conformational plasticity and its alteration
Heeschen, Christopher	Vascular regeneration in the 21st century – definition of the optimal cell therapy protocol for patients with cardiovascular diseases
Lietha, Daniel	From the molecular study of growth signalling and cellular adhesion to the drug discovery
Losada, Ana	Animal models for the study of cohesine functions
Malumbres, Marcos	Cellular and physiological consequences of mitotic deregulation in mammals
Méndez, Juan	MCM complex functions in the DNA replication and the genetic stability
Montoya, Guillermo	Structural biology of macromolecular machines involved in chromosome dynamics
Ortega, Sagrario	New murine genetic models for the angiogenesis and lynphangiogenesis in tumours
Osorio, Ana	Implications of the type of germline mutation in the prognosis and treatment of patients with hereditary breast cancer carrying mutations in the *BRCA1* gene
Pérez de Castro, Ignacio	Aurora A, *in vivo* essential functions, anti-tumoural target validation and identification of new regulatory mechanisms
Pérez-Moreno, Mirna	Links between adhesion proteins, inflammation and cancer in skin
Ramón-Maiques, Santiago	Structural determination of the architecture of CAD; an antitumoural target that controls the biosynthesis of pyrimidines
Real, Francisco X.	Pancreatic adenocarcinoma: Role of the acinar and ductal components and development of animal models
Real, Francisco X.	Transcriptional control of acinar cell differentiation and pancreatic cancer
Rodríguez, Cristina	Identification of predictive genetic markers of haematologic toxicity
Sánchez-Carbayo, Marta	Profiling bladder cancer epigenetic events
Serrano, Manuel	New mouse models for the study of cancer and ageing
Soengas, María S.	Cellular stress in melanoma progression and chemoresistance
Valencia, Alfonso	Gene groups functions

SUB-PROGRAMME EURORESEARCH / *SUBPROGRAMA EUROINVESTIGACIÓN*

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Fernández-Capetillo, Óscar	Is the DNA damage response a tumour barrier?

SUB-PROGRAMME OF ACTIONS RELATED TO INTERNATIONAL SCIENTIFIC INFRASTRUCTURES / *SUBPROGRAMA DE ACTUACIONES RELATIVAS A INFRAESTRUCTURAS CIENTÍFICAS INTERNACIONALES*

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Morente, Manuel M.	Establishment of the Spanish Biobank BBMRI.[es] platform for promoting the integration of the European BBMRI platform and its transition to BBMRI-ERIC

SUB-PROGRAMME OF SUPPORT TO TECHNOLOGY TRANSFER CAPACITIES 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*

COMPLEMENTARY ACTIONS / *ACCIONES COMPLEMENTARIAS*

PRINCIPAL INVESTIGATORS	PROJECT TITLES
Lietha, Daniel	CNIO Frontier Meeting on allosteric regulation of cancer cell signalling
Real, Francisco X.	*Genómica del Cáncer de vejiga urinaria: Validación a gran escala de nuevos candidatos*

SUB-PROGRAMME OF SUPPORT TO 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'*

MUTUA MADRILEÑA FOUNDATION / *FUNDACIÓN MUTUA MADRILEÑA*

PRINCIPAL INVESTIGATORS	PROJECT TITLES
García, María José	*Caracterización inmunohistopatológica de los tumores de ovario epiteliales hereditarios y esporádicos: Vías de alteración comunes y específicas. Estatus de BRCA1 y/o biomarcadores asociados como predictores de pronóstico y respuesta a la quimioterapia*
Martínez, Beatriz	*Identificación de miRNAS como biomarcadores de diagnóstico precoz en cáncer de mama hereditario*
Osorio, Ana	*Análisis de polimorfismos en genes candidatos como modificadores del fenotipo en pacientes portadoras de mutaciones en los genes de alta susceptibilidad para el desarrollo del cáncer de mama BRCA1 y BRCA2*
Rodríguez, Cristina	*Estudio de microRNAs como predictores de respuesta a Sunitinib en pacientes con carcinoma renal*
Sánchez-Carbayo, Marta	*Perfiles epigenéticos como marcadores diagnósticos y pronóstico en cáncer de vejiga*
Guinea-Viniegra, Juan	*JunB/AP-1, supresor tumoral en la piel. Mecanismos moleculares e interacción funcional con p53*

RAMÓN ARECES FOUNDATION / *FUNDACIÓN RAMÓN ARECES*

PRINCIPAL INVESTIGATORS	PROJECT TITLES
Guerra, Carmen	*Implicación de los oncogenes RAS en el desarrollo de los síndromes Costello y Noonan*
Malumbres, Marcos	*Base genética y celular del síndrome de microdeleción 16p11.2-p12.2 y de los trastornos neurales relacionados*
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*

SANDRA IBARRA FOUNDATION / *FUNDACIÓN SANDRA IBARRA*

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Martínez, Beatriz	*Identificación de miRNAs como biomarcadores de diagnóstico precoz en sangre y suero de pacientes con cáncer de mama hereditario*

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

PRINCIPAL INVESTIGATORS	PROJECT TITLES
Benítez, Javier	*Diseccionando las bases genéticas del cáncer de mama hereditario*
González-Neira, Anna	*Farmacogenética en tumores infantiles*

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

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Quintela-Fandino, Miguel	*Validación de una estrategia novedosa en la identificación de genes implicados en la evolución de cáncer de mama hormonopositivo*

PRIVATE SPONSORS

WE WOULD LIKE TO THANK ALL OUR SPONSORS AND DONORS
FOR THE GENEROUS SUPPORT THAT WE RECEIVED FROM THEM IN 2012.
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 ***Fundación BBVA*** generously supports the BBVA Foundation-CNIO Cancer Cell Biology Programme, headed by Professor Erwin F. 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.

The **Spanish Association Against Cancer *(Asociación Española Contra el Cáncer, AECC)***, through its Science Foundation, awarded five grants aimed at supporting cancer research. The grants add up to a total of over 700,000 EUR out of the 1.5 million EUR that the *AECC* offered for the funding of research projects in 2012. These grants are aimed to support scientists and clinicians who work intensively in the field of oncology. The CNIO scientists who were honoured this year are Andrés J. López-Contreras, María Salazar, David Olmos, Chiara Ambrogio and Pablo J. Fernández-Marcos.

The ***Fundación Marcelino Botín*** is committed to supporting scientific research and knowledge transfer from academia to the market through science programmes; this transfer is regarded as one of the main driving forces for Spain's economic and social development. The *Fundación Marcelino Botín* collaborates with CNIO in this regard by supporting the Research Groups led by Manuel Serrano and Maria A. Blasco.

Fundación CRIS is dedicated to the promotion and development of research with the aim of eliminating the serious health threat of cancer. In 2012, CNIO signed a contract with *Fundación CRIS* to set up a new Clinical Research Unit to study Genitourinary Tumours. This new laboratory is headed by David Olmos.

AVON, funds the Breast Cancer Clinical Research Unit, led by Miguel Quintela-Fandino, since 2010. The Research Project "Avon-CNIO" on breast cancer research has the main goal of advancing the personalised treatment of 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. Thanks to the support received from this foundation, a new laboratory with a clear translational vocation was created; it will focus on the identification of markers for brain tumours as its principal activity. This new laboratory is integrated within the BBVA Foundation-CNIO Cancer Cell Biology Programme and is headed by Massimo Squatrito.

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 beneficiaries of the *Jesús Serra* Foundation's Visiting Researcher Programme in 2012 were Maria Sibilia (from the Institute of Cancer Research of the Medical University of Vienna) and Robert Benezra (from the Memorial Sloan – Kettering Cancer Center in New York).

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.

And last but not least, there are several private sector organisations that provide financial support for CNIO scientists to carry out their research through privately-funded projects. These include the ***Fundación Mutua Madrileña, Fundación Ramón Areces, Fundación Sociedad Española de Oncología Médica (SEOM).***

The ***Fundación Banco Santander*** funds the Banco Santander Foundation – CNIO Fellowships for Young Researchers. This year the *Fundación Banco Santander* provided its support to a new CNIO initiative; a course on Management Fundamentals and Skills for Scientists and Researchers, which has been developed by the IE Business School.

OTHER SPONSORS

Our activities 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 Fundación Antoni Serra, Grupo Fressia de Cambrils y Perelló, Asociación de Familias de Niños con Cáncer de Castilla y la Mancha (AFANION), Fundación Instituto Roche, Laboratorios Gebro Pharma, Asociación Benéfica Monterrubiana (ABEMONT), Amalie Petroquímica.***

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.

TRAINING PROGRAMMES

The CNIO is highly dedicated to providing an excellent training environment for the next generation of researchers and professionals in the health sector at all career levels. To achieve this goal, the CNIO collaborates with public and private academic institutions all over the country. In 2012, the CNIO has expanded its collaborations with academia through the signing of collaborative agreements with 11 different academic entities from 6 Spanish Universities (*Universidad de Alcalá de Henares, Universidad CEU San Pablo, Universidad Complutense de Madrid, Universidad de Lleida, Universidad Politécnica de Madrid, Universidad Pompeu Fabra)*, as well as 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)*, the Madrid Science Park (*Parque Científico de Madrid, PCM*), and the Centre for Biomedical Studies (*Centro de Estudios Biosanitarios, CEB)*.

NUMBER OF TRAINEES

	2007	2008	2009	2010	2011	2012
Laboratory Training For BSc/MSc Students	24	31	39	54	46	42
Training of PhD Students	130	144	133	132	123	121
Postdoctoral Training	45	69	75	95	83	81
Laboratory Training for Technicians	19	27	22	29	26	26
Training for MDs	25	18	24	25	20	16

LABORATORY TRAINING FOR 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 3 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 (320 hours). During this time the students actively participate in research projects in one of the CNIO groups. During 2012, 8 students from 5 countries participated in this programme.

→ Within the framework of the MIT-Spain Program at the Massachusetts Institute of Technology (MIT), the CNIO hosts undergraduate students from all of the five MIT schools for a 2-month internship. In 2012, 2 MIT students participated in this internship 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, 42 students participated in these programmes, of which 7 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, 16 students obtained their PhD degrees in 2012 and 21 joined the CNIO in that same year. Almost half of the 121 students working at the CNIO in 2012 were graduates from foreign universities, thus contributing to the internationalisation of the Centre.

In 2008, the *Fundación "la Caixa"* initiated an innovative programme that 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. Until 2012, the 4-year programme supported 10 fellows per year; they were selected after an international call and a competitive selection process. The 2012 call was very successful, attracting around 263 eligible applications of undergraduates from 44 different countries.

In 2012, the *Jesús Serra* Foundation (*Catalana Occidente* Group) awarded a fellowship for 1 PhD student, allowing for the finalisation of relevant studies beyond the planned thesis project.

FUNDING OF PHD TRAINING	FELLOWSHIPS
SPANISH ENTITIES	99
Ministry of Economy and Competitiveness / *Ministerio de Economía y Competitividad* (RD projects)	9
Ministry of Economy and Competitiveness / *Ministerio de Economía y Competitividad* (FPI fellowships)	18
Ministry of Education, Culture and Sport / *Ministerio de Educación, Cultura y Deporte* (FPU fellowships)	10
Health Research Fund / *Fondo de Investigaciones Sanitarias* (FPI fellowships)	5
Autonomous Community of Madrid / *Comunidad Autónoma de Madrid* (RD projects)	1
Autonomous Community of Madrid / *Comunidad Autónoma de Madrid* (FPI fellowships)	1
Government of the Basque Country / *Gobierno del País Vasco* (pre-doctoral fellowship)	1
Scientific Foundation of the Spanish Association Against Cancer / *Fundación Científica de la AECC* (RD projects)	2
CIBERER	2
Ferrer Group / *Grupo Ferrer*	1
"la Caixa" Foundation / *Fundación "la Caixa"*	48
CNIO-CNIC Agreement / *Acuerdo* CNIO-CNIC	1
INTERNATIONAL ENTITIES	22
EU Framework Programme	8
European Research Council	8
Fulbright Programme	1
American Institute for Cancer Research	1
Howard Hughes Medical Institute	2
National Institutes of Health	1
Novartis	1
TOTAL	**121**

The distribution of students across the CNIO's Research Programmes in 2012 was as follows: Molecular Oncology Programme (36% of students), the BBVA Foundation-CNIO Cancer Cell Biology Programme (8%), the Structural Biology and Biocomputing Programme (16%), the Molecular Pathology Programme (11%), the Human Cancer Genetics Programme (20%), the Clinical Research Programme (8%) and the Biotechnology Programme (1%).

POST-DOCTORAL TRAINING

One of 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 2012, 81 postdoctoral fellows worked at the CNIO. Notably 60% of these fellows were from outside of Spain, many coming from very prestigious international institutions.

In 2008, the CNIO launched the CNIO-Caja Navarra International Postdoctoral Programme. During 2012, this 2-year programme supported 7 postdoctoral fellows at the CNIO (5 of them were still at the CNIO by end of 2012); they were selected through an international call and a competitive selection process.

In 2012, the *Fundación Banco Santander* funded a highly competitive fellowship programme aimed to support outstanding young scientists who have been trained in the UK and wish to start or continue their postdoctoral training at the CNIO. Two young scientists from the University of Leicester and the London Research Institute – Cancer Research UK were awarded a Santander Foundation-CNIO Fellowship; one of them already started his fellowship in 2012.

FUNDING OF POST-DOCTORAL TRAINING	FELLOWSHIPS
SPANISH ENTITIES	54
Ministry of Economy and Competitiveness / *Ministerio de Economía y Competitividad* (RD projects)	4
Ministry of Economy and Competitiveness / *Ministerio de Economía y Competitividad* (fellowships)	7
Ministry of Education, Culture and Sport / *Ministerio de Educación, Cultura y Deporte* (mobility programme)	1
Health Research Fund / *Fondo de Investigaciones Sanitarias*	7
Autonomous Community of Madrid / *Comunidad Autónoma de Madrid*	1
Scientific Foundation of the Spanish Association Against Cancer / *Fundación Científica de la AECC*	9
CIBERER	1
Caja Navarra Foundation / *Fundación Caja Navarra*	5
Banco Santander Foundation / *Fundación Banco Santander*	1
CNIO	18
INTERNATIONAL ENTITIES	27
EU Framework Programme	11
European Research Council	6
German Research Foundation / *Deutsche Forschungsgemeinschaft*	1
Association for International Cancer Research	1
Federation of European Biochemical Societies	2
Howard Hughes Medical Institute	1
AXA-CNIO Chair in Molecular Oncology	1
Open Phacts	2
Boehringer Ingelheim	1
F. Hofmann-La Roche	1
TOTAL	**81**

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 en 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 de Henares (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 9 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 19 weeks (710 hours) of laboratory training for students. Additionally, the CNIO offers real-life work experience to 2 Analytical Assays and Quality Control students (370 hours), 2 Medical Archiving and Recording students for 13 weeks (440 hours), and 1 Clinical Diagnosis student for 12 weeks (380 hours). Of the 26 students who participated in this programme in 2012, 3 were hired by the CNIO.

MOLECULAR PATHOLOGY AND FAMILIAL CANCER TRAINING FOR MDS

In line with CNIO's commitment to bridge the "bench to bedside" gap, the Centre offers excellent training opportunities in molecular diagnostics and familial cancer genetics to MDs and other health care professionals; this initiative is a collaborative effort with the Spanish Ministry of Health (now *Ministerio de Sanidad, Servicios Sociales e Igualdad*). Training usually consists of a 3-month period during residency. For 2012, 16 medical residents from 12 different hospitals enjoyed the benefits of rotations within the different Groups and Units of the CNIO's Molecular Pathology, Human Cancer Genetics and Clinical Research Programmes.

ADVANCED TRAINING OF SCIENTISTS THROUGH EXTRAMURAL PROGRAMMES

During 2012, 12 scientists were supported by the *Ramón y Cajal* Programme. This initiative – established in 2001 by the former Spanish Ministry of Science and Technology (now *Ministerio de Economía y Competitividad*) – aims to encourage scientists who work abroad to complete their scientific training in Spanish research institutions. 30 other scientists were funded by similar programmes, including the *Miguel Servet* (6), *Sara Borrell* (4) and *Río Hortega* (3) programmes, funded by the *Fondo de Investigaciones Sanitarias* del *Instituto de Salud Carlos III*, the *Juan de la Cierva* programme (8), funded by the Spanish Ministry of Economy and Competitiveness, as well as the *Ayudas para Investigadores en Oncología* (9) funded by the *Asociación Española Contra el Cáncer*.

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. The beneficiaries of the *Jesús Serra* Foundation's Visiting Researcher Programme in 2012 were Maria Sibilia from the Institute of Cancer Research of the Medical University of Vienna and Robert Benezra from the Memorial Sloan-Kettering Cancer Center in New York.

FUNDACIÓN JESÚS SERRA
Grupo CATALANA OCCIDENTE

DEAN'S OFFICE

MARÍA S. SOENGAS DEAN FOR ACADEMIC AFFAIRS

At the CNIO we take particular pride in the relevance of our scientific Programmes as well as in their international projection; these factors have led us to be amongst the world's top research institutions in the cancer field. Key to our mission is to nurture and foster the development of our scientists in-training, so that they themselves can become future leaders. Since its inception, the CNIO has dedicated particular attention to establishing agreements with various foundations that have generously funded highly competitive PhD and Postdoctoral fellowships. Also very successful, are our undergraduate summer internships, as well as diverse exchange and visitor programmes with national and international universities. Additional collaborations are in place with multiple hospitals, which further help to contribute towards a vibrant community of young and active investigators. As these educational activities expand, the CNIO Director and the Programme Directors' Committee have decided to better integrate all efforts through the establishments of an Office for Academic Affairs. I am honoured to be the first Dean of this Office.

The Dean's Office is busily working on various fronts. Our primary objective is to help our trainees achieve the most competitive educational background as possible. Seminar series and workshops are being organised to cover key aspects that will ensure success in academia. These range from scientific reasoning, to grant writing, job interviewing or team building, among others. Moreover, we acknowledge that science extends beyond the bench; therefore journalism, management of intellectual property, and even the creation of start-ups or spin-offs, are part of additional topics that constitute our curriculum. These activities are performed in concertation with the CNIO's Training Programmes, Innovation and Communication Offices that are deeply committed to providing the best environment for our personnel. Also central to our endeavours, is the mentoring aspect, and faculty members are readily available for consultation and guidance. We also continue to greatly value our alumni, and we are currently very interested in pursuing the creation of a network to integrate past, present (and future) researchers.

Importantly, our activities are not just destined for trainees. We very much encourage CNIO scientists to participate and I am glad that they have taken this challenge to heart and that they have responded with great enthusiasm. After internal voting and election procedures, the CNIO Student Association (CNIOSA) and the Postdoc Association (CNIOPDA) were created. They are leading many initiatives to help their peers, and represent positive stimulation for the Dean's Office. We held our First CNIO Science and Arts contest that resulted in revealing several talented photographers. This led to the creation of an inspiring calendar used for our fundraising purposes. Members of CNIOSA and CNIOPDA have also created an educational "Video for Kids" that presents the daily life at our Centre in a very comprehensible manner. We are also preparing other outreach activities, as we believe that an informed society will be better prepared to understand (and if needed, face) the diseases that constitute human cancer. ■

SCIENTIFIC EVENTS

CNIO FRONTIERS MEETINGS

CNIO Frontiers Meetings are the main international conferences that are organised by the CNIO. They focus on specific, cutting-edge aspects of cancer research, thus providing a unique platform for an intensive and dynamic exchange and debate of scientific ideas. The invited speakers – 20 internationally renowned leaders in oncology – present their latest findings for a period of 2 and a half days. Up to 100 additional participants are selected, via a widely publicised call for applications, based on their potential to make relevant contributions to the conference by presenting hot topics as posters or short talks.

ALLOSTERIC REGULATION OF CELL SIGNALLING, SEPTEMBER 17-19, 2012.

ORGANISERS

- **Francesco L. Gervasio**, CNIO, Madrid, Spain
- **Ermanno Gherardi**, MRC Laboratory of Molecular Biology, Cambridge, UK
- **Daniel Lietha**, CNIO, Madrid, Spain
- **Giulio Superti-Furga**, CeMM, Vienna, Austria

SESSIONS

- Allosteric signalling mechanisms
- Dynamics and modelling of allosteric systems
- Allosteric inhibition

SPEAKERS

- **Tom Blundell**, University of Cambridge, UK
- **Françoise Bono**, Sanofi R&D, Toulouse, France
- **Jean Pierre Changeux**, *Collège de France, Institut Pasteur*, Paris, France
- **Nikolay Dokholyan**, University of North Carolina at Chapel Hill, USA
- **Michael Eck**, Dana-Farber Cancer Institute, Harvard Medical School, Boston, USA
- **Doriano Fabbro**, Novartis Pharma AG, Basel, Switzerland
- **Markus Grütter**, University of Zurich, Switzerland
- **Gerhard Hummer**, NIDDK, NIH, Bethesda, USA
- **Yvonne Jones**, Wellcome Trust Centre for Human Genetics, University of Oxford, UK
- **Dorothee Kern**, Brandeis University, Waltham, USA
- **Daniel Leahy**, Johns Hopkins University School of Medicine, Baltimore, USA
- **Ruth Nussinov**, National Cancer Institute at Frederick, USA
- **Jose Onuchic**, Center for Theoretical Biological Physics at Rice and UCSD, La Jolla, USA
- **Jeffrey Peterson**, Fox Chase Cancer Center, Philadelphia, USA
- **Timothy Springer**, Harvard Medical School, Boston, USA
- **Roger Sunahara**, University of Michigan Medical School, USA
- **Susan Taylor**, University of California, San Diego, La Jolla, USA
- **Alfred Wittinghofer**, Max Planck Institute for Molecular, Physiology, Dortmund, Germany

In addition, 10 short talks were selected among participants' contributions and 27 posters were presented.

OTHER INTERNATIONAL MEETINGS & CONFERENCES

In addition to the CNIO Frontiers Meetings, the CNIO annually hosts various international meetings and conferences. Within this category, the 3 international events in 2012 focused on recent advances in the areas of familial cancer, bladder cancer and adrenal tumours.

5th ESO-CNIO FAMILIAL CANCER CONFERENCE, JUNE 7-8, 2012.

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
- Rare tumours
- New technologies applied to familial cancer studies

SPEAKERS

- **Antonis Antoniou**, University of Cambridge, UK
- **Javier Benítez**, CNIO, Madrid, Spain
- **Gabriel Capellá**, Catalan Institute of Oncology, Barcelona, Spain
- **Alberto Cascón**, CNIO, Madrid, Spain
- **Antoni Castells**, *Clinic Hospital*, Barcelona, Spain
- **Françoise Dantzer**, University of Strasbourg, France
- **Diana Eccles**, Southampton University Hospital Trust, UK
- **Rosalind Eeles**, The Royal Marsden Hospital, Sutton, UK
- **Thierry Frebourg**, Rouen University Hospital, France
- **María José García**, CNIO, Madrid, Spain
- **David Goldgar**, University of Utah, Salt Lake City, USA
- **Nelleke Gruis**, Leiden University Medical Center, Leiden, The Netherlands
- **Carmen Guerra**, CNIO, Madrid, Spain
- **Shirley Victoria Hodgson**, St. George's Hospital Medical School, London, UK
- **Fred Menko**, VU University Medical Center, Amsterdam, The Netherlands
- **Roger L. Milne**, CNIO, Madrid, Spain
- **Ana Osorio**, CNIO, Madrid, Spain
- **José Palacios**, *Ramón y Cajal* University Hospital, Madrid, Spain
- **Rosario Perona**, Biomedical Research Institute, Madrid, Spain
- **Barbara Rivera**, CNIO, Madrid, Spain
- **Mercedes Robledo**, CNIO, Madrid, Spain
- **Dimitrios Roukos**, Ionnanina University School of Medicine, Ioannina, Greece
- **Jordi Surrallés**, Autonomous University of Barcelona, Spain
- **Hans Vasen**, Leiden University Medical Centre, Leiden, The Netherlands

In addition, 8 short talks were selected among participants' contributions and 35 posters were presented.

5th INTERNATIONAL CONSORTIUM OF BLADDER CANCER MEETING, OCTOBER 8-9, 2012.

ORGANISERS

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

SESSIONS

- 'OMICS'
- Clinical Challenges
- Next-generation Sequencing

SPEAKERS

- **Joaquim Bellmunt**, *Hospital del Mar*, Barcelona, Spain
- **José Costa**, Yale University, New Haven, USA
- **Manel Esteller**, *ICO-IDIBELL*, Barcelona, Spain
- **Ivo Gut**, Spanish National Centre for Genome Analysis (*CNAG*), Barcelona, Spain
- **Bart Kiemeney**, Radboud University Nijmegen Medical Centre, The Netherlands
- **Isidro Masana**, Agilent Technologies, Spain

- **Francisco X. Real**, CNIO, Madrid, Spain
- **Nigel Skinner**, Agilent Technologies, UK
- **Mark Teo**, Leeds Institute of Molecular Medicine, University of Leeds, UK
- **Fred Witjes**, University Hospital Nijmegen, The Netherlands

ENS@T 11th SCIENTIFIC MEETING, NOVEMBER 23-24, 2012.

ORGANISERS

- **Javier Aller**, *Puerta de Hierro* University Hospital, Madrid, Spain
- **Mercedes Robledo**, CNIO, Madrid, Spain

SESSIONS

- ENS@T Projects on APA/NAPACA/ACC
- ENS@T Projects on PCC/PGL

SPEAKERS

- **Guillaume Assié,** *Université Paris Descartes, INSERM U567, Institut Cochin*, Paris, France
- **Felix Beuschlein**, Ludwig-Maximilians-University Munich, Germany
- **Sheerazed Boulkroun**, *INSERM*-Cardiovascular Research Centre, Paris, France
- **Tarik Bozoglu**, Ludwig-Maximilians-University Munich, Germany
- **Nadia Cherradi**, *INSERM U1036, Biologie du Cancer et de l'Infection, Commissariat à l'Energie Atomique*, Grenoble, France
- **Vasileios Chortis**, University of Birmingham, UK
- **Aguirre A. de Cubas**, CNIO, Madrid, Spain
- **Tanja Dekkers**, Radboud University Medical Center, Nijmegen, The Netherlands
- **Graeme Eisenhofer**, University Hospital Carl Gustav Carus, Dresden, Germany
- **Judith Favier**, *INSERM*-Cardiovascular Research Centre, Paris, France
- **Constanze Hantel**, Ludwig-Maximilians-University Munich, Germany

- **Camilo Jiménez**, The University of Texas MD Anderson Cancer Centre, Houston, USA
- **Enzo Lalli**, *Institut de Pharmacologie Moleculaire et Cellulaire CNRS*, Valbonne, France
- **Rosella Libé**, *INSERM Unité 1016*, Paris, France
- **Giuseppe Opocher**, University of Padova, Italy
- **Lindsey Oudijk**, Erasmus Medical Centre, Rotterdam, The Netherlands
- **Giada Poli**, University of Florence, Italy
- **Martin Reincke**, Ludwig-Maximilians-University Munich, Germany
- **Cristina Ronchi**, University of Milan, Italy
- **Jyotsna Upendra Rao**, Radboud University Medical Center, Nijmegen, The Netherlands

In addition, 25 posters were presented during this conference.

TRAINING COURSES AND WORKSHOPS

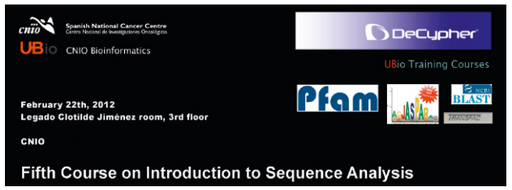
The CNIO is committed to disseminating the results of state-of-the-art cancer research to the wider community, including medical professionals and junior scientists, enabling them to stay abreast of recent developments in specialised techniques. This is achieved through international training courses and hands-on workshops organised by CNIO scientists and technologists.

LABORATORY ANIMAL SCIENCE COURSE FOR RESEARCHERS, CATEGORY C
JANUARY 23 – FEBRUARY 9, 2012.

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

SPEAKERS
- **Ignacio Álvarez**, University Complutense of Madrid, Spain
- **Jean-Louis Bequet**, Charles River, France
- **Isabel Blanco**, CNIO, Spain
- **Argelia Castaño**, National Institute of Healt Carlos III
- **Ernesto de la Cueva**, *Vivo*tecnia, Spain
- **Colin Dunn**, Charles River, UK
- **Ricardo Feinstein**, National Veterinary Department, Sweden
- **Michael Festing**, Animal Procedures Committee, UK
- **Javier Guillen**, AAALAC International, Spain
- **Bryan Howard**, University of Sheffield, UK
- **Marcos Malumbres**, CNIO, Spain
- **Jose M. Morgado**, CNIC, Spain
- **David Morton**, University of Birmingham, UK
- **Francisca Mulero**, CNIO, Spain
- **José M. Orellana**, University of Alcalá, Spain
- **Sagrario Ortega**, CNIO, Spain
- **Belén Pintado**, CNB, Spain
- **Nieves Salvador**, Cajal Neuroscience Institute, Spain
- **Graham Tobin**, Harlan Laboratories Inc.
- **Patri Vergara**, Autonomous University of Barcelona, Spain

FIFTH COURSE ON INTRODUCTION TO SEQUENCE ANALYSIS, FEBRUARY 22, 2012.



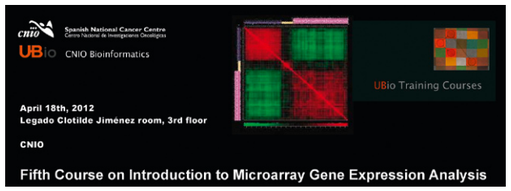
ORGANISERS
- **Osvaldo Graña**, CNIO, Spain
- **David G. Pisano**, CNIO, Spain

SPEAKERS
- **Osvaldo Graña**, CNIO, Spain
- **Daniel Rico**, CNIO, Spain

FIFTH COURSE ON INTRODUCTION TO MICROARRAY GENE EXPRESSION ANALYSIS, APRIL 18, 2012.

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

SPEAKERS
- **Gonzalo Gómez**, CNIO, Spain
- **Daniel Rico**, CNIO, Spain



SECOND HANDS-ON INTRODUCTION TO R, JUNE 6, 2012.

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

SPEAKERS
- **Ramón Díaz Uriarte**, CNIO, Spain

THE MADRID-MIT M+VISIÓN CONSORTIUM COURSES ON MEDICINE, IMAGING, AND INNOVATION: ONCOLOGY-FROM BENCH TO BEDSIDE, JULY 17, 2012.

ORGANISER
- **Francisca Mulero**, CNIO, Spain

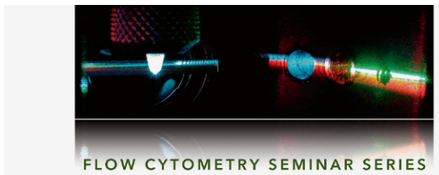
SPEAKERS
- **Fernando Peláez**, CNIO, Spain
- **María S. Soengas**, CNIO, Spain
- **Miguel Quintela-Fandino**, CNIO, Spain
- **Marisol Quintero**, CNIO, Spain
- **Rajiv Gupta**, Harvard Medical School, USA

Madrid-MIT m+VISIÓN CONSORTIUM

FIRST EDITION OF CNIO'S FLOW CYTOMETRY SEMINAR SERIES, SEPTEMBER 5-24, 2012.

ORGANISER
- **Lola Martínez**, CNIO, Madrid, Spain

SPEAKERS
- **Lola Martínez**, CNIO, Madrid, Spain
- **Kylie M. Price**, Malaghan Institute of Medical Research, Wellington, New Zealand



ACCESS TO GENES AND GENOMES WITH ENSEMBL 2012. OCTOBER 3, 2012.

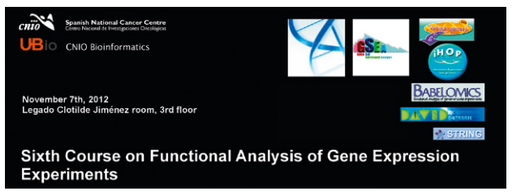
ORGANISERS
- **Osvaldo Graña**, CNIO, Spain
- **David G. Pisano**, CNIO, Spain

SPEAKERS
- **Denise Carvalho-Silva**, European Bionformatics Institute (EBI), UK

SIXTH COURSE ON FUNCTIONAL ANALYSIS OF GENE EXPRESSION EXPERIMENTS, NOVEMBER 7, 2012.

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

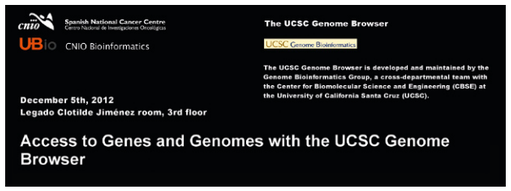
SPEAKERS
- **Gonzalo Gómez**, CNIO, Spain
- **Daniel Rico**, CNIO, Spain



ACCESS TO GENES AND GENOMES WITH THE UCSC GENOME BROWSER, DECEMBER 5, 2012.

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

SPEAKER
- **Osvaldo Graña**, CNIO, Spain



OTHER MEETINGS (held in Spanish)

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

JORNADAS CIENTÍFICAS-ESTUDIO BIBF 1120 NEOADYUVANCIA, FEBRUARY 21, 2012.

ORGANISERS
- **Miguel Quintela-Fandino**, CNIO, Madrid, Spain
- **Ramón Colomer**, CNIO, Madrid, Spain

NUEVAS APLICACIONES Y NUEVAS TECNOLOGÍAS-III EDICIÓN, OCTOBER 25-26, 2012.

ORGANISERS
- **Diego Megías**, CNIO, Madrid, Spain
- **Margarita Fité**, Leica Microsystems

CURSO DE ESTADÍSTICA APLICADA A LA INVESTIGACIÓN BIOMÉDICA CON R, APRIL 25-27, 2012.

ORGANISERS
- **Núria Malats**, CNIO, Madrid, Spain
- **Jesús Herranz Valera**, CNIO, Madrid, Spain

CURSO DE ESTADÍSTICA APLICADA A LA INVESTIGACIÓN BIOMÉDICA CON R, DECEMBER, 17-19, 2012.

ORGANISERS
- **Núria Malats**, CNIO, Madrid, Spain
- **Jesús Herranz Valera**, IMDEA Institute, Madrid, Spain

CNIO DISTINGUISHED SEMINARS

The purpose of the Distinguished Seminars 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 usually held on Friday noon in the CNIO Auditorium throughout the year, with the exception of holidays and the July to September summer break. Each Distinguished Seminar series includes world-leading scientists who address topics that are of general interest to the CNIO faculty. In total, the CNIO hosted 33 distinguished speakers in 2012, among which, 2 Nobel Prize winners.

This year, the Distinguished Seminars Series has widened its scope and spectrum of topics by including: i) young scientists with an outstanding scientific track-record and who work on hot-topics in cancer research; ii) out-of-the-box speakers who address topics that are not necessarily related to cancer research, but that provide novel perspectives and ideas that contribute to the CNIO's intellectually challenging trans-disciplinary environment; and iii), senior clinical investigators with renowned expertise in translational cancer research who hold leading positions in institutions of the health system.

DATE	SPEAKER	ORGANISATION
JANUARY		
13/01/2012	**Yoshiaki Ito**	Cancer Science Institute of Singapore (CSI Singapore), Centre for Life Sciences, Singapore
20/01/2012	**Hans Lehrach**	Max Planck Institute for Molecular Genetics, Berlin, Germany
27/01/2012	**Pasi A. Janne**	Dana-Farber Cancer Institute, Boston, USA
FEBRUARY		
03/02/2012	**Valentín Fuster**	Spanish National Cardiovascular Research Centre (CNIC), Madrid, Spain
10/02/2012	**Richard Treisman**	Cancer Research UK London Research Institute, UK
24/02/2012	**Manuel Patarroyo**	Karolinska Institute, Stockholm, Sweden
MARCH		
09/03/2012	**Tom Blundell**	University of Cambridge, UK
16/03/2012	**Andrea Musacchio**	IFOM-IEO European Institute of Oncology, Milan, Italy
23/03/2012	**Wei Yang**	LMB, NIDDK, National Institutes of Health, Bethesda, USA
30/03/2012	**Thomas W. Glover**	University of Michigan, Ann Arbor, USA
APRIL		
12/04/2012	**Irving Weissman**	Institute for Stem Cell Biology and Regenerative Medicine, Stanford University School of Medicine, USA
13/04/2012	**Rudi Balling**	Luxembourg Centre for Systems Biomedicine, University of Luxembourg
20/04/2012	**Rune Toftgård**	Karolinska Institute, Huddinge, Sweden
27/04/2012	**Patricia Dahia**	University of Texas Health Science Centre at San Antonio, USA
MAY		
04/05/2012	**Philip Beachy**	Stanford Cancer Centre, Stanford School of Medicine, USA
11/05/2012	**Michael Eck**	Dana-Farber Cancer Institute, Boston, USA
18/05/2012	**Jürgen Knoblich**	Institute of Molecular Biotechnology (IMBA), Vienna, Austria
JUNE		
01/06/2012	**Tak Mak**	The Campbell Family Institute for Breast Cancer Research – Ontario Cancer Institute, Princess Margaret Hospital, Toronto, Canada
15/06/2012	**John Ioannidis**	Prevention Research Centre, Stanford School of Medicine, USA
22/06/2012	**Daniel Gerlich**	Institute of Molecular Biotechnology IMBA, Vienna, Austria
29/06/2012	**Anton Berns**	The Netherlands Cancer Institute, Antoni van Leeuwenhoek Hospital, Amsterdam, The Netherlands

SEPTEMBER		
06/09/2012	**Robert Huber**	Max Planck Institute of Biochemistry, Martinsried, Germany
07/09/2012	**Peter Campbell**	The Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, UK
14/09/2012	**Kari Stefansson**	deCODE Genetics, Reykjavik, Iceland
21/09/2012	**Cristóbal Belda**	University Hospital Madrid Sanchinarro, Madrid, Spain
OCTOBER		
05/10/2012	**Geoffrey M. Wahl**	Gene Expression Laboratory, The Salk Institute for Biological Studies, La Jolla, USA
19/10/2012	**Eamonn Maher**	Institute of Biomedical Research, University of Birmingham School of Medicine, Birmingham, UK
26/10/2012	**George Thomas**	*IDIBELL*, Barcelona, Spain
NOVEMBER		
16/11/2012	**Dan Littman**	Skirball Institute of Biomolecular Medicine, New York, USA
23/11/2012	**Paul Nurse**	London Research Institute, London, UK
30/11/2012	**Keith Baggerly**	The University of Texas M. D. Anderson Cancer Centre, Houston, USA
DECEMBER		
14/12/2012	**Nancy Hynes**	*Friedrich Miescher* Institute for Biomedical Research, Basel, Switzerland
21/12/2012	**Juan Carlos Izpisúa Belmonte**	Centre for Regenerative Medicine, Barcelona, Spain & Gene Expression Laboratory, The Salk Institute for Biological Studies, La Jolla, USA

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 54 *ad-hoc* seminars were organised by CNIO researchers in 2012.

DATE	SPEAKER	ORGANISATION
FEBRUARY		
07/02/12	**Ivan Diaz-Padilla**	UHN Princess Margaret Hospital, Toronto, Canada
09/02/12	**Eduardo Orias**	University of California, Santa Barbara, USA
10/02/12	**Javier Herrero**	European Bioinformatics Institute, Cambridge, UK
13/02/12	**Albert Lowenfels**	New York Medical College, USA
16/02/12	**Marc A. Marti-Renom**	National Centre for Genomic Analysis (*CNAG*), Barcelona, Spain
17/02/12	**Mª Luisa Alonso Núñez**	Paterson Institute for Cancer Research, The University of Manchester, UK
21/02/12	**Kristel Van Steen**	University of Liège, Belgium
23/02/12	**Françoise Bono**	Sanofi-Aventis Research & Development, Toulouse, France
28/02/12	**Thanos D. Halazonetis**	University of Geneva, Switzerland
29/02/12	**Eugenia Meiler**	Complutense University of Madrid, Spain
MARCH		
01/03/12	**Eleonora Lapi**	Cancer Research UK, London, UK
12/03/12	**Ronald Wolf**	Ludwig-Maximilian University, Munich, Germany
21/03/12	**Javier Muñoz**	Utrecht University, The Netherlands
22/03/12	**Daniel Leahy**	Johns Hopkins University School of Medicine, Baltimore, USA
22/03/12	**Maria Ana Gómez Ferreria**	Samuel Lunenfeld Research Institute, Toronto, Canada
23/03/12	**David C. Whitcomb**	Giant Eagle Foundation, University of Pittsburgh and University of Pittsburgh Medical Center, USA
27/03/12	**Marco Antonio Fontelos López**	Institute for the Mathematical Sciences – *ICMAT*, Madrid, Spain
30/03/12	**Terry Lechler**	Duke University Medical Centre, Durham, USA

MAY		
16/05/12	**Pamela Feliciano**	Nature Publishing Group – Nature Genetics, New York, USA
17/05/12	**Yves Allory**	Henri Mondor Hospital, Créteil, France
18/05/12	**Razqallah Hakem**	Ontario Cancer Institute, University Health Network and Department of Medical Biophysics, University of Toronto, Canada
22/05/12	**Lucas Moreno**	The Royal Marsden Hospital & The Institute of Cancer Research, Sutton, UK
24/05/12	**Ivo Gut**	Spanish National Centre for Genome Analysis (*CNAG*), Barcelona, Spain
25/05/12	**Salvador Aznar Benitah**	Centre for Genomic Regulation, Barcelona, Spain
30/05/12	**Petros Papadopoulos**	VU University Medical Centre, Amsterdam, The Netherlands

JUNE		
05/06/12	**Juan M. Vaquerizas**	Max Planck Institute for Molecular Biomedicine, Münster, Germany
12/06/12	**David Olmos**	The Institute of Cancer Research and The Royal Marsden Hospital, Sutton, United Kingdom
19/06/12	**Ludmila Prokunina**	National Institutes of Health – National Cancer Institute, Bethesda, USA
19/06/12	**Héctor Peinado**	Cornell University, New York, USA
27/06/12	**Amir Aharoni**	Ben-Gurion University of the Negev, Israel
28/06/12	**Marilyn M. Li**	Baylor College of Medicine, Houston, Texas, USA

JULY		
05/07/12	**Douglas Hanahan**	Head of the Swiss Institute for Experimental Cancer Research, Lausanne, Switzerland
12/07/12	**Serge Scherrer**	Genomatix Software GmbH, Munich, Germany
26/07/12	**Stefan Rose-John**	Christian-Albrechts University Kiel, Germany

AUGUST		
20/08/12	**Paul C. Boutros**	Ontario Institute for Cancer Research, Toronto, Canada

SEPTEMBER		
10/09/12	**Kevin Ryan**	The Beatson Institute for Cancer Research, Glasgow, UK
17/09/12	**Manuel Fernández-Rojo**	Institute for Molecular Bioscience, University of Queensland, Queensland, Australia
25/09/12	**Maria García-Parajo**	ICFO, Barcelona, Spain
25/09/12	**Melike Lakadamyali**	ICFO, Barcelona, Spain
28/09/12	**Emmanuelle Génin**	*Inserm U1078 Génétique, Génomique fonctionnelle et Biotechnologies*, Brest, France
28/09/12	**Peter Bryant**	IE Business School, Madrid, Spain
28/09/12	**Kristel Van Steen**	University of Liège, Belgium
28/09/12	**Richard Redon**	*Institut du Thorax*, Nantes University, France

OCTOBER		
10/10/12	**Silvio Gutkind**	NIDCR, NIH, Bethesda, USA
15/10/12	**Patricia Ruíz-Noppinger**	Metanomics Health GmbH, Berlin, Germany

NOVEMBER		
08/11/12	**Kresten Lindorff-Larsen**	Copenhagen Biocentre, University of Copenhagen, Denmark

DECEMBER		
04/12/12	**Xosé Bustelo**	Cancer Research Centre, Salamanca, Spain
18/12/12	**Mariona Graupera García Mila**	*IDIBELL*, Durán and Reynals Hospital, Barcelona, Spain

SCIENTIFIC DIVULGATION

SCIENCE WEEK

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 *(XII Semana de la Ciencia,* November 5 –18, 2012). In 2012, approximately 180 people participated in guided visits to the Centre's research facilities over the course of 3 days.

SCIENCE IN ACTION

This year, the CNIO supported, together with 5 other Spanish research institutions, the XIII[th] edition of Science in Action (*Ciencia en Acción*). More than 15,000 people participated in activities aimed at promoting science at the *Ciencia en Acción* fair held at the Science Museum *CosmoCaixa* in Alcobendas-Madrid from October 5 to 7. Francisco X. Real, Manuel Serrano and Oscar Fernández-Capetillo were the CNIO researchers in charge of the opening presentation titled "We are mosaics, we can reprogramme ourselves and we need magic bullets". The CNIO further contributed to the organisation of the meeting by dedicating 8 in-house experts who pre-selected the activities presented at the fair and who acted as the jury for awarding the best contributions.

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 2012, more than 300 people participated in such guided visits, most of them were *ESO* and *Bachillerato* student groups, but also professionals in the health sector such as the participants of the OMNIPREX Workshop '*Oncología: Medicina Personalizada en la Oncología Traslacional*'.

COMMUNICATION

JUAN J. GÓMEZ COMMUNICATION DIRECTOR

Nuria Noriega ESP

At the beginning of 2012, CNIO created a Communications Department in order to better promote and relate the Centre's work to society at large, to highlight its vocation for service, as well as to further encourage private donations.

Our main aim was to improve the visibility of the Centre, to inform the public about its considerable activities as a research centre of excellence, and to increase awareness of its endeavours through the media and via social networks.

The results have been very positive. Despite the broader context of the global media crisis, CNIO has almost doubled its number of media appearances compared to the previous year; particularly, its growing international impact is noteworthy.

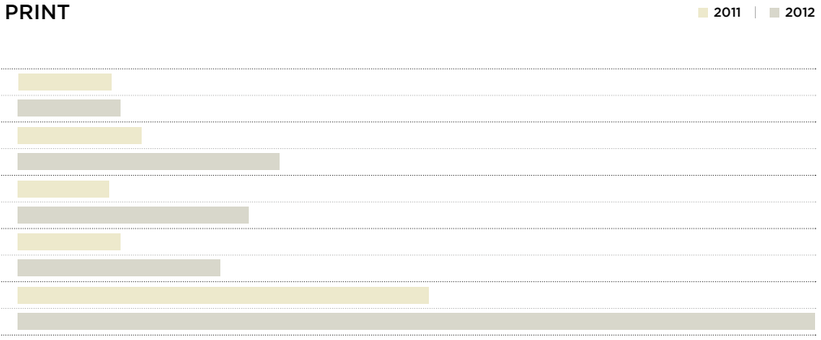
An especially successful media relationship has been developed with Spain's public broadcasting service *Televisión Española* (TVE), which has allowed CNIO's scientific knowledge to reach millions of Spaniards thanks to dozens of appearances on the country's most watched public channel.

Another goal was to increase CNIO's presence on social networks. The ambitious challenge of reaching 2,000 Twitter followers was achieved before the year ended and the launch of our own YouTube channel has shown rapid growth with thousands of visitors being attracted to the site.

The Communications Department has also brought more visibility to the CNIO thanks to other activities such as the dissemination of the prestigious Distinguished Seminars Series, the support of guided visits to the institution, and the promotion of the book "Desarmando al Cáncer". ■

"BECOMING MORE VISIBLE TO BECOME MORE VALUED."

CNIO APPEARANCES IN PRINT AND DIGITAL MEDIA



*More than 194% when compared to 2011.

THE TOP 10 CNIO STORIES SPREAD AROUND THE WORLD (2012)

Since March 2012, CNIO submits press releases to EurekAlert! for publication. This online, global news service, is operated by the American Association for the Advancement of Science (AAAS). Up to December 2012, the CNIO has submitted 22 press releases which received a total of **44,558** visits. This means that, on average, there were more than 2,000 visits for each press release submitted during this time; thus, majorly boosting CNIO's visibility amongst universities, medical centres, journals, government agencies, corporations and other organisations engaged in research all around the world.

PRESS RELEASE	PRINCIPAL INVESTIGATOR	DATE OF PUBLICATION	PAGE VIEWS*
CNIO scientists successfully test the first gene therapy against ageing-associated decline	Maria A. Blasco	14/05/12	4,298
CNIO researchers develop new databases for understanding the human genome	Alfonso Valencia	17/12/12	3,077
New insights on cell competition	Eduardo Moreno	14/09/12	3,224
A novel oncogenic network specific to liver cancer initiation	Erwin F. Wagner	05/10/12	2,988
CNIO researchers describe a new target for developing anti-angiogenic and anti-tumoural therapies	Jorge L. Martínez-Torrecuadrada	10/05/12	2,748
CNIO team discovers the first real indicator of longevity in mammals	Maria A. Blasco	27/09/12	2,689
Bioinformatics Experts at the CNIO explore additional coding potential hidden in the human genome	Alfonso Valencia	18/06/12	2,686
CNIO researchers discover a new therapy that prevents lung cancer growth in mice	Manuel Serrano	13/08/12	2,198
High levels of vitamin D in plasma protects against bladder cancer	Núria Malats	30/10/12	1,897
CNIO participates in the ENCODE project: a stride forward in biomedical research	Alfonso Valencia	06/09/12	1,792

*Data provided by EurekAlert!

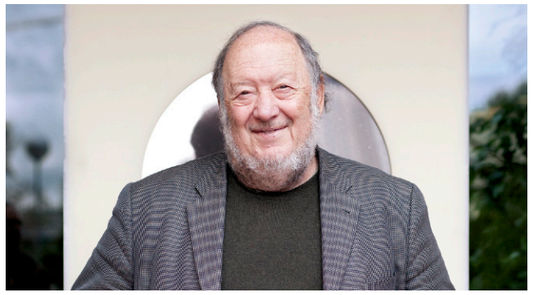
PRESS CLIPPINGS

1 *Diario Médico*. March 16, 2012
2 *El País*. March 16, 2012
3 *Diario Médico*. April 3, 2012
4 *ABC*. April 23, 2012
5 *Informe Semanal, TVE*. April 28, 2012
6 *Diario Médico*. June 4, 2012
7 *Diario Médico*. June 13, 2012

8 *Diario Médico*. June 19, 2012
9 *La Opinión*. July 9, 2012
10 *El Economista*. July 28, 2012
11 *Heraldo de Aragón*. August 14, 2012
12 *La Razón*. September 6, 2012
13 *ABC*. September 15, 2012
14 *El País*. September 16, 2012

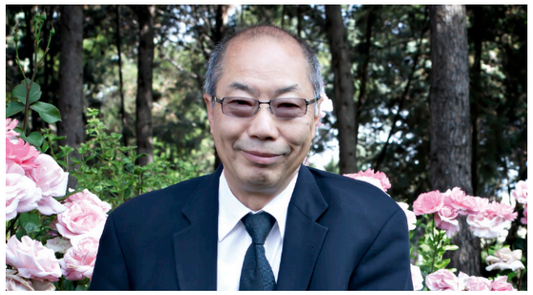
15 *El País*. September 25, 2012
16 *The New York Times*. September 25, 2012
17 *El País*. October 5, 2012
18 *La Razón*. October 14, 2012
19 *Diario de Almería*. November 3, 2012
20 *El Mundo*. December 2, 2012
21 *La Opinión*. December 30, 2012
22 *SINC*. November 20, 2012

INVITED GUEST SPEAKERS (Distinguished Seminar Series)



Irving Weissman. April 12, 2012

Jürgen Knoblich. May 18, 2012



Tak Mak. June 1, 2012

Robert Huber. September 6, 2012

Peter Campbell. September 7, 2012

Paul Nurse. November 11, 2012

SOCIAL EVENTS

LEFT Maria A. Blasco outlined the institution's most significant strengths to the UK Ambassador in Spain, Giles Paxman. March 8, 2012.

BELOW Representatives of the *Seve Ballesteros* Foundation visited the CNIO in order to formalise their support for the Centre's research on brain tumours. April 25, 2012.

ABOVE Maria A. Blasco invited Spanish media to celebrate her first year as CNIO Director. June 19, 2012.

LEFT First meeting of the members of the WISE CNIO Office (Women in Science Office at CNIO), created in 2012 to foster the career development of female scientists at the Centre. October 11, 2012.

ADMINISTRATION

BOARD OF TRUSTEES

→ **Honorary President**

· **Luis de Guindos Jurado**
Minister of Economy and Competitiveness
Ministro de Economía y Competitividad

→ **President**

· **Carmen Vela Olmo**
Secretary of State for Research, Development and Innovation
Secretaria de Estado de Investigación, Desarrollo e Innovación

→ **Vice-President**

· **Joaquín Arenas Barbero**
Director of the National Institute of Health Carlos III
Director del Instituto de Salud Carlos III

→ **Appointed Members**

· **Pilar Farjas Abadía**
Secretary General for Health and Consumer Affairs
Secretaria General de Sanidad y Consumo

· **Juan María Vázquez Rojas**
Director General for Scientific and Technical Research
Director General de Investigación Científica y Técnica

· **María Fernández Pérez**
Director of the Technical Secretariate of the Executive Committee for Economic Affairs, Economic Affairs Office of the President of the Government
Directora de la Secretaría Técnica de la Comisión Delegada para Asuntos Económicos de la Oficina Económica del Presidente del Gobierno

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

· **Cristina Ibarrola Guillén**
Director General of Health, Health Council of the Government of Navarre
Directora General de Salud de la Consejería de Salud del Gobierno de Navarra

· **Javier Paz Esquete**
Deputy Director General for Research, Academic Affairs and Innovation, Galician Health Service (*SERGAS*)
Subdirector General de Investigación, Docencia e Innovación, SERGAS

· **César Pascual Fernández**
Managing Director, Valdecilla University Hospital
Director Gerente del Hospital Universitario de Valdecilla

· **Luis Rosel Onde**
Managing Director, Aragon Institute of Health Sciences
Director Gerente del Instituto Aragonés de Ciencias de la Salud

→ **Elected Members**

· **Rafael Pardo Avellaneda**
Director General, BBVA Foundation
Director General de la Fundación BBVA

· **Enric Banda Tarradellas**
Director of Science, Research and the Environment, *"la Caixa"* Foundation
Director del Área de Ciencia, Investigación y Medio Ambiente de la Fundación "la Caixa"

· **Ignacio Polanco Moreno**
Chairman, Group PRISA
Presidente del Grupo PRISA

· **Pío Díaz de Tuesta Vázquez**
Director, Caja Madrid Foundation
Director de la Fundación Caja Madrid

→ **Secretary**

· **Javier Arias-Díaz**
Deputy Director General for Cell Therapy and Regenerative Medicine, National Institute of Health Carlos III
Subdirector General de Terapia Celular y Medicina Regenerativa, Instituto de Salud Carlos III

→ **Legal Advisor**

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

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  Memorial Sloan-Kettering Cancer Center
  New York, USA

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  Full Professor
  of Biochemistry and Molecular Biology
  University of Oviedo
  Oviedo, Spain

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  Head of the Developmental Neurobiology Unit
  Neuroscience Institute (*CSIC-UMH*)
  Alicante, Spain

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  Scientific Director
  Biomedical Research Institute of Salamanca
  Salamanca, Spain

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  University of Helsinki, Finland

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  Institut Curie
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  Medical Research Council Laboratory
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  Cambridge, United Kingdom

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  Director
  G.W. Hooper Research Foundation
  San Francisco, USA

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  Institute of Cancer Biology
  Danish Cancer Society
  Copenhagen, Denmark

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  Translational Diagnostics
  Yale University School of Medicine
  New Haven, USA

- **Sara Courtneidge,** PhD, DSc (hc)
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  Sanford-Burnham Medical Research Institute
  La Jolla, USA

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  Clare Hall Laboratories
  London, United Kingdom

- **Denise Galloway,** PhD
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  Human Biology Division
  Fred Hutchinson Cancer Research Center
  Seattle, USA

- **Scott W. Lowe,** PhD
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  Geoffrey Beene Cancer Research Center at
  Memorial Sloan-Kettering Cancer Center
  New York, USA

- **Janet M. Thornton,** PhD
  Director
  European Bioinformatics Institute (*EMBL-EBI*)
  Hinxton, United Kingdom

- **Karen H. Vousden,** PhD
  Director
  The Beatson Institute for Cancer Research
  Cancer Research UK
  Glasgow, United Kingdom

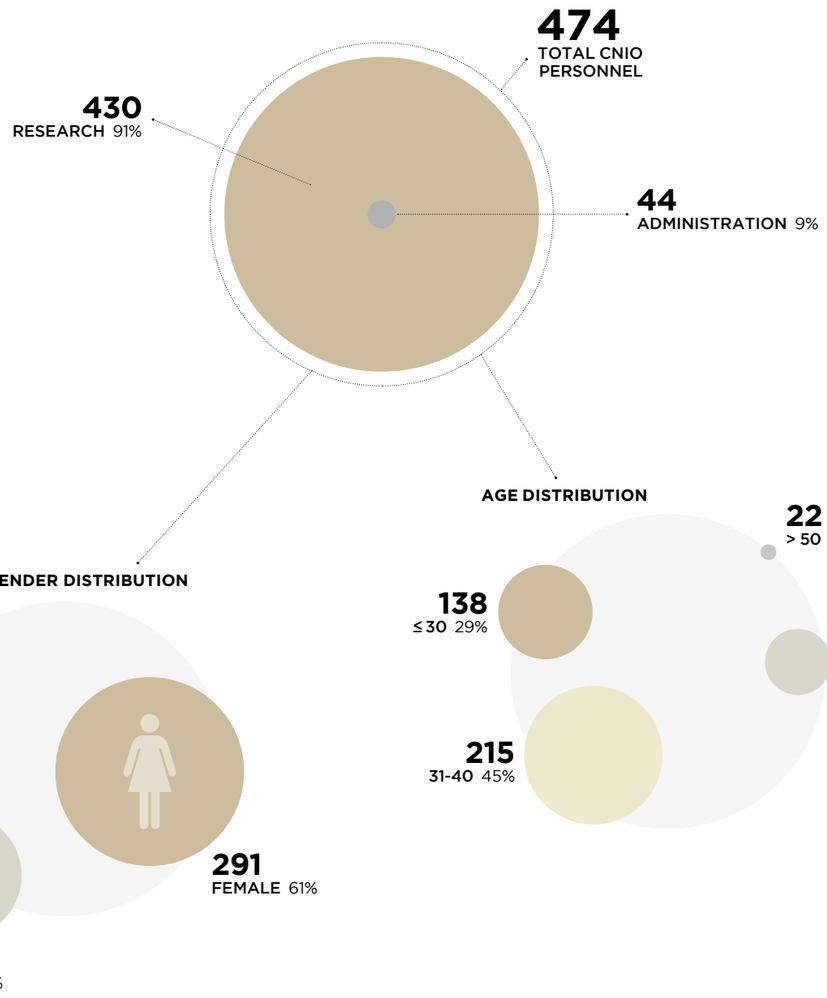
- **Alfred Wittinghofer,** PhD
  Director
  Department of Structural Biology
  Max Planck Institute for Molecular Physiology
  Dortmund, Germany

MANAGEMENT

DIRECTOR	**Blasco, Maria A.**		
	SECRETARIATE	**Alcamí, María Jesús**	
DIRECTOR'S OFFICE	**Peláez, Fernando**		
COMMUNICATION	**Gómez, Juan J.** Director	**Noriega, Nuria** (since June)	
INNOVATION	**Quintero, Marisol** Director		
	TECHNOLOGY TRANSFER & VALORISATION	**Sanz, Anabel** (since May)	
SCIENTIFIC MANAGEMENT	**Klatt, Peter** Director		
	PROJECTS & CONSORTIA	**Liébanes, M. Dolores** Head	Almendro, Aránzazu (since March) **Manukyan, Lilit** (since March)
	EDUCATION & TRAINING PROGRAMMES	**Molina, Juan Ramón** Head	
	SCIENTIFIC EVENTS	**Moro, Mercedes** Head	García-Sabaté, Carolina (until February) **de la Cruz, Virginia** (since April)
	SCIENTIFIC PUBLISHING	**Cerdá, Sonia** Head	**Merino, Ana** (since May)
	LIBRARY & ARCHIVES	**López, Victoria** Head	**Merino, Ana** (until May)

MANAGING DIRECTOR	**Arroyo, Juan**		
	SECRETARIATE	**Ámez, María del Mar**	
MANAGING DIRECTOR'S OFFICE	**Hernández, Jesús** (until June)		
SAP	**Ferrer, Alfonso**		
QUALITY MANAGEMENT	**Martín, Carmen** Head	**Cañizares, Ana M.**	
FINANCE & ADMINISTRATION	**Fontaneda, Manuela** Director		
	PURCHASING	**Araujo, Miguel Ángel** Head **Baviano, Marta** **Carmona, Cristina**	**Galindo, José Antonio** **García-Andrade, Javier** **Rodríguez, Raquel**
	HUMAN RESOURCES	**Pérez, José Lorenzo** Head **Bardají, Paz**	**Carbonel, David** **Sotorres, Luisa** (until December)
	ECONOMIC MANAGEMENT	**Salido, M. Isabel** Head **Hernando, M. Elena**	**Rodríguez, M. José** **García, Juan J.**
	AUDIT	**Bergua, Lucrecia Isabel** Head (until June)	**Domínguez, Pilar** **García-Risco, Silvia**
INFRASTRUCTURE MANAGEMENT	**de Dios, Luis Javier** Director		
	MAINTENANCE	**Vicente, Miguel** Head	
	PREVENTION & BIOSECURITY	**Cespón, Constantino** Head	**Bertol, Narciso**
	INFORMATION TECHNOLOGIES	**Fernández, José Luis** Head **de Miguel, Marcos**	**Fernández-Vera, Jaime Pedro** (until September)
EXTRAMURAL CLINICAL RESEARCH	**López, Antonio** Director		

CNIO PERSONNEL 2012



SCIENTIFIC PERSONNEL 2012

TOTAL SCIENTIFIC PERSONNEL 100% **430**

DISTRIBUTION BY PROGRAMMES

MOLECULAR ONCOLOGY 24%	102
BBVA FOUNDATION-CNIO CANCER CELL BIOLOGY 8%	33
STRUCTURAL BIOLOGY AND BIOCOMPUTING 16%	69
MOLECULAR PATHOLOGY 9%	39
HUMAN CANCER GENETICS 15%	65
CLINICAL RESEARCH 10%	42
BIOTECHNOLOGY 11%	47
EXPERIMENTAL THERAPEUTICS 7%	33

DISTRIBUTION BY PROFESSIONAL CATEGORY

PRINCIPAL INVESTIGATORS 11%	46
STAFF SCIENTISTS 17%	73
POST-DOCTORAL FELLOWS 16%	68
GRADUATE STUDENTS 25%	106
TECHNICIANS 31%	137

GENDER DISTRIBUTION BY PROFESSIONAL CATEGORY

PRINCIPAL INVESTIGATORS	FEMALE	35%	**16**
	MALE	65%	**30**
STAFF SCIENTISTS	FEMALE	66%	**48**
	MALE	34%	**25**
POST-DOCTORAL FELLOWS	FEMALE	49%	**33**
	MALE	51%	**35**
GRADUATE STUDENTS	FEMALE	68%	**72**
	MALE	32%	**34**
TECHNICIANS	FEMALE	70%	**96**
	MALE	30%	**41**

DISTRIBUTION BY PROFESSIONAL CATEGORY IN: BASIC RESEARCH

TOTAL SCIENTIFIC PERSONNEL 100% **430**

PRINCIPAL INVESTIGATORS 9%	18
STAFF SCIENTISTS 18%	36
POST-DOCTORAL FELLOWS 22%	44
GRADUATE STUDENTS 33%	67
TECHNICIANS 18%	38
TOTAL 100%	**203**

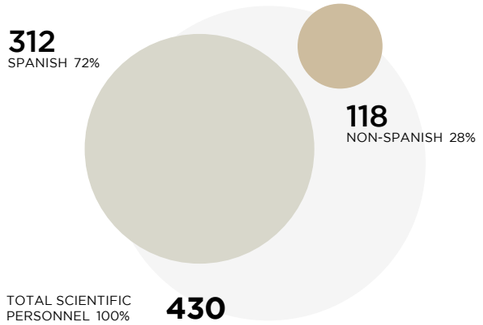
DISTRIBUTION BY PROFESSIONAL CATEGORY IN: TRANSLATIONAL RESEARCH

PRINCIPAL INVESTIGATORS 10%	15
STAFF SCIENTISTS 15%	22
POST-DOCTORAL FELLOWS 16%	23
GRADUATE STUDENTS 26%	39
TECHNICIANS 33%	48
TOTAL 100%	**147**

DISTRIBUTION BY PROFESSIONAL CATEGORY IN: INNOVATION

PRINCIPAL INVESTIGATORS 16%	13
STAFF SCIENTISTS 19%	15
POST-DOCTORAL FELLOWS 1%	1
TECHNICIANS 64%	51
TOTAL 100%	**80**

SCIENTIFIC PERSONNEL: NATIONAL ORIGIN

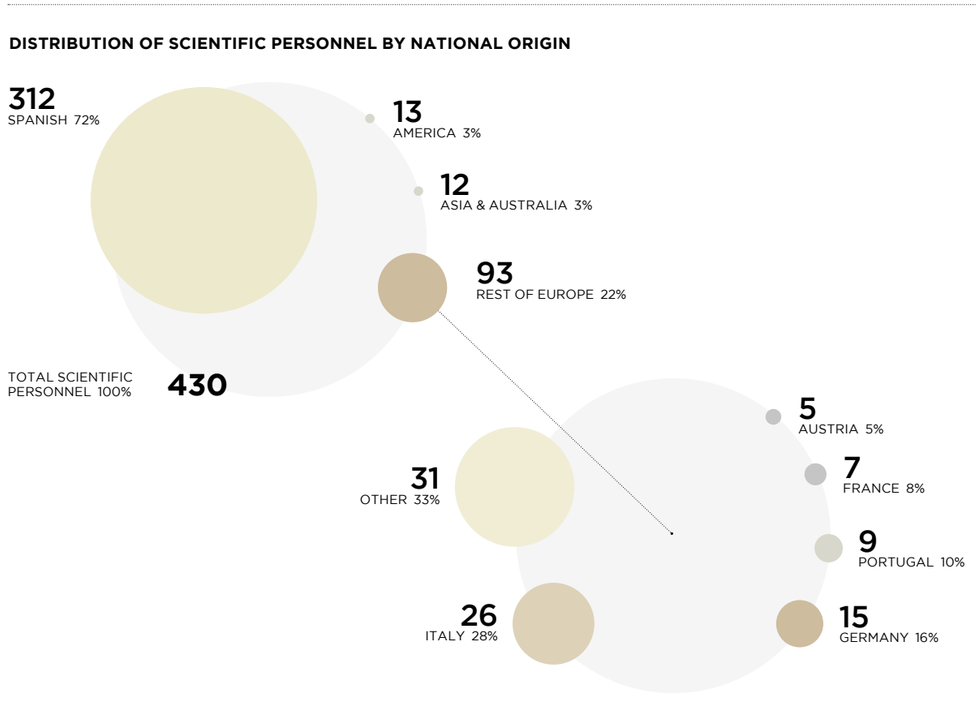


FOREIGN SCIENTIFIC PERSONNEL: DISTRIBUTION BY PROFESSIONAL CATEGORY

PRINCIPAL INVESTIGATORS 17%	8
STAFF SCIENTISTS 18%	13
POST-DOCTORAL FELLOWS 60%	41
GRADUATE STUDENTS 43%	46
TECHNICIANS 7%	10

Total foreign scientific personnel 118
Percent values represent percentages of foreign employees of the total CNIO personnel in each category

DISTRIBUTION OF SCIENTIFIC PERSONNEL BY NATIONAL ORIGIN



*For details please see sections in the report within their respective Research Group/Unit/Section (names of countries are represented by 3-letter country codes according to the ISO3166-1 alpha 3 standard).

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

"... HOW MUCH OF WHAT IS REPRESENTED IN A PORTRAIT PERTAINS OR REFERS TO THE INNERMOST BEING OF THE PERSON BEING PHOTOGRAPHED, AND HOW MUCH CORRESPONDS TO THE PHOTOGRAPHER'S OWN GAZE, HIS INNER WORLD, AND TO WHAT HE SEEKS TO COMMUNCIATE ABOUT HIMSELF USING THE OTHER AS MEDIUM?

I TRY WITH DESPERATE SINCERITY TO ALLOW COMPLETE FREEDOM TO THE "OTHERS" AND AVOID PROJECTING MY SELFHOOD ONTO THEIRS, BUT I SUPPOSE IT IS INEVITABLE THAT A SMALL PART OF WHAT IS REPRESENTED SHOULD REVEAL SOMETHING ABOUT MYSELF..."

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

The Madrid design studio Underbau emerged from the partnership formed in 2008 by Juanjo Justicia and Joaquín Labayen; two freelance designers with years of shared experience in the field of corporate design, publishing and advertising. From the very beginning, the studio has sought to maintain its primary focus on art and culture, working together with Spanish and international bodies such as the *Cervantes* Institute, the Andalusian regional authority's department of Culture, the National Council for Scientific Research, and the Sports Council. Underbau's total-design approach puts the emphasis on efficiency and coherency. To achieve that, the studio assumes full responsibility for the entire creative process, from the initial concept to the final product.

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