

**ANNUAL
REPORT 2017**

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“The year 2017 has been a record year in terms of CNIO publications in top journals.”

MARIA A. BLASCO
Director

FOREWORD

Maria A. Blasco Director

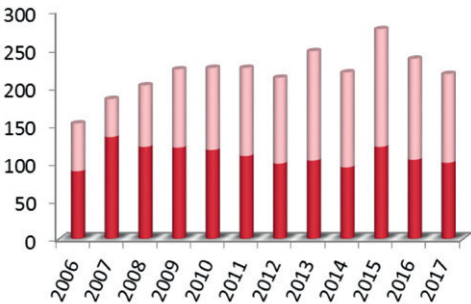
This year, once again, the data on CNIO’s scientific performance illustrate that we are doing a good job in contributing towards scientific breakthroughs. During 2017, the CNIO authored a total of 217 papers, 26 of which were published in journals with impact factors (IF) ranging from 10 to 15, and 44 papers in journals with impact factors greater than 15. Comparison with previous years (2006-2017) indicates that CNIO has continued to increase the numbers of papers it published in top journals with IF greater than 15; in fact, 2017 is the year that counts the most publications in these journals in our Centre’s history.

Our excellence in research has been bolstered by an institutional effort to establish new alliances and collaborations with important partners all over the world, both from the public and private sectors. The creation of these ties has enabled us to forge a robust research and innovation network that will help the CNIO to retain its position as a key centre of reference in cancer research within the international community. We continue to focus on the importance of attracting new talent from abroad and to safeguarding our strong, competitive position in Europe, opening up our centre to the realm of excellent science worldwide. In this regard, we are implementing new institutional initiatives that will, on one hand, provide better support to our investigators for achieving their objectives, and on the other hand, help us to coordinate all endeavours with the aim of better aligning our research agenda, efforts and strategy with the work programme and goals of the European Commission for research and innovation.

We have not forgotten our duties and responsibilities towards other centres in Spain and our own Spanish society; in this light, we have welcomed the launch of the *Severo Ochoa* and *Maria de Maetzu* Alliance, *SOMMa*, an important Spanish research network, for which we are actively contributing to its mission. With every step we take, we get a bit closer to achieving great impact through science; our institutional strategy delineates a clear path to meet this goal.

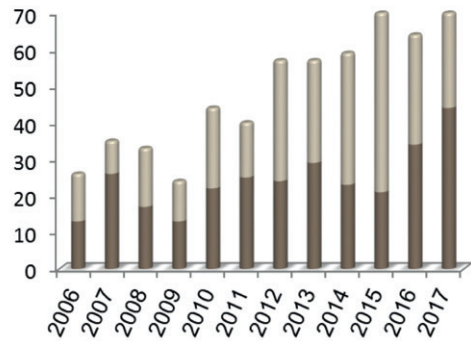
In 2017, Manuel Serrano, from the Molecular Oncology Programme, and Alfonso Valencia, from the Structural Biology and Biocomputing Programme, left the CNIO after many years

Total Number of Publications



CNIO first/last author
CNIO as co-author

Top Papers



IF > 15
IF 10-15

of excellent work and dedication to the CNIO. We want to wholeheartedly thank them for their contribution in making the CNIO a centre of excellence of international reputation and we wish them all the best in their new ventures.

I am also happy to mention that we have attracted new talent to the CNIO. During 2017, we incorporated 3 new Groups within the Structural Biology Programme, with the aim of boosting the structural biology activities at the CNIO. We hired Óscar Llorca as the new Director of CNIO's Structural Biology Programme. Óscar is an international leader in the cryo-EM field and his joining the CNIO has served to re-design this key programme for the Centre, thereby integrating this exciting technology within our existing portfolio; the Programme is now equipped with a new electron microscope that allows for atomic resolution. Under the leadership of Óscar Llorca, we have hired 2 new Junior Group Leaders for the Programme: Iván Plaza Menacho, former Senior Research associate at the Structural Biology Laboratory at the Biozentrum, University of Basel, Switzerland; and Rafael Fernández Leiro, coming from the MRC Laboratory of Molecular Biology, Cambridge, UK.

The valorisation of the research results generated by CNIO scientists, with the aim of turning them into high-potential diagnostic or therapeutic products and services, is one of our many ways of creating added value for society and boosting public benefit through improving cancer patient outcomes. At CNIO, our scientists perform research on the key biological processes underlying the disease; these include structural biology, drug development, molecular and cellular biology, animal models, human genetics, and clinical collaborations with hospitals. Importantly, the number of scientists that have joined forces to develop novel drugs relying on the Experimental Therapeutics Programme capacity has grown most notably in 2017. Each one of these activities can lead to know-how and/or goods that can be beneficial to the patients and to the health sector beyond the boundaries of academic research – It is the role of the Direction of Innovation to gear up this transition. This activity is essential to translate our discoveries and to make an impact on the patient community as well as on the health and economic system. Additionally, it contributes to the generation of income for CNIO in the form of royalties, which help the CNIO, its groups and the inventors themselves to further pursue their goals.

In 2017, a remarkable step was taken under the leadership of Manuel Serrano; the start-up of a new company based on his research in the field of senescence, which has applications in cancer treatment and other diseases such as pulmonary fibrosis, took place. A patent based on discoveries made by Nabil Djouder's group was licensed to the Spanish company *Stemtek Therapeutics* for the development of compounds to treat and prevent hepatocellular carcinoma and other tumours. CNIO has applied for 5 novel patents covering a wide range of applications such as novel targets, tissue regeneration, nano-systems to deliver cancer drugs to the tumour site, and methods to treat metastasis in the brain. We continue to strengthen our ties with industry,

and to build research collaborations to advance research results towards the generation of novel products useful to patients. In 2017, scientists at the CNIO, in collaboration with local industry leaders, presented 5 innovative projects for competitive funding under private-public partnerships.

Commercialisation and return on investment in research remain priorities. Royalty income in 2017 raised more than 550 thousand euros. This includes revenues from patent licences as well as from commercialisation of research tools such as monoclonal antibodies. A total of 52 inventors, nearly 15% of the researches at CNIO, have contributed and benefited from this achievement.

The CNIO External Scientific Advisory Board (SAB), currently chaired by Mariann Bienz, is of utmost importance for guiding the strategic plans of the CNIO as well as for the review of our research groups. In June 2017, 2 Research Programmes were evaluated by the SAB, namely the Clinical Research Programme (CRP) and the Cancer Genetics Programme (CGP). As a result of this evaluation, we also conducted a review procedure to promote Nabil Djouder from Junior Group Leader to a full CNIO Group Leader position. Congratulations Nabil!

I would like to take this opportunity to thank all those who have helped the CNIO by sponsoring our students, postdoctoral programmes and the stays of several researchers. I hereby extend my gratitude to the *Banco Santander* Foundation for funding postdoctoral stays at the CNIO and the IE Business School course; the *"la Caixa"* Foundation for fostering international PhD fellowships as well as for sponsoring the CNIO Frontiers Meetings; the *Seve Ballesteros* Foundation that supports the Seve-Ballesteros Foundation-CNIO Brain Tumour Group; and the *Jesus Serra* Foundation for supporting the Visiting Scientists Programme as well as the Dean's Office. During 2017, we hosted Raul Rabadan, Associate Professor of the Department of Biomedical Informatics at the Columbia University of New York, USA; and Wolfgang Weninger, Head of the Department of Dermatology at the Royal Prince Alfred Hospital of Sydney, Australia.

I also wish to thank the Foundation *Banc Sabadell* for sponsoring the Distinguished Seminars hosted at the CNIO and given by 'outside-the-box speakers' who provided novel perspectives that contribute to the CNIO's transdisciplinary environment. During 2017, we had the privilege to listen to: Tom Kirkwood, from the Newcastle University Institute for Ageing, UK; Vera Gorbunova, from the University of Rochester, New York, USA; José Luis Sanz from the Autonomous University of Madrid (UAM), Spain; Nuria Oliver from the Data-Pop Alliance, New York, USA; and Óscar Marin, Director of the MRC Centre for Developmental Neurobiology at King's College, London, UK.

I would also like to thank the British Embassy of Madrid for sponsoring a seminar given by Ricardo Baptista, of the Cell and Gene Therapy Catapult, UK.

Furthermore, I would like to highlight the work that is being carried out by the CNIO Women and Science (WISE) Office. During 2017, we had the pleasure of listening to Maria Ángeles Durán, ad honorem Professor of the Center for Human and Social Sciences (CSIC); Belén Yuste and Sonnia L. Rivas-Caballero, of *Rocaviva Eventos*, Madrid; Teresa Jurado and Mariano Nieto of the *Plataforma por Permisos Iguales e Intransferibles de Nacimiento y Adopción*, Madrid; Ana Botella, Politician and ex-Mayor of Madrid; Natalia Flores Sanz, ex player of the Spanish National indoor Football team and Director of the Woman and Sport programme of the *Consejo Superior de Deporte*; Katharina Miller, Founder Partner of the 3CCompliance, Madrid; Ana Requena, Journalist and Columnist at "El Diario"; and Margarita de Cos, Head of the Major Donors Relations WWF Spain and CEO of Riansoft SL, Madrid. Moreover, on the occasion of International Women's Day, a theatre play was performed at the CNIO, namely "Una Habitación Propia", based on Virginia Woolf's speech, with Maria Ruiz as stage director and Clara Sanchis as actress.

In 2017, advances and discoveries made at the CNIO were very well received and attracted broad media coverage, with over 2,300 appearances in the press (on line and on paper), and nearly 200 appearances on radio and TV, representing an increase of 63% compared to the previous year. Throughout the year, the featured stories received nearly 52,222 hits (EurekAlert! news service) from around the world. During this year, the CNIO established an agreement with the TV & Media Group *Atresmedia*, one of the leading media groups in Spain, and the AXA Foundation to disseminate scientific knowledge and to foster scientific values. Through this agreement, CNIO celebrated World Cancer Research Day with the event 'Present and Future of Cancer Research', which took place at *El Matadero* – one of the most vibrant and cultural venues in Madrid – and enjoyed the participation of Nobel Prize-winner Harald zur Hausen as guest of honour, as well as Ángela Nieto, from the Institute of Neurosciences (CSIC-UMH), Pilar Garrido, Chief Oncologist at the *Hospital Ramón y Cajal* and myself.

The year 2017 has been the year in which we reaffirmed and renewed our image. We adopted a new logo, which can be found on the CNIO website and our social media accounts. This new image is another way of transmitting the values and mission of the CNIO, the picture on our passport. Therefore, we said goodbye to the logo that has served us well since the Centre's inception, and we have welcomed a new one that is elegant, clear-cut and faithful to our origins at the Carlos III Health Institute. The renewed CNIO branding matches the leading-edge nature of our research and brings our image into the present.

The 'CNIO Friends' initiative, devoted to raising funds for cancer research at the CNIO, celebrated its first 3 years of existence at the end of 2017. At that time, the initiative had about 900 Friends who showed their unwavering commitment at all times. Thanks to them, we were able to put 2 more cancer research grants in place in 2017. In addition to the first 2 'CNIO Friends' grants and the

Juegaterapia Foundation grant, 2 more researchers were awarded with these two CNIO Friends Grants: Sebastian Thompson, from the Growth Factors, Nutrients and Cancer Group, and Carolina Maestre, from the Cell Division and Cancer Group.

Beyond other activities, the CNIO presented the book *Excelentes* that features artistic photographs and personal stories by some of the most influential people who have visited the Centre in recent years, such as the Nobel laureates Elizabeth Blackburn and Paul Nurse, the physicist Ignacio Cirac or the palaeontologist Juan Luis Arsuaga. The funds collected through this book, on sale at stores like *El Corte Inglés*, VIPS and the CNIO store, go directly to the 'CNIO Friends' initiative, which motivates us even more to keep working on the important cause of cancer research. In December, CNIO launched a campaign with the participation of 2 of Spain's best humourists, José Mota and Mago More, who offered their image and ingenuity to the service of CNIO Friends. Mota and More made their message clear: "If many of us provide a bit, we will all do a lot."

The CNIO has a rich history when it comes to *science outreach* activities. In 2017 we launched a new project that aims to centralise all of CNIO's efforts in terms of bringing science and research to the general public. CNIO & The City seeks to encourage creativity and scientific vocations by providing secondary school students with direct access to science, as well as providing new tools for teachers to consolidate this goal. During its first edition, more than 150 students and teachers took part in this initiative.

Last but not least, I would like to thank all the CNIO volunteers who make it possible for us to move our mission forward, and of course, to the entire CNIO Friends community. Combining society's efforts with the endeavours of the research community can make a significant difference for the future of cancer treatment. Finally, I would like to thank all of those who have once again collaborated on the elaboration of this Annual Report, with especial thanks to Sonia Cerdá who is responsible for this CNIO publication, as well as to our collaborators: the visual artist Amparo Garrido and the Underbau graphic design team.





OSCAR FERNÁNDEZ-CAPETILLO
Vice-Director

“During 2017, CNIO scientists have made important contributions that help us to understand why cancer occurs, how we can prevent it, and ultimately how we can treat it if prevention fails.”

The end of the year is a good time to look back and reflect on the overall progress that has been made – with a bit of a panoramic view, I can only say that our scientists have, one more year, made amazing contributions to cancer research. We now have mice that can predict where metastasis will emerge, and a better knowledge of how the protein content in the diet or cholesterol levels influence cancer development. We have discovered new therapeutic approaches for cancers with poor prognosis and found that certain cancer treatments might be bad for our hearts, thereby providing clinicians with an important alert of what to watch for in patients treated with these agents. We have understood the paradox of why certain oncogenes lead to tumour

development upon their inactivation, and found new mutations that cause neuroendocrine hereditary cancers. We have made progress in understanding the consequences of abnormal DNA replication for mammalian health, and generated new tools to model cancer mutations in the lab. These are all great indicators of the excellence of our science, and illustrate that CNIO is still a superb place to be a scientist. Last but certainly not least, I want to acknowledge that these and other important works would not have been possible without the continuous support provided by all the CNIO personnel who facilitate our daily activities. We are all CNIO. Thank you for your help.

ORGANISATION OF RESEARCH

MARIA A. BLASCO DIRECTOR

ÓSCAR FERNÁNDEZ-CAPETILLO VICE-DIRECTOR

BASIC RESEARCH

MOLECULAR ONCOLOGY PROGRAMME

Manuel Serrano Programme Director (until April)

Manuel Serrano (until April)
Tumour Suppression Group

Mariano Barbacid
Experimental Oncology Group

Maria A. Blasco
Telomeres and Telomerase Group

Marcos Malumbres
Cell Division and Cancer Group

Óscar Fernández-Capetillo
Genomic Instability Group

Ana Losada
Chromosome Dynamics Group

Juan Méndez
DNA Replication Group

María S. Soengas
Melanoma Group

Héctor Peinado
Microenvironment and Metastasis Junior Group

Manuel Valiente
Brain Metastasis Junior Group

Alejo Efeyan
Metabolism and Cell Signalling Junior Group

CANCER CELL BIOLOGY PROGRAMME

Erwin F. Wagner Programme Director

Erwin F. Wagner
Genes, Development and Disease Group

Francisco X. Real
Epithelial Carcinogenesis Group

Mirna Pérez-Moreno
Epithelial Cell Biology Junior Group

Nabil Djouder
Growth Factors, Nutrients and Cancer Junior Group

Massimo Squatrito
Seve Ballesteros Foundation-CNIO Brain Tumour Junior Group

STRUCTURAL BIOLOGY PROGRAMME

Óscal Llorca (since July) / Alfonso Valencia (until February) Programme Director

Oscar Llorca (since July)
Macromolecular Complexes in DNA Damage Response Group

Alfonso Valencia (until February)
Structural Computational Biology Group

Daniel Lietha
Cell Signalling and Adhesion Junior Group

Santiago Ramón-Maiques (until February)
Structural Bases of Genome Integrity Junior Group

Ivan Plaza-Menacho (since May)
Kinases, Protein Phosphorylation and Cancer Junior Group

Rafael Fernández Leiro (since September)
Genome Integrity and Structural Biology Junior Group

Ramón Campos-Olivas
Spectroscopy and Nuclear Magnetic Resonance Unit

Fátima Al-Shahrour
Bioinformatics Unit

Salvador J. Capella Gutierrez (until June)
National Bioinformatics Institute Unit

Jasminka Boskovic
Electron Microscopy Unit

Inés Muñoz
Crystallography and Protein Engineering Unit

Martin Krallinger
Biological Text Mining Unit

TRANSLATIONAL RESEARCH

HUMAN CANCER GENETICS PROGRAMME

Javier Benítez Programme Director

Javier Benítez
Human Genetics Group

Mercedes Robledo
Hereditary Endocrine Cancer Group

Núria Malats
Genetic and Molecular Epidemiology Group

Miguel Urioste
Familial Cancer Clinical Unit

Sandra Rodríguez
Moleculra Cytogenetics and Genome Editing Unit

Anna González-Neira
Human Genotyping-CEGEN Unit

CLINICAL RESEARCH PROGRAMME

Miguel Quintela-Fandino Acting Programme Director

Miguel Quintela-Fandino
Breast Cancer Junior Clinical Research Unit

David Olmos
Prostate Cancer Junior Clinical Research Unit

Luis J. Lombardía
Molecular Diagnostics Unit

Joaquín Martínez-López
H12O-CNIO Haematological Malignancies Clinical Research Unit

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

BIOBANK

Manuel M. Morente Director

INNOVATION

ÓSCAR FERNÁNDEZ-CAPETILLO DIRECTOR OF INNOVATION

BIOTECHNOLOGY PROGRAMME

Fernando Peláez Programme Director

Orlando Domínguez
Genomics Core Unit

Sagrario Ortega
Transgenic Mice Core Unit

Giovanna Roncador
Monoclonal Antibodies Core Unit

Francisca Mulero
Molecular Imaging Core Unit

Lola Martínez
Flow Cytometry Core Unit

Diego Megías
Confocal Microscopy Core Unit

Javier Muñoz
Proteomics Core Unit

Vacant
Histopathology Core Unit

Isabel Blanco
Animal Facility (Vivotecnia Management & Services)

EXPERIMENTAL THERAPEUTICS PROGRAMME

Joaquín Pastor Programme Director

Sonia Martínez
Medicinal Chemistry Section

Carmen Blanco
Biology Section

Susana Velasco
CNIO -Lilly Cell Signalling Therapies Section

María José Barrero
CNIO -Lilly Epigenetics Section

TECHNOLOGY TRANSFER AND VALORISATION OFFICE

Anabel Sanz Director

Basic Research

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Structural Computational Biology Group	98
National Bioinformatics Institute Unit	99

MOLECULAR ONCOLOGY PROGRAMME

MANUEL SERRANO (until April) Programme Director



The time has come for me to say “good bye”. I will not be far away and I will often be around, so rather than saying an actual farewell, what I really want to put into words is a big “THANK YOU” to the CNIO in general and, in particular, to my colleagues in the Molecular Oncology Programme.

I have been with the CNIO since 2003, and looking back at all my time in the Centre, I am amazed by how much I have managed to accomplish scientifically at the CNIO. The CNIO put to my service the best scientific facilities that I could ever have imagined. I am deeply grateful to the superb scientists of the Biotechnology Programme. The Centre has also given me high-level visibility through the constant organisation of meetings, conferences and seminars. But most importantly, I have been surrounded by the best possible colleagues, creative, ambitious and always helpful. The scientists at the CNIO have been my main source of

inspiration for new ideas and bestowed me with the necessary confidence for exploring new fields of research.

It has been a great satisfaction for me to have served as the Director of the Molecular Oncology Programme since 2012. Despite the complicated times in terms of funding and hiring, with everyone’s help, we have been able to grow with the addition of three new Junior Groups that have brought new topics, new ideas and enthusiasm. Thank you for having joined us!

I will continue to bother you all to ask for your help, advice or reagents; thanks in advance! Needless to say, count on me whenever I can be of help to any of you, I’ll do my best!

Let’s keep in touch!

The Molecular Oncology Programme is the largest basic research programme at the CNIO, encompassing a total of 8 Research Groups and 3 Junior Research Groups. The Programme focuses on the study of some of the most active areas of research in cellular oncology, including DNA and chromosome stability (Maria A. Blasco, Óscar Fernández-Capetillo, and Ana Losada), oncogenes and cell cycle kinases (Mariano Barbacid), DNA replication (Juan Méndez), mitosis (Marcos Malumbres), tumour suppressors (Manuel Serrano), molecular mechanisms in melanoma (María S. Soengas), metabolism and cell signalling (Alejo Efeyan), and metastasis (Manuel Valiente and Héctor Peinado).

This year, the Molecular Oncology Programme has continued to be on the frontline of oncology research. The top-level quality of the research conducted by each of these Groups is detailed in the following pages. In 2017, the Programme has authored a total of 11 papers in *Nature* journals (*Nature* 5×, *Nature Medicine*, *Nature Reviews Cancer*, *Nature Cell Biology* and *Nature Communications* 3×) and 7 papers in *Cell* Journals (*Cancer Cell*, *Cell*, *Cell Stem Cell* 2x, *Cell Metabolism* 2x and *Trends in Cell Biology*); this trend continues with *New England Journal of Medicine*, *Circulation Research*, *JACS*, *The Journal of Clinical Investigation* and *PNAS*. 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.

Over the year to come we hope to continue to make many exciting discoveries, further increase the quality of scientific production and strive to impact on cancer research and cancer therapeutics.

We would like take this opportunity to thank our former Programme Director, Manuel Serrano, who has served as the Director of the Molecular Oncology Programme since 2012. Manuel Serrano and his group left the CNIO in April 2017 to join the Institute for Research in Biomedicine (*IRB Barcelona*). We are thankful to him for his important contributions as well as for his unconditional support in bringing new ideas and challenges to the Programme. Thank you Manuel for having been a part of our CNIO community and we wish you the best in your new position!

A new Director for the Molecular Oncology Programme will be appointed soon. We are confident that, under a new leadership, the Programme will continue to make significant discoveries and generate knowledge that can be translated into better care for cancer patients.

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

TUMOUR SUPPRESSION GROUP

Manuel Serrano
Group Leader

Associate Investigator (since May, from the *IRB Barcelona*)

Staff Scientists
Susana Llanos,
Bárbara Martínez (until April),
Cristina Pantoja (until April)



Post-Doctoral Fellows
Timothy Cash (until July), Cian J. Lynch (until April), Gianluca Varetto (until April), Maté Maus (until April)

Graduate Students
Noelia Alcázar, Raquel Bernad (until April), Selim Chaib (until April), Dafni Chondronasiou (until July), Lluç Mosteiro (until June), Miguel Rovira (until July)

Technicians
Maribel Muñoz (until April) (TS)*, Yolanda Durán (since May)

**Titulado Superior (Advanced Degree)*

OVERVIEW

Our Group upholds the unifying concept that tumour suppressor genes protect against many types of damage, no matter what the potential detrimental consequences of the damage might be. Tumour suppressors, therefore, confer protection from damage even if that damage is not going to lead to cancer, but to a degenerative disease instead. Cancer protection would thus be just one of the outcomes of tumour suppressors, with other outcomes being protection from degenerative diseases, from nutritional overload, from tissue injuries, or from ageing. We aim to achieve an integrated understanding of the protection provided by tumour suppressors.

Our goals are to:

- Understand tumour suppression mechanisms and identify new tumour suppressor regulators.
- Study the interplay between tumour suppression and ageing.
- Characterise cellular senescence as a tumour suppression mechanism.
- Investigate cellular pluripotency and the involvement of tumour suppressors in the regulation of reprogramming to pluripotent stem cells.
- Explore the role(s) of cell plasticity in cancer, tissue regeneration, and ageing.
- Search for new frontiers in cell plasticity.

“We have developed a strategy for the targeted delivery of drugs that is therapeutically efficient against tumours treated with senescence-inducing chemotherapies.”

RESEARCH HIGHLIGHTS

A novel RNA Polymerase II regulator critical for cell identity

The mechanisms underlying cell plasticity are poorly understood. The activation or silencing of enhancers is thought to be at the core of cell identity and, therefore, changes in enhancers underlie cell plasticity. The Mediator complex is critical for the function of enhancers, serving to communicate the enhancers with the RNA polymerase II at promoters. We have characterised a novel regulator of RNA Polymerase II called RPAP1 (RNA Polymerase II Associated Protein 1), which is critical for the interaction between Pol II and Mediator, as well as for the recruitment of the key factor Gdown1 (FIGURE 1). RPAP1 is essential for the expression of cell identity genes and for viability. Depletion of RPAP1 erases cell identity gene expression and triggers cell de-differentiation, facilitates reprogramming towards pluripotency, and impairs differentiation. We have concluded that Mediator-RPAP1-Pol II is an ancient module, conserved from plants to mammals, which is critical for establishing and maintaining cell identity.

A new therapeutic strategy for drug delivery into senescent cells

Severe or unrepairable cellular damage often triggers a stereotypical cellular response known as senescence. Senescent cells accumulate in many ageing-associated diseases, contributing actively to their pathological manifestations. Genetic ablation of senescent cells delays and ameliorates some ageing-associated diseases, reverts long-term degenerative processes associated to chemotherapy, and extends longevity. The pharmaceutical targeting of senescent cells is emerging as a promising therapeutic approach. We have exploited the characteristic high levels of lysosomal β -galactosidase activity that exist in senescent cells as a vulnerable trait in order to design a drug delivery system that targets senescent cells. This system is based on the encapsulation of drugs with galacto-oligosaccharides (FIGURE 2). We have shown that gal-encapsulated drugs are preferentially released within senescent cells in mice, and that this targeted delivery strategy is therapeutically efficient *in vivo*. In a model of cancer chemotherapy, gal-encapsulated cytotoxic drugs target tumour cells undergoing chemotherapy-induced senescence and contribute to tumour xenograft regression. In the context of pulmonary fibrosis in mice, gal-encapsulated cytotoxics target senescent cells, reducing collagen deposition and ameliorating pulmonary function. Importantly, gal-encapsulation reduces the systemic toxicity of the cytotoxic drugs. Drug delivery into senescent cells opens up new diagnostic and therapeutic applications in senescence-associated disorders.

p16^{INK4a} and IL6 play a critical role in the interplay between senescence and reprogramming

Cellular senescence is a damage response aimed to orchestrate tissue repair. We have recently reported that cellular senescence, through the production of interleukin-6 and other soluble factors, strongly favours cellular reprogramming by Oct4, Sox2, Klf4 and cMyc (OSKM). The expression of an OSKM transgene results in senescence and reprogramming in different cell populations, both processes occurring in close proximity. In this system, upon OSKM activation, *Ink4/Arf*-null tissues cannot undergo senescence, fail to produce IL6 and cannot reprogram efficiently; whereas the absence of *p53* results in extensive tissue damage, high levels of senescence and IL6, and efficient reprogramming. We have explored some genetic determinants of *in vivo* reprogramming. We have found that *Ink4a*, but not *Arf*, is critical for OSKM-induced senescence and, thereby, for the paracrine stimulation of reprogramming. Interestingly, *p53* deficiency renders IL6 production and reprogramming independent of *Ink4a*, as implied by the high levels of IL6 and reprogramming in triply deficient *p53/Ink4a/Arf* mice. We have also observed that *Il6*-deficient mice present profoundly reduced levels of reprogramming, therefore, providing genetic proof for the critical role of IL6 in reprogramming. Finally, young female mice present lower efficiency of *in vivo* reprogramming compared to male mice, and this gender difference disappears with ageing, which is consistent with the anti-inflammatory effect of oestrogens. These findings regarding the interplay between senescence and reprogramming may conceivably apply to other contexts of tissue damage. ■

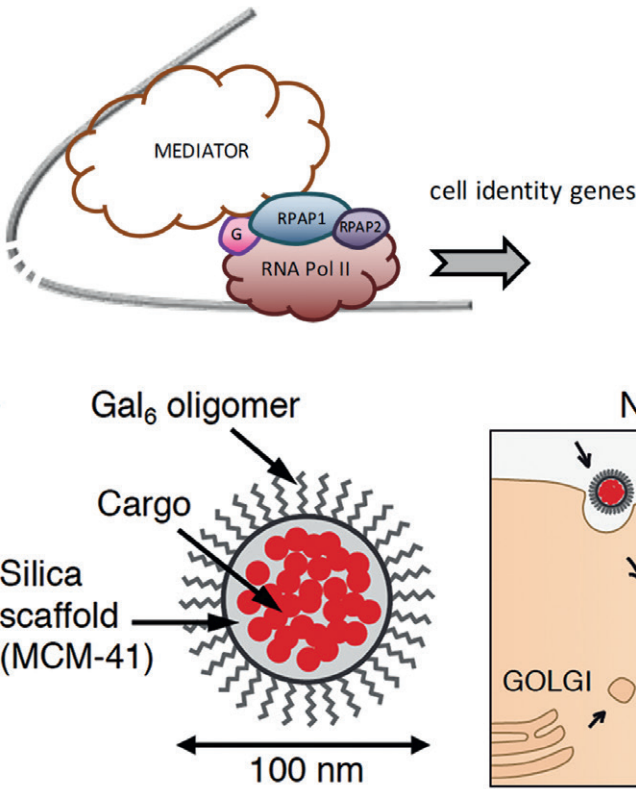


Figure 1 Model for RPAP1 function. RPAP1 exists in complex with RNA Pol II, playing an essential role in the Mediator-Pol II regulatory axis. Loss of RPAP1 impairs the association

between Mediator and Pol II affecting the ability of enhancers to activate Mediator target genes, thereby, disrupting cell identity.

Figure 2 Senescent cells targeted drug delivery system. GalNP beads consist of a mesoporous silica scaffold loaded with different cargoes encapsulated by a coat of galacto-oligosaccharides. Cellular uptake of the GalNP beads occurs via endocytosis and after fusion with lysosomal vesicles the beads are secreted by exocytosis. The release of the cargo requires β -galactosidase-mediated hydrolysis of the cap and this occurs preferentially in senescent cells due to their high levels of lysosomes and β -galactosidase activity.

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OVERVIEW

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

“We have demonstrated that the **Capicua** transcription factor is a tumour suppressor capable of inducing thymomas. We have also demonstrated that kinase dead **B-Raf** mutants are oncogenic. Tumours harbouring these **BRAF** mutants are sensitive to the inhibitors of receptor tyrosine kinases, hence offering novel treatment options for these cancer patients.”

RESEARCH HIGHLIGHTS

A B-Raf kinase inactive mutant is a driver oncogene in lung adenocarcinoma

The initiating oncogenic event in almost half of human lung adenocarcinomas is still unknown; a fact that complicates the development of selective targeted therapies. We have demonstrated that expression of an endogenous B-Raf^{D631A} kinase inactive isoform, present in a significant percentage of human lung tumours, triggers lung adenocarcinoma *in vivo*, indicating that BRAF inactivating mutations represent novel oncogenic drivers. Moreover, these BRAF mutations have also been identified in a subset of K-RAS driven human lung tumours. Co-expression of K-Ras^{G12V} and B-Raf^{D631A} mutations dramatically enhances tumour initiation, a phenomenon mediated by c-Raf kinase activity and that effectively accelerates tumour progression in advanced lung adenocarcinomas. Concomitant ablation of the wild type *B-Raf* allele prevents lung adenocarcinoma by inducing oncogenic toxicity. Loss of B-Raf expression in this scenario also induces trans-differentiation of Clara cells, leading to the development of intrabronchiolar lesions that result in the rapid death of the mice. We have also shown that this class of BRAF mutants are sensitive to ERK-mediated feedback and that their activation of signalling is dependent on RAS activation. The dependence of these mutants on RAS suggests that their activation of ERK signalling requires incomplete feedback inhibition of RAS activation. Indeed, these are sensitive to inhibition of RAS activation, which can be achieved by using available inhibitors of tyrosine protein kinase receptors.

Inactivation of Capicua in adult mice causes T cell lymphoblastic lymphoma

Capicua (CIC) is a transcriptional repressor negatively regulated by RAS/MAPK signalling. CIC is inactivated in a

variety of human tumours and has recently been implicated in lung metastasis. Here, we describe a mouse model in which we inactivate Cic by blocking its transcriptional repressor activity. Cic function is essential for embryonic development but not for adult homeostasis. Systemic inactivation of Cic in adult mice results in T-cell acute lymphoblastic lymphoma (T-ALL) with high penetrance. Although Cic inactivation induces a large transcriptional program, T-ALL is mediated by the selective expression of the Etv4 transcription factor. We also show that a genetic signature indicative of Cic inactivation is enriched in Ras-induced T-ALL. Indeed, Cic mutations render T-ALL insensitive to MEK inhibitors. More importantly, human T-ALL also displays this signature, indicating that CIC inactivation plays a key role in this human malignancy and may confer resistance to inhibitors of the MAPK pathway.

In vivo oncogenic conflict triggered by co-existing KRAS and EGFR activating mutations in lung adenocarcinoma

Activating mutations in *KRAS* and *EGFR*, the two most frequent oncogenic drivers in human lung adenocarcinoma, occur in a mutually exclusive manner suggesting functional redundancy and implying lack of positive selection. By means of a mouse model engineered to induce expression of mutant *EGFR*^{L858R} in advanced tumours driven by a resident *Kras*^{G12V} oncogene we show that, instead, their co-expression is detrimental for the progression of lung adenocarcinoma. *In vivo*, expression of *EGFR*^{L858R} in *Kras*^{G12V}-driven tumours triggers an immediate response with hallmarks of replicative stress resulting in apoptosis. Yet, a fraction of tumour cells survive but enter a transient cytostatic state incompatible with tumour development that is fully reversible upon discontinuation of *EGFR*^{L858R} expression. Ultimately, continuous co-expression of both mutants results in the attenuation of the overall oncogenic signalling to levels

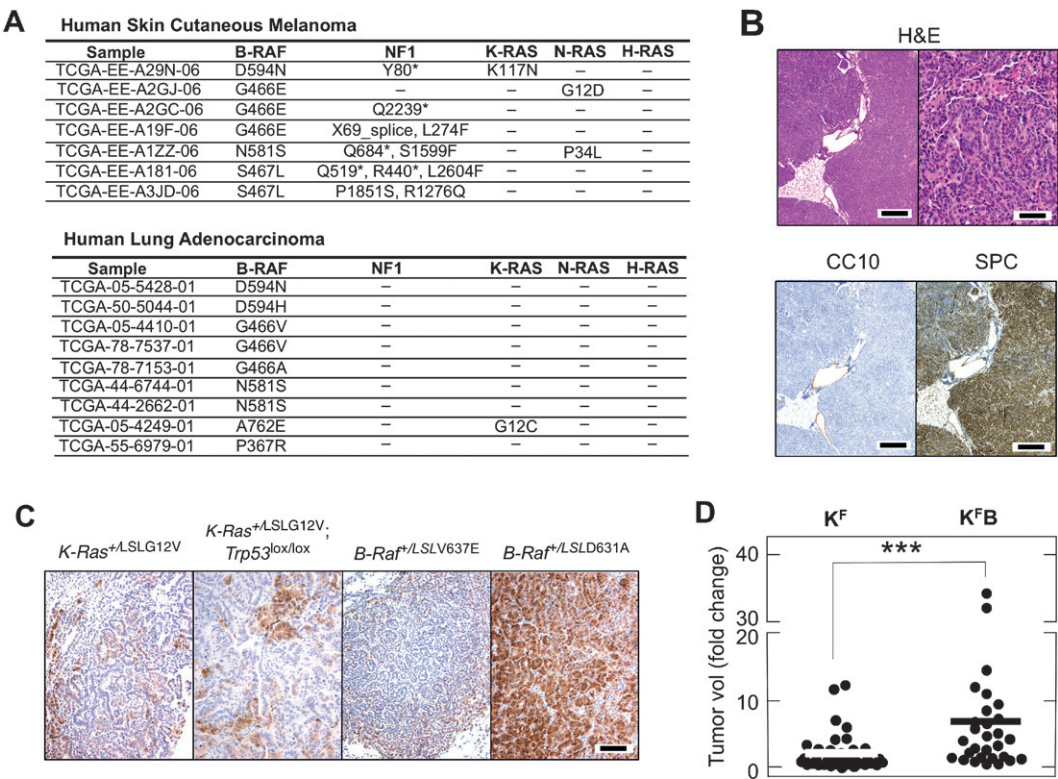


Figure *B-Raf*^{D631A} is a driver oncogene in lung adenocarcinoma. **(A)** Co-occurrence of *B-Raf* hypoaactive mutants with *NF1* and *RAS* mutations in human skin cutaneous melanoma and lung adenocarcinoma patients. Sample ID as well as the precise mutation/s identified are indicated. **(B)** Representative images of H&E and SPC and CC10 immunostaining of paraffin embedded lung sections from Ad-Cre-infected *B-Raf*^{D631A} mice sacrificed at humane end-point. Scale bar 500 mM and 50 mM (top right panel). **(C)** Representative images of pErk1/2 immunostaining of paraffin embedded lung sections from Ad-Cre-infected *K-Ras*^{+/LSLG12Vglo}, *K-Ras*^{+/LSLG12Vglo}, *Trp53*^{lox/lox}, *B-Raf*^{+/LSLV637E} or *B-Raf*^{+/LSLD631A} mice. Scale bar 100 mM. **(D)** Mice carrying a *K-Ras*^{+/FSFG12V} allele were infected intratracheally with Ad-Flp and tumour formation was monitored by CT. Mice bearing CT+ tumours were fed *ad libitum* a tamoxifen-containing diet to activate expression of the B-Raf^{D468A} kinase dead isoform. Tumour volume increase (measured as fold change) was calculated after 8 weeks in continuous diet in *K-Ras*^{+/FSFG12V}; *B-Raf*^{+/LSLD631A}; *Tg.hUbCre-ERT2*^{+/T}; (K^FB, n=29) and control *K-Ras*^{+/FSFG12V}; *Tg.hUbCre-ERT2*^{+/T} (K^F, n=38) cohorts.

compatible with cell proliferation and tumour growth. In sum, our results indicate that the mutual exclusivity of *KRAS* and *EGFR* activating mutations occurs as a combination of cellular

toxicity and signal adjustment that results in the lack of selective advantage for those cells expressing both oncogenes. ■

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AWARDS AND RECOGNITION

Burkitt Medal, Trinity College Dublin, Ireland.

Lección Conmemorativa Teófilo Hernando, Madrid, Spain.

Chair, Gordon Research Conference on “Cell Growth & Proliferation”, Mount Snow, VT, USA.

Keynote Speaker, Gulbenkian & Brabham Institutes Joint Retreat, Vimeiro, Portugal.

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OVERVIEW

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

Our research focuses on:

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

“We have designed an effective therapeutic strategy for the treatment of glioblastoma multiforme based on targeting telomeres by small molecule inhibition of telomeric protein TRF1.”

RESEARCH HIGHLIGHTS

The PI3K/AKT pathway modulates telomere protection

We have described a role for PI3K/AKT in the regulation of TRF1, an essential component of the shelterin complex. Small molecule inhibitors of PI3K and AKT significantly reduce TRF1 telomeric foci and lead to increased telomeric DNA damage and fragility. We identified PI3K α as the PI3K isoform responsible for this TRF1 inhibition. We found TRF1 is phosphorylated at different residues by AKT and that these modifications regulate TRF1 protein stability and TRF1 *in vitro* binding to telomeric DNA (FIGURE 1), and that they are important for *in vivo* TRF1 telomere location and cell viability. Patient-derived xenograft (PDX) breast cancer mouse models that responded effectively to a PI3K α -specific inhibitor showed decreased TRF1 levels and increased telomeric DNA damage. Our discovery that the telomeric protein TRF1 is regulated by the PI3K signalling pathway not only functionally connects two of the hallmarks of cancer and ageing, but also pinpoints PI3K and AKT as novel targets for telomere-based therapies in cancer and age-related diseases.

Targeting telomeres as a therapeutic strategy for Glioblastoma Multiforme (GBM)

Glioblastoma multiforme is a deadly and common brain tumour. Its poor prognosis is linked to high proliferation and cell heterogeneity, including glioma stem cells (GSCs). Disruption of telomere maintenance is among the most frequent alterations found in human glioblastoma, but no previous studies had validated telomeres as a good target to arrest GBM growth. TRF1 is a shelterin protein essential for both telomere protection and adult and pluripotent stem cells. We demonstrated that disrupting telomere capping through direct inhibition of TRF1 is a promising strategy for the treatment of GBM. We showed that inhibition of TRF1 blocks GBM in both mouse and human models (FIGURE 2). We also established the striking effectiveness of TRF1 inhibition in impairing the growth of glioma stem cells. Our results have a potential impact in cancer treatment as current GBM therapies are unable to kill these cells and patients die because of the strong recurrence of the tumours.

TRF1-based gene therapy rescues reduced TRF1 levels with ageing and prolongs mouse health span

TRF1 deficiency in the context of different mouse tissues leads to loss of tissue homeostasis due to impaired stem cell function. We have now shown that TRF1 levels decrease during organismal ageing, both in mice and in humans. We also showed that increasing TRF1 expression in both adult and old mice using

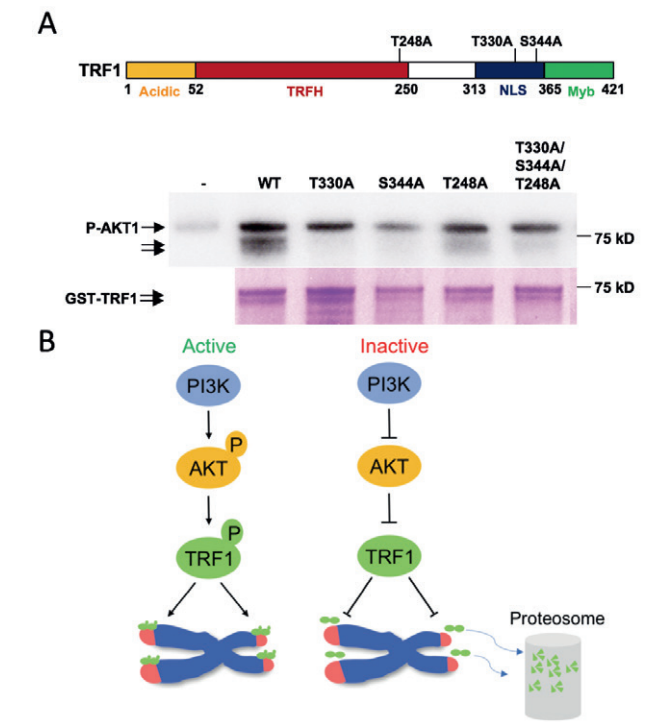


Figure 1 Phosphorylation of TRF1 by AKT stabilises TRF1 telomeric foci. (A) AKT phosphorylates TRF1 at T248, T330 and S344 residues. (B) Model: PI3K activation leads to AKT-dependent phosphorylation of TRF1 regulating its turn-over and its binding to telomeres. Inhibition of the PI3K pathway results in TRF1 release from telomeres and degradation.

gene therapy can delay age-associated pathologies. We used the non-integrative adeno-associated serotype 9 vector (AAV9), which allows for moderate and transient TRF1 overexpression. AAV9-TRF1 gene therapy prevented age-related decline in neuromuscular function, glucose tolerance, cognitive function, maintenance of subcutaneous fat, and chronic anaemia. We also found a lower abundance of short telomeres and of telomere-associated DNA damage in some tissues. Rescuing naturally decreased TRF1 levels during mouse ageing results in an improved mouse health span.

Common telomere changes during *in vivo* reprogramming and early stages of tumourigenesis

We studied whether tissue dedifferentiation induced by *in vivo* reprogramming involves changes at telomeres. In the reprogrammed areas, we found telomerase-dependent telomere elongation and telomere length-independent highly upregulated expression of TRF1. TRF1 inhibition reduced *in*

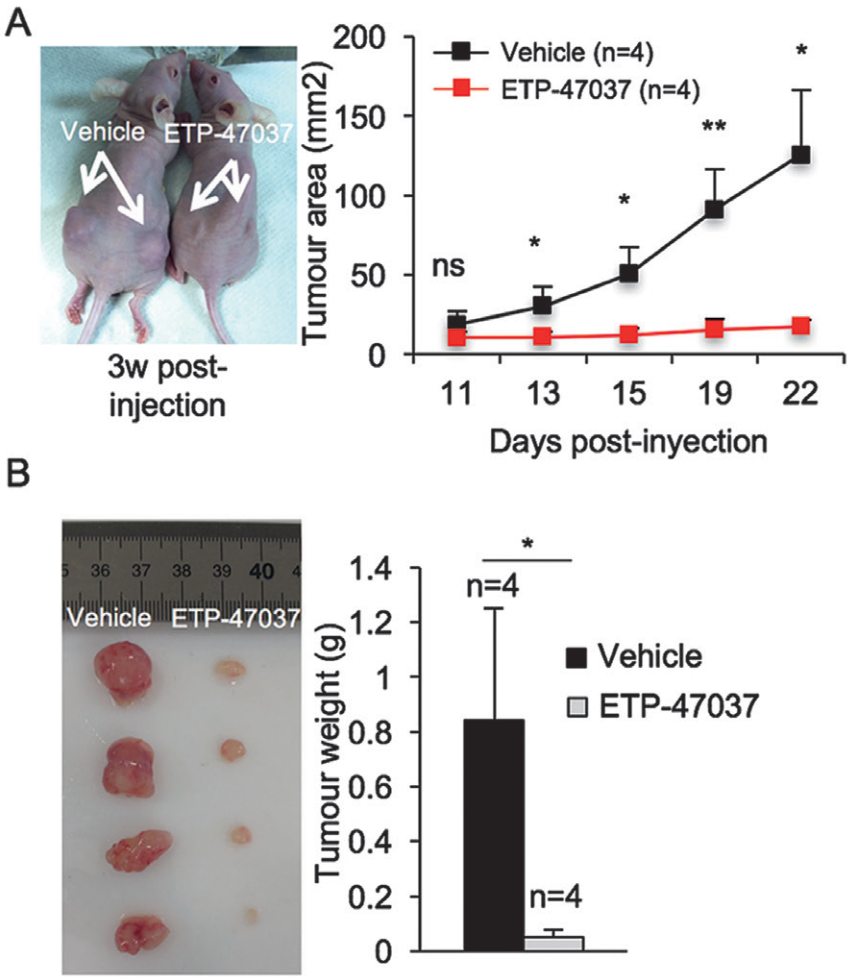


Figure 2 TRF1 chemical inhibitors reduce xenograft tumour growth. (A) Longitudinal tumour growth follow-up in treated/untreated xenograft models (right), representative tumours (left). (B) Tumour weight (right), representative tumours post-mortem.

vivo reprogramming efficiency. We extended the finding of TRF1 upregulation to pathological tissue dedifferentiation associated with neoplasias, specifically, to pancreatic acinar-to-ductal metaplasia, a K-Ras driven process that involves

transdifferentiation of adult acinar cells into ductal-like cells. Our findings place telomeres as important players in cellular plasticity both during *in vivo* reprogramming and in pathological conditions associated with increased plasticity, such as cancer. ■

PUBLICATIONS

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AWARDS AND RECOGNITION

- Scientific Merit Award, *Generalitat Valenciana*, Spain.
- XIII Health Science Award, *Fundación Caja Rural de Granada*, Spain.
- XXXVI Lección Memorial Fernández-Cruz Award, Fernández-Cruz Foundation, Spain.
- Doctorate Honoris Causa, *Universidad de Alicante*, Alicante, Spain.

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Salvador (since June), Belén Sanz
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Elisabet Zapatero (TS)**

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**Titulado Superior (Advanced Degree)

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OVERVIEW

The Cell Division and Cancer Group is interested in deciphering the mechanisms by which cell division and cell proliferation are regulated in mammalian cells. During the last few years, we have used different mouse models to understand the relevance of cell cycle regulators, including cell cycle kinases and phosphatases, as well as of proteins involved in ubiquitin-dependent degradation, in the control of cell division and tissue physiology. Our interests are: i) to understand the basic control mechanisms that regulate the cell division cycle; ii) to characterise the physiological and therapeutic consequences of cell cycle deregulation; iii) understanding the function of microRNAs in stem cell biology and tumour development, and iv) to understand how progenitor cells and cancer stem cells control their self-renewal and proliferative properties. As a final goal, we aim to generate information that may be useful for improving therapeutic strategies against cancer cell proliferation.

“During 2017, we uncovered a new function for the cell cycle kinase Plk1 in vascular postmitotic cells, suggesting unexpected side effects of Plk1 in the treatment of human tumours.”

RESEARCH HIGHLIGHTS

Recent research in our laboratory has focused on several aspects of the control of cancer cell proliferation by the cell cycle machinery. We are currently studying the relevance of critical cell cycle kinases such as cyclin-dependent kinases, Plk1 or the PP2A-inhibitory kinase Mastl in cancer cells from different origins. Although mostly overlooked in the past, we are also studying major phosphatases such as PP2A or Cdc14 using genetic models. In addition, we have generated mouse models with gain-of- or loss-of-mutations in miR-203, a microRNA with relevant properties in the control of self-renewal and differentiation properties of stem cells (Patent EP 17382304.8).

The cell cycle kinase Plk1 controls vascular homeostasis

Among cell cycle enzymes, Polo-like kinase 1 (Plk1) is an essential kinase with multiple roles in centrosome maturation and separation, DNA replication, chromosome segregation, and cytokinesis. In human cancer, Plk1 is upregulated and its expression frequently correlates with poor prognosis in a variety of tumour types such as breast or lung cancer, among others. Pioneering work in model organisms such as flies or mammalian cells in culture demonstrated that inhibition of this kinase resulted in prometaphase arrest due to monopolar spindles or

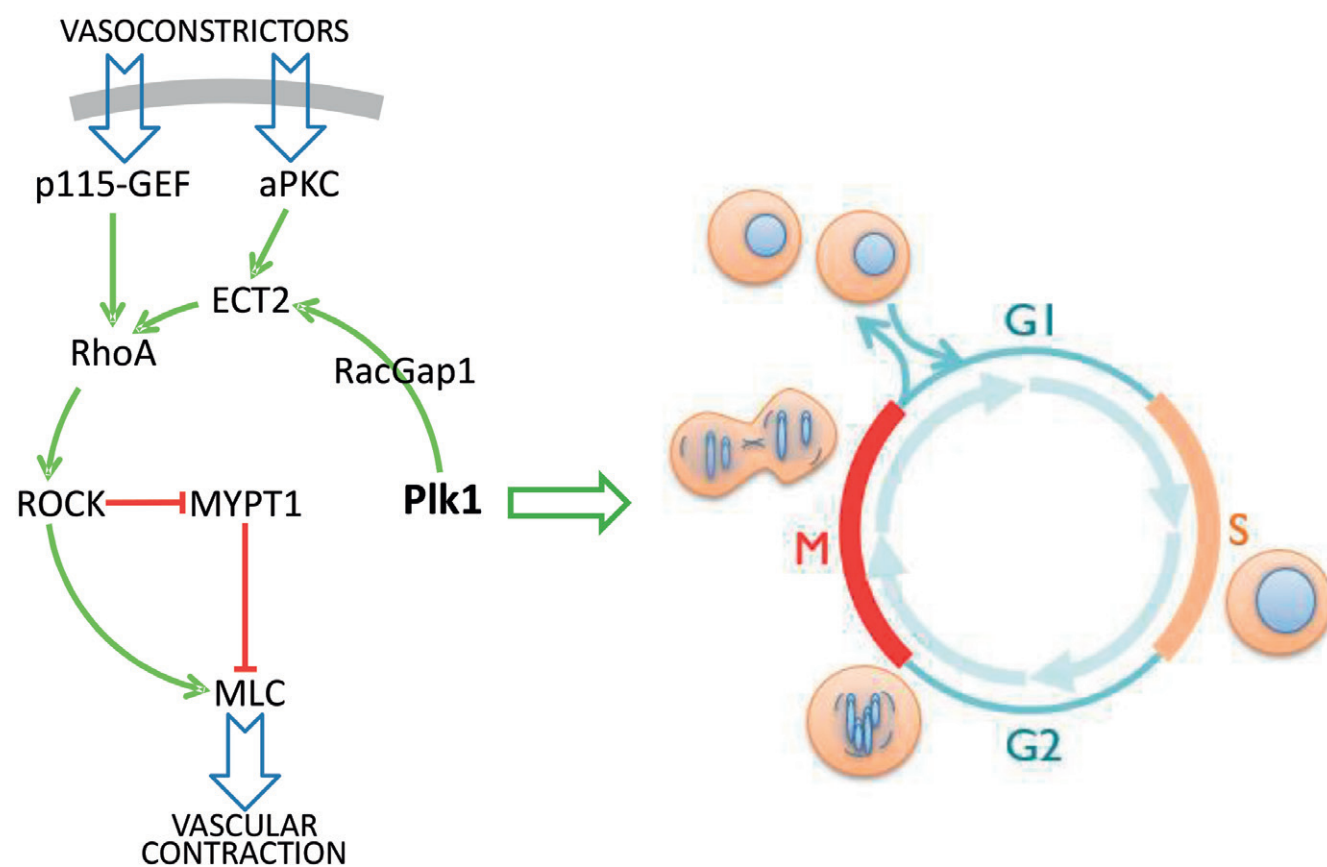


Figure Schematic representation of the involvement of Plk1 in the response to vasoconstrictors and in the cell division cycle. Plk1 participates in the recruitment of the RacGap1-Ect2 complex, thereby leading to the activation of RhoA. This process is mediated by atypical PKCs. Activation

of RhoA leads to changes in the actomyosin cytoskeleton required for contraction. In parallel, Plk1 regulates several processes required for mitosis (M) such as centrosome maturation, chromosome segregation and cytokinesis, which also involves RhoA-mediated actomyosin contraction.

misaligned chromosomes, as well as in specific defects during cytokinesis. Not surprisingly, a number of Plk1 small-molecule inhibitors are currently under scrutiny in clinical trials for cancer therapy. One of these inhibitors, volasertib, recently received the Breakthrough Therapy designation by the FDA owing to its therapeutic effect in acute myeloid leukaemia.

We have recently analysed the relevance of this kinase using genetically engineered mouse models. Our results uncovered a new function for this protein in the maintenance of the proper structure of the arteries. Plk1 regulates the contractile response of smooth muscle cells to changes in blood pressure. Mechanistically, this kinase regulates the activation of the RhoA GTPase, a central node in the actomyosin changes required for cell contractility (FIGURE). This function is actually related to the known role of Plk1 in controlling actomyosin dynamics during the later steps of mitosis and cytokinesis. In fact, the control of myosin contractility by Polo-like kinases is conserved through evolution from yeast to humans. Similarly to its genetic inactivation, the use of Plk1 inhibitors resulted in defects in vascular structure and function in mice. These data do not preclude the use of Plk1 inhibitors in the clinic but suggest that understanding their toxicity effects will be critical for the proper design of therapeutic strategies against cancer. In addition, as RhoA is a critical mediator of major oncogenic and metastatic pathways, the use of low doses of Plk1 inhibitors could be considered as a potential therapeutic strategy to limit the activation of these pathways in cancer cells.

Mastl-PP2A in the cell cycle and cancer

During the last few years, the cell cycle kinase Mastl (also known as Greatwall) has emerged as a key player in the regulation of the PP2A phosphatase during mitosis. Mastl phosphorylates two small proteins, endosulfine (ENSA) and ARPP19, which in their phosphorylated form bind and inhibit PP2A-B55 complexes. In vertebrates, PP2A-B55 complexes counteract the phosphorylation of CDK substrates, and Mastl-dependent inhibition of PP2A-B55 prevents the activity of this phosphatase during mitosis, thus contributing to the phosphorylation of mitotic phospho-proteins.

PUBLICATIONS

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- de Cárcer G, Wachowicz P, Martínez-Martínez S, Oller J, Méndez-Barbero N, Escobar B, González-Loyola A, Takaki T, El Bakkali A, Cámara JA, Jiménez-Borreguero LJ, Bustelo X, Cañamero M, Mulero F, Sevilla MdA, Montero MJ, Redondo JM, Malumbres M (2017). Plk1 regulates contraction of postmitotic smooth muscle cells and is required for vascular homeo-

stasis. *Nat Med* 23, 964-974.

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- Ha K, Ma C, Lin H, Tang L, Lian Z, Zhao F, Li J-M, Zhen B, Pei H, Han S, Malumbres M, Jin J, Chen H, Zhao Y, Zhu Q, Zhang P (2017). The anaphase promoting complex impacts repair choice by protecting ubiquitin signaling at DNA damage sites. *Nat Commun* 8, 15751.
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(2017). Programmed mitophagy is essential for the glycolytic switch during cell differentiation. *EMBO J* 36, 1688-1706.

PATENT

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AWARDS AND RECOGNITION

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OVERVIEW

The Genomic Instability laboratory centres its research on understanding how cells respond to a specific type of DNA damage known as replication stress (RS), which is the main source of genomic rearrangements in cancer cells. In mammals, RS is sensed and suppressed by a signalling cascade initiated by ATR and CHK1 kinases. Throughout the years, our laboratory has developed a wide battery of cellular and animal tools for the study of RS. These tools include mice with enhanced or limited function of ATR/CHK1 kinases, cell lines in which the RS-response pathway can be activated at will, and chemical inhibitors of ATR. Our studies have enhanced our understanding of the impact of RS on cancer and ageing, and have led to novel drugs with antitumour potential that can exploit the presence of RS in cancer cells. Altogether, our main aim is to understand how genome maintenance is safeguarded – particularly during replication – and to exploit this knowledge as a way to fight against cancer.

“In 2017, we have invested a significant part of our activities in trying to improve the existing technologies for forward genetic screenings in mammalian cells, and have used them (among others) to identify new determinants of the sensitivity to ATR inhibitors.”

RESEARCH HIGHLIGHTS

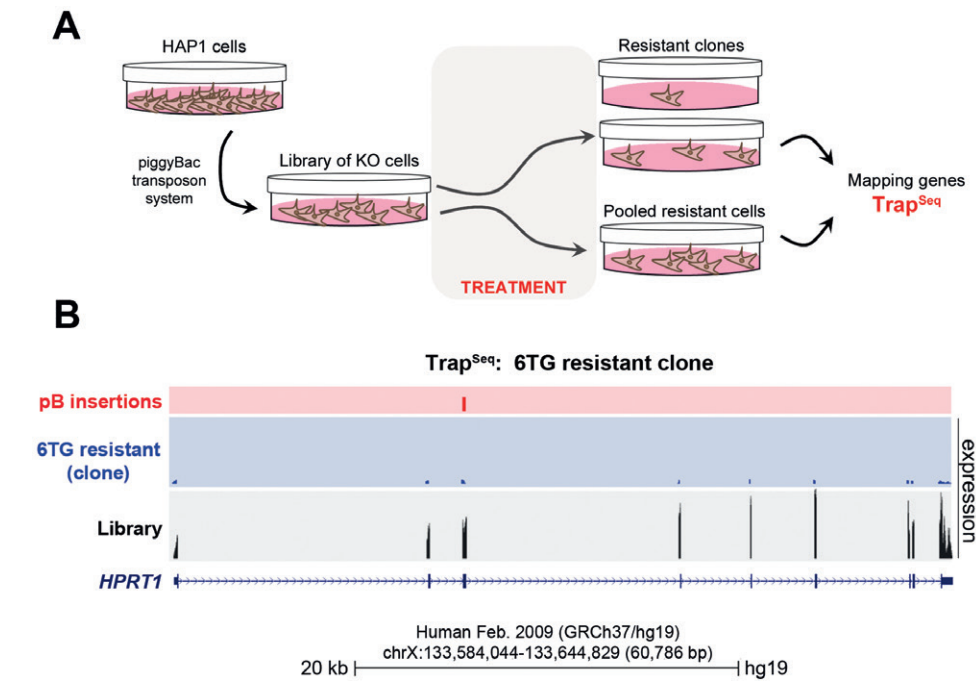


Figure 1 Pipeline for genetrap-based genetic screenings with TrapSeq. (A) Scheme followed for the mutagenesis and genetic screenings on the human haploid cell line HAP1. (B) Example of the output of TrapSeq in an individual HAP1 clone that was

selected for resistance against 6TG. The first track (red) indicates the insertion site, and the other ones the expression of the trapped gene (*HPRT*), which is downregulated in the mutant clone.

Forward genetic screening (FGS) represents one of the most powerful methods for the discovery of new pathways and/or mechanisms of disease. Haploid organisms, such as yeast, have long been the ideal platform for FGS, since mutations in one allele can suffice to reveal a phenotype. The relevance of this approach is exemplified by the high number of Nobel Prizes that have been awarded in the recent decades to investigations that started with yeast studies. While many biological questions are intrinsic to mammals and cannot be approached through yeast, the presence of a diploid genome (with two copies per gene) has significantly limited FGS approaches in mammalian cells. The recent development of CRISPR-Cas9 gene editing technologies, together with the isolation of mammalian haploid cell lines, has recently changed this landscape. During 2017, our laboratory contributed to this field by finding ways to stabilise the haploid state in mammalian cells, as well as by developing a new method for the identification of genetrap insertions by RNA sequencing that can be coupled to FGS approaches.

TrapSeq: A new method for genetrap-based genetic screenings in mammals

Genetraps are one of the most widely used methods to conduct genetic screenings in mammals. In these studies, identifying the genetrap insertion site was an essential step, which was accomplished via inverse PCR-based methods that (a) are prone to biases and artefacts, and (b) while they are able to identify the insertion site, they do not provide information as to how the insertion affects the expression of the targeted gene. To overcome these limitations, we have now developed an RNA sequencing-based method (TrapSeq) that provides a fast, direct and cost-effective pipeline for the identification of a gene-trap insertion mutation, and which also reveals the impact of the genetrap on the expression of the mutated gene (FIGURE 1). We have now used TrapSeq to conduct several genetic screenings in haploid mammalian cells, including the discovery of mutations that increase the resistance to ATR inhibitors. This screening confirmed the key role of the mitosis-promoting phosphatase CDC25A in the response to ATR inhibition that we had previously identified in CRISPR-based screenings (Ruiz *et al.*, *Mol Cell* 2016),

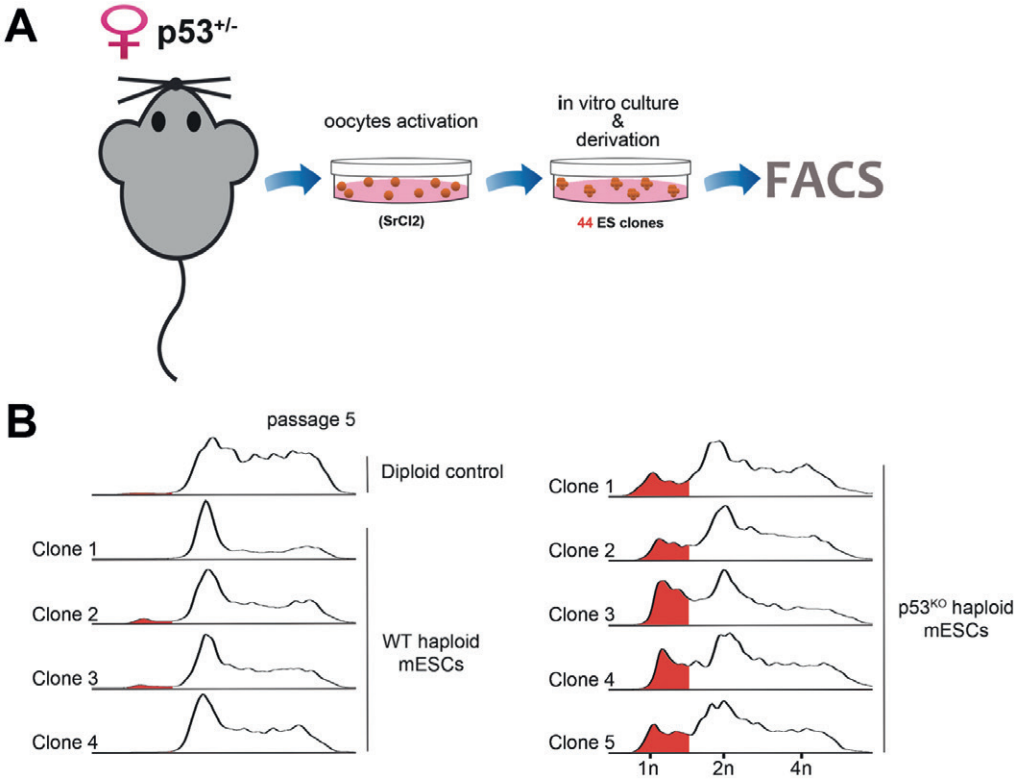


Figure 2 p53 deficiency increases the percentage of haploidy in mouse embryonic stem cells. (A) Mouse haploid embryonic stem cells (mhaESC) were derived by inducing parthenogenesis with SrCl₂ on oocytes from p53^{-/-} female mice. (B) While, as previously reported, mhaESC cultures derived from wild type oocytes presented very small percentages of haploid cells (red), this was significantly increased in p53-deficient lines.

as well as identified new determinants of the sensitivity to these chemicals such as the oncogene Epithelial Cell Transforming 2 (ECT2). Studies aiming to understand the mechanisms by which the identified mutations alter the response to ATR inhibition are currently underway.

Identification of a ‘Haploidy Checkpoint’

One important limitation of mammalian haploid cell lines is the rapid loss of the haploid state, resulting in cultures becoming rapidly enriched in diploid cells. This phenomenon has been previously assumed to be due to the ‘diploidization’ of the haploid genomes, although how this occurs has remained poorly understood. We have now revealed that the so-called ‘diploidization’ is a consequence of a growing disadvantage of haploid cells, which are outcompeted by the few diploids that are present in these cultures. In support of this, single-cell sorting can significantly stabilise the haploid state. We have also discovered that the reduced fitness of mammalian haploid cells arises as a consequence of problems during chromosome

segregation in mitosis, which subsequently lead to the activation of a cytotoxic p53-dependent response. Consequently, p53-deletion can increase the stability of haploid cultures in human HAP1 cells or mouse embryonic stem cells. Due to the similarities between our findings and those previously reported in aneuploid or polyploid cells, we propose the existence of a unified p53-dependent ‘ploidy’ checkpoint, which is activated as a consequence of the difficulties in segregating a suboptimal chromosomal content during mitosis. ■

- PUBLICATIONS**
- Olbrich T, Mayor-Ruiz C, Vega-Sendino M, Gomez C, Ortega S, Ruiz S, Fernandez-Capetillo O (2017). A p53-dependent response limits the viability of mammalian haploid cells. *Proc Natl Acad Sci USA* 114, 9367-9372.
 - Mayor-Ruiz C, Dominguez O, Fernandez-Capetillo O (2017). Trap(Seq): An RNA sequencing-based pipeline for the identification of gene-trap insertions in mammalian cells. *J Mol Biol* 429, 2780-2789.
- AWARDS AND RECOGNITION**
- Erik K. Fernström Award.

CHROMOSOME DYNAMICS GROUP

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OVERVIEW

Our research focuses on a protein complex named cohesin that embraces DNA and is essential for chromosome organisation. Cohesin mediates sister chromatid cohesion and, thereby, ensures faithful DNA repair by homologous recombination and proper chromosome segregation during cell division. It also plays a major role in the spatial organisation of the genome by promoting long-range DNA looping, which in turn contributes to transcriptional regulation, organisation of DNA replication factories and locus rearrangement by recombination. Mutations in cohesin have been found in several tumour types, most prominently in bladder cancer and acute myeloid leukaemia. Mutations in cohesin and its regulatory factors are also at the origin of a group of human syndromes collectively known as cohesinopathies.

Our goal is to understand how cohesin works, how it is regulated and how its dysfunction contributes to cancer and other human diseases. In particular, we are intrigued by the existence of different versions of the cohesin complex in somatic cells. We use mouse models carrying *knock out* alleles of genes encoding cohesin subunit to investigate their functional specificity both at the cellular level and in the context of an organism. We also take advantage of the *Xenopus* egg cell-free system to explore additional aspects of cohesin regulation.

“We have defined the specific contributions of cohesin-SA1 and cohesin-SA2 to genome organisation. Our work may uncover vulnerabilities in cancer cells carrying mutations in the gene encoding SA2, which is one of the twelve genes most mutated in cancer.”

RESEARCH HIGHLIGHTS

Cohesin-SA1 and cohesin-SA2 have distinct roles in 3D genome organisation

Cohesin consists of four core subunits, SMC1, SMC3, RAD21 and SA. There are two versions of the SA subunit in vertebrate somatic cells, SA1 and SA2. Loss of function mutations in the *STAG2* gene encoding SA2 have been identified in bladder cancer, Ewing sarcoma and other tumour types. Cells lacking cohesin-SA2 can proliferate because cohesin-SA1 performs the essential function of cohesin in cohesion. This explains why *STAG2* mutant tumours are not aneuploid. However, it is likely that cohesin-SA1 cannot accomplish the other functions of cohesin-SA2 related with chromatin organisation and gene regulation, thereby providing some advantage to the tumour. Importantly, lack of cohesin-SA2 may also generate vulnerabilities that could be exploited in cancer therapy. To characterise the specific functions of the two variant complexes in chromatin architecture and gene regulation we are pursuing two strategies. The first one is the characterisation of cells derived from mouse embryos deficient for SA1 or SA2. A *STAG1* knockout allele was obtained a few years ago (Remeseiro *et al.*, 2012), while a conditional *STAG2* knockout allele has been generated more recently in collaboration with Francisco X. Real (CNIO Epithelial Carcinogenesis Group). The second strategy makes use of non-transformed human cell lines before and after downregulation of SA1 or SA2. In these cells, we have analysed the genome-wide distribution of the two cohesin complexes as well as their transcriptomes. Moreover, in collaboration with M. A. Martí-Renom (CRG-CNAG), we have interrogated the genome architecture by Hi-C. From these studies, and in line with our previous work, we conclude that cohesin-SA1 collaborates with CTCF in the demarcation of domain boundaries (FIGURE 1). In contrast, a more dynamic cohesin-SA2 complex promotes cell type-specific interactions between enhancers and promoters within contact domains (or TADs, for Topologically Associated Domains) independently of CTCF. Loss of SA2 rewires local chromatin contacts and alters gene expression. We are currently exploring the molecular mechanisms underlying these functional specificities of cohesin-SA1 and cohesin-SA2.

Pds5 proteins regulate cohesin distribution and dynamics

Two factors associate with chromatin-bound cohesin, Pds5 and Wapl. Wapl promotes cohesin unloading, and in its absence there is an excess of cohesin on chromatin and chromosome organisation is altered, both in interphase and mitosis. The role of Pds5 is less clear. Moreover, there are two versions of Pds5 present in vertebrate cells, Pds5A and Pds5B. In order to explore their specific functions we previously generated murine knock out (KO) alleles for these two genes. We showed that both Pds5A and Pds5B contribute to cohesion establishment during S phase by promoting cohesin acetylation and Sororin binding, with Pds5B being specifically required for cohesion at centromeres (Carretero *et al.*, 2013). Now we have analysed how these proteins regulate cohesin distribution and dynamics. We have found that the presence of Pds5A or Pds5B does not specify cohesin localisation, since either one can be found at most cohesin binding sites. However, genome wide distribution of cohesin clearly becomes restricted in the absence of both (FIGURE 2). Under this condition, the dynamic association of cohesin to chromatin, measured in Fluorescence Recovery After Photobleaching (FRAP) experiments, is significantly decreased. Much milder effects are observed in cells lacking only Pds5A or Pds5B. Aberrant accumulation of cohesin in axial structures known as *vermicelli*, previously described in Wapl depleted cells (Tedeschi *et al.*, 2013), can be observed only in the absence of the two Pds5 proteins, although overall accumulation of the complex on chromatin is not as dramatic. From these studies we conclude that Wapl and Pds5 work together in cohesin unloading, but that Pds5 has additional functions. Our current hypothesis is that cohesin acetylation, which is carried out by Cohesin Acetyl Transferases that are recruited to cohesin through Pds5, is essential not only for cohesion establishment during S phase but also for proper cohesin dynamics throughout the cell cycle as well as in non-dividing cells. ■

PUBLICATION

Messina G, Attarrato MT, Prozzillo Y, Piacentini L, Losada A, Dimitri P (2017). The human Cranio Facial Development Protein

1 (Cfdp1) gene encodes a protein required for the maintenance of higher-order chromatin organization. *Sci Rep* 7, 45022.

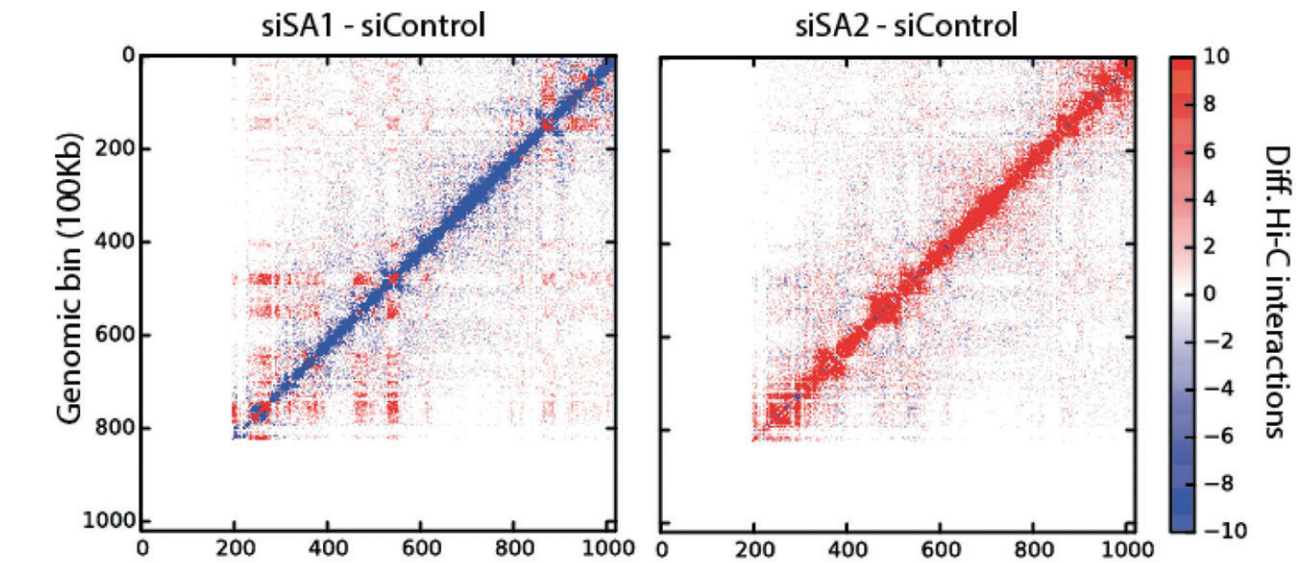


Figure 1

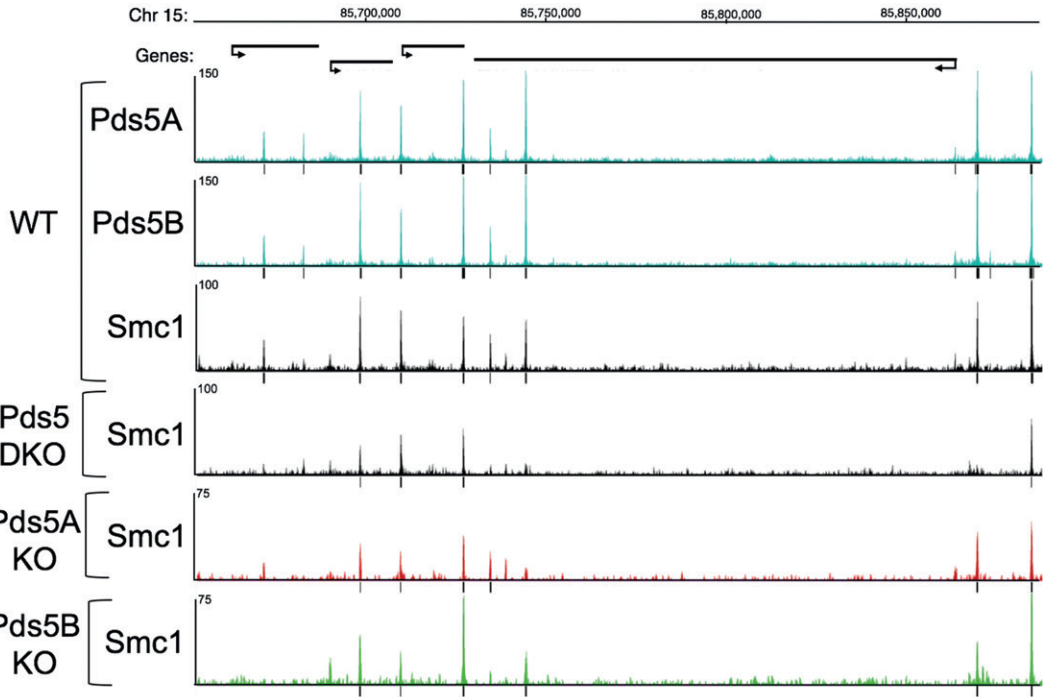


Figure 2

Figure 1 Matrices showing differential interactions along human chromosome 15 comparing control MCF10A cells (mock transfected) with cells downregulated for SA1 or SA2 by siRNA (siSA1 and siSA2, respectively) by means of Hi-C analyses.

The absence of cohesin-SA1 increases long range interactions between TADs (red patches outside the diagonal) whereas the absence of cohesin-SA2 increases interactions between neighbouring TADs (close to the diagonal).

Figure 2 Snapshot of the UCSC browser showing the distribution of Pds5A and Pds5B in a 200-kb long region of chromosome 15 in wild type (WT) mouse embryo fibroblasts, as well as the distribution of cohesin (SMC1)

in the presence and absence of either or both Pds5 proteins (WT, Pds5A/B double KO or Pds5 DKO, Pds5A KO and Pds5B KO), as obtained by ChIP-seq analyses.

DNA REPLICATION GROUP

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OVERVIEW

Up to two thirds of the total mutations detected in tumours are caused by inaccurate DNA replication. Our laboratory studies the process of DNA replication using a combination of biochemistry, cell biology and mouse genetics. Our current interests include: (1) the genetic and epigenetic elements that define replication origins; (2) the cellular responses to replicative stress (RS) caused by endogenous elements (e.g. natural decay of DNA; collisions between replication and transcription proteins) or exogenous factors such as UV radiation or DNA-damaging chemical agents. One of the responses to RS is the activation of ‘dormant’ origins, whose regulation remains poorly understood; (3) the mechanisms that limit DNA replication to only one round in each cell cycle, and the physiological consequences of their malfunction; and (4) the role of PrimPol, a recently characterised enzyme with primase and polymerase activities, in the ‘tolerance’ mechanisms that enable replication through damaged DNA.

“We have reported that deregulation of CDC6 and CDT1 proteins – a frequent occurrence in cancer cells – leads to DNA over-replication and causes lethal tissue dysplasias.”

RESEARCH HIGHLIGHTS

Genome-wide analysis of replication origin usage upon replicative stress

Mammalian DNA replication starts at tens of thousands of points called ‘origins’ whose frequency of activation is flexible. Our laboratory reported, several years ago, that stalled replication forks induce the activation of extra origins as a backup mechanism (Ibarra *et al.*, *Proc Natl Acad Sci USA*, 2008). In collaboration with the group of Dr María Gómez (*Centro de Biología Molecular “Severo Ochoa”*, Madrid), we have used a technique called SNS-Seq (isolation of short nascent DNA strands followed by DNA sequencing) to map the genomic positions and global efficiency of origins in mouse embryonic stem cells under experimental conditions that trigger extra origin activation. This approach allows a comparative analysis of ‘constitutive’ origins that are active in all the tested conditions, as well as the analysis of ‘responsive’ origins that become stimulated under stress. We have found that constitutive origins are more frequently associated with open chromatin marks, CpG islands, bivalent promoters and transcription factors than responsive ones. In addition, many replication origins increase their frequency of activation following stress. In collaboration with computational biologists at CNIO and the Barcelona Supercomputing Center, we are working on integrating the linear origin maps into three-dimensional chromatin networks, in order to shed light on the complex hubs of nuclear DNA replication factories.

Lethal tissue dysplasias caused by DNA re-replication

Activation of oncogenes such as c-Myc affects replication origins through mechanisms that are not well understood. It has been reported that oncogenic stress may lead to origin hyper-activation, which leads to DNA over-replication and possibly gene amplification. Origin activity is largely controlled by the CDC6 and CDT1 proteins responsible for the loading of the MCM DNA helicase onto DNA. In normal cells, the activity of CDC6 and CDT1 is strictly regulated to prevent origin reactivation, but both factors are overexpressed in multiple types of cancer.

To investigate the effects of deregulated expression of CDC6 and CDT1 *in vivo*, we have designed mouse strains that allow the inducible expression of both proteins, alone or in combination. This year, we have reported that simultaneous, but not individual, deregulation of CDC6 and CDT1 is lethal to adult mice due to a striking dysplasia of the intestinal epithelium. The regeneration of intestinal epithelia is driven by transit-amplifying cells derived from stem cell niches located at the bottom of the intestinal crypts. Upon CDC6 and CDT1 deregulation, transit-amplifying progenitors undergo DNA over-replication and cell death, causing rapid intestinal failure (FIGURE).

A mechanistic insight derived from this study is that lethal DNA over-replication only occurred when CDC6 and CDT1 were deregulated at the same time. Therefore, unleashing CDT1 activity could kill tumour cells that generally express high levels of CDC6, but have no effect on the surrounding tissue that display normal CDC6 expression. At this time, CDT1 stimulation can be achieved by targeting GMN, a CDT1 inhibitor, or by the use of neddylation inhibitors (e.g. MLN4924) that stabilise CDT1. The mouse strains characterised in our study reveal the marked cytotoxicity of DNA re-replication *in vivo* and may therefore become useful models in pre-clinical studies.

PrimPol: a damage tolerance protein with potential applications in cancer therapy

We continue to investigate the functions of PrimPol, an enzyme that facilitates replication through damaged DNA. We have found that genetic ablation of PrimPol by Crispr/Cas9 sensitises cells to UV and chemical agents that induce DNA damage. Because PrimPol facilitates cell survival in the presence of DNA lesions, its inhibition could enhance the efficacy of cytotoxic chemotherapeutic agents that trigger cell death by inducing DNA damage. ■

► **PUBLICATIONS**

► Muñoz S, Búa S, Rodríguez-Acebes S, Megías D, Ortega S, De Martino A, Méndez J (2017). *In vivo* DNA re-replication elicits lethal tissue dysplasias. *Cell Rep* 19, 928-938.

► Garzón J, Rodríguez R, Kong Z, Chabes A, Rodríguez-Acebes S, Méndez J, Moreno S, García-Higuera I (2017). Shortage of dNTPs underlies altered replication dynamics and DNA breakage in the absence of the APC/C cofactor Cdh1. *Oncogene* 36, 5808-5818.

► Muñoz S, Méndez J (2017). DNA replication stress: from molecular mechanisms to human disease. *Chromosoma* 126, 1-15.

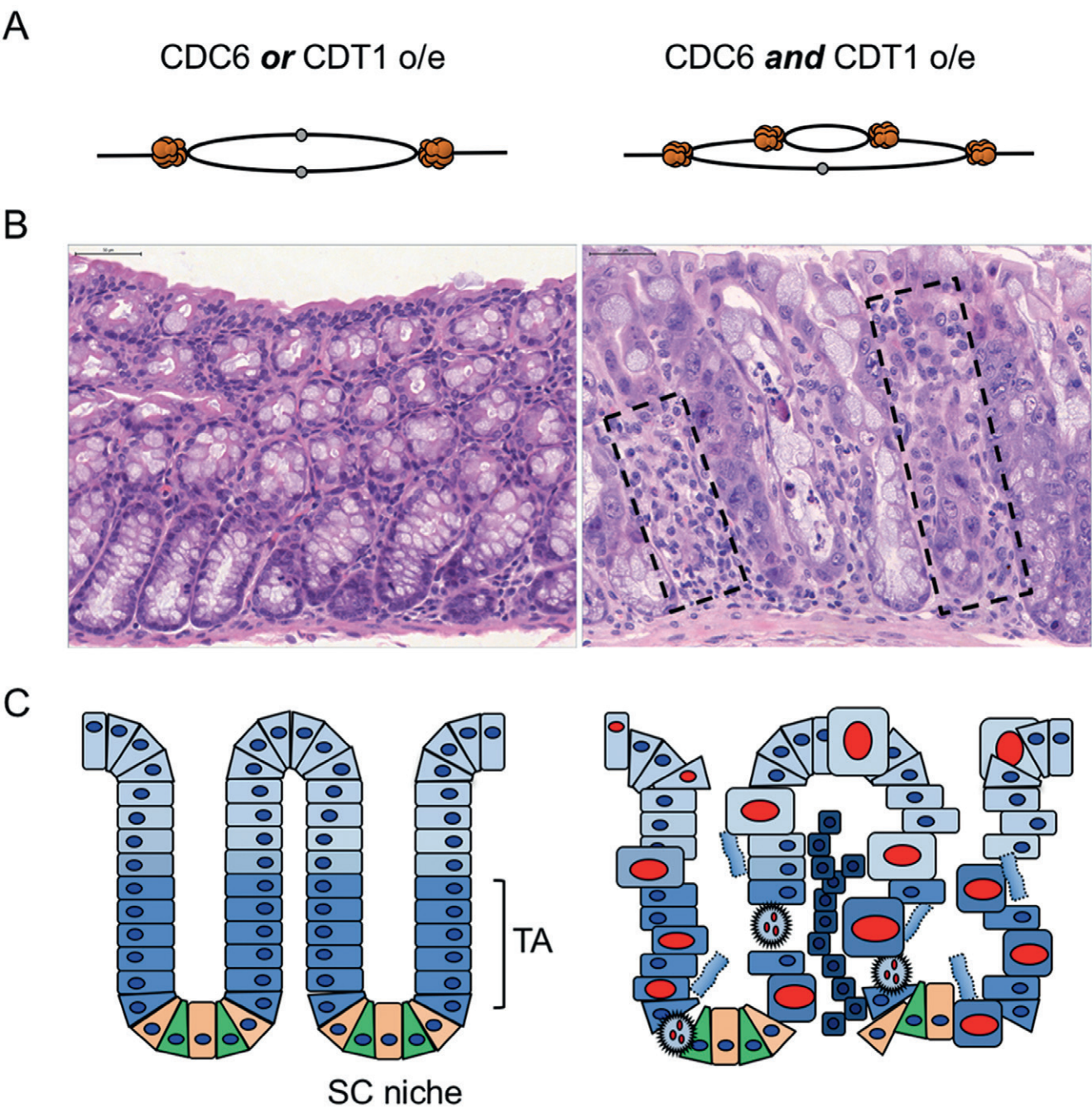


Figure DNA re-replication elicits lethal tissue dysplasia. (A) Left, bidirectional DNA replication forks starting from an origin, as observed in control cells or after CDC6 or CDT1 deregulation. Right, aberrant origin reactivation caused by simultaneous CDC6 and CDT1 deregulation. (B) Haematoxylin-eosin staining in colon sections of control (left) or CDC6 and CDT1-overexpressing mice (right). Boxes mark areas of inflammatory infiltration. (C) Schematic of normal intestinal crypts (left) or dysplastic crypts (right). SC, stem cells. TA, transit-amplifying cells. Adapted from Muñoz *et al.* (2017).

MELANOMA GROUP

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OVERVIEW

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

“We have developed the first-in-class lymphoreporter mouse models of melanoma for *in vivo* imaging and pharmacological testing of new metastatic agents.”

RESEARCH HIGHLIGHTS

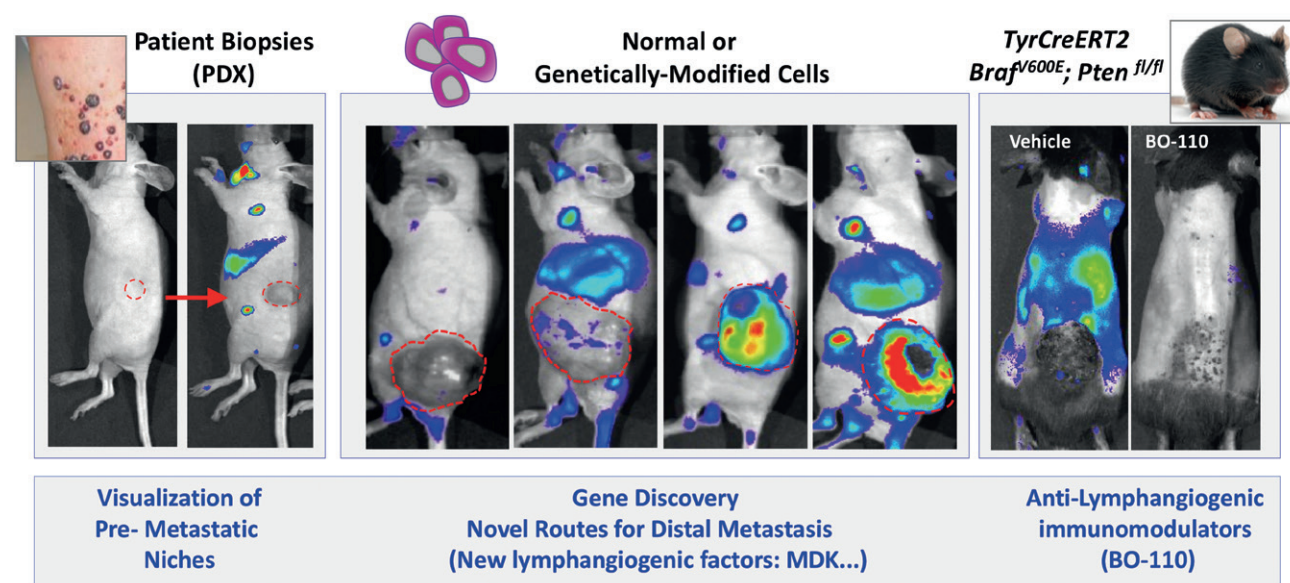


Figure 1 *Vegfr3^{lac}* (MetAlert) mice to test PDX, melanoma cell lines or genetically engineered mice for the visualisation of pre-metastatic niches

and as a platform for gene discovery and drug testing (here shown to visualise the efficacy of the dsRNA-based nanoparticle BO-110).

CNIO Melanoma Group: Objectives and model systems

Melanomas are aggressive solid tumours and a prime example of how integrated basic and clinical research has significantly improved patient prognosis. Nevertheless, despite great success with targeted and immune-based therapies, sustained clinical responses are still limited. Moreover, the field lacks molecular markers of diagnosis, and the knowledge on how melanomas progress and metastasise is largely incomplete. In addition, one of the main hurdles to advance in this disease is the lack of animal models to monitor melanoma initiation and progression *in vivo*.

To this end, our Group focuses on three main areas of research (FIGURE 1):

- **Aim 1.** Oncogenic pathways selectively deregulated in melanoma that may represent new diagnostic indicators.
- **Aim 2.** Risk factors and prognostic markers.
- **Aim 3.** Animal models that allow for non-invasive monitoring of pre-metastatic niches.

Lineage-specific oncogenic dependencies in melanoma

One of the long-term objectives of the Melanoma Group is to discover **new melanoma drivers**. We have previously identified

a cluster of endolysosomal-associated genes that distinguish melanoma from over 35 additional malignancies (Alonso-Curbelo *et al.*, *Cancer Cell* 2014; Alonso-Curbelo *et al.*, *Oncotarget* 2015a and *Oncotarget* 2015b). Further analyses of lysosomal-dependent pathways also revealed unique features of autophagy genes (ATG5) in melanoma (García-Fernández *et al.*, *Autophagy* 2016). Additional contributions of autophagy to melanoma cell survival and response to targeted therapy were generated in collaboration with the Ashani Weeraratna group at the Wistar Institute (USA) (Ndoye *et al.*, *Cancer Res* 2017).

Other melanoma-enriched regulatory mechanisms were identified by focusing on RNA binding proteins (RBPs). We selected RBPs (a family of over 950 members) because they are largely unexplored in melanoma, although this is a tumour characteristically associated with a plethora of changes in mRNA gene expression profiles. Performing a series of genome wide studies (i.e. genomic, transcriptomic, proteomic and interactomic analyses) we uncovered new roles of the RBPs CPEB4 and CUGBP1 in the modulation of mRNA stability, with unexpected targets involving master specifiers of the melanocyte lineage (Perez-Guijarro *et al.*, *Nat Commun* 2016; Cifdaloz *et al.*, *Nat Commun* 2017).

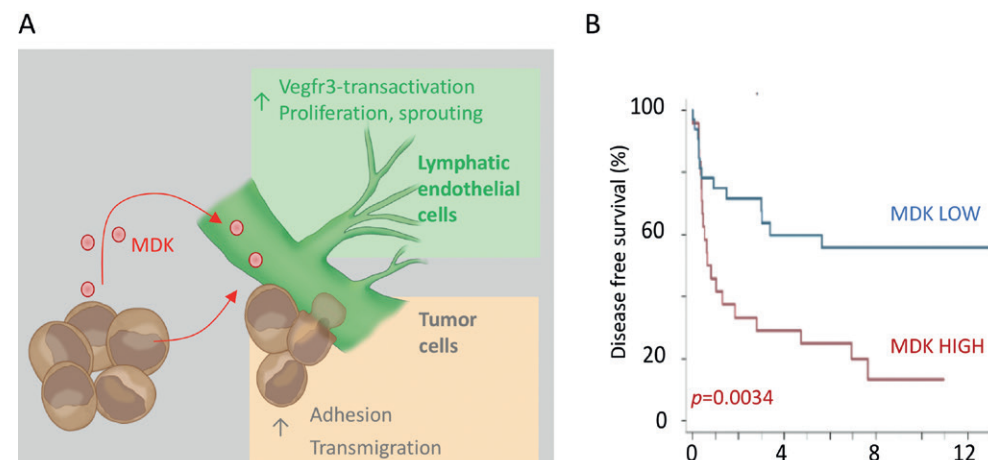


Figure 2 MIDKINE (MDK) as pro-metastatic driver and marker of poor prognosis in melanoma. (A) Schematic depicting newly-described roles of tumour-secreted MDK on tumour cells and the lymphatic vasculature. (B) Kaplan-Meier survival curves of melanoma patients (Stage II-IV) classified on the basis of MDK expression in sentinel lymph nodes.

‘MetAlert’ mice for the visualisation of premetastatic niches in melanoma and as a platform for gene discovery and target validation

We have also made great progress regarding one of the most pressing needs in the melanoma field, namely, the mechanisms that enable melanoma cells to disseminate already from lesions of barely 1 mm in depth. In collaboration with Sagrario Ortega at the CNIO, we have generated a series of mouse models of melanoma that have the unique feature of revealing how these cells act ‘a distance’ from very early stages of tumour development, activating the lymphatic vasculature and preparing metastatic niches before their colonisation (Olmeda *et al.*, *Nature* 2017; see the versatility of these mice for gene discovery and pharmacological analyses in FIGURE 1). Using these ‘MetAlert’ animals we found the growth factor MIDKINE as a new driver of lymphangiogenesis and melanoma metastasis (FIGURE 2, A). The physiological relevance of these data was validated in

human clinical biopsies where MIDKINE expression correlated with poor patient prognosis (FIGURE 2, B). This paper of Olmeda *et al.* was highlighted in *Nature*, *Cancer Discovery*, *Developmental Cell* and other scientific journals, and was awarded the *Premio “Constantes y Vitales”* by the *Fundación AtresMedia* for the Best Publication in Biomedical Research in 2017. This article was also considered as being among the *Top 10 publications in Spain in 2017* by the news agency *EFE*. This publication, together with others from the Soengas Group, was recognised by the *Estela Medrano Memorial Award* by the *Society of Melanoma Research*, which honours the most influential female leaders in the melanoma field. In addition, clinical trials with the compound BO-112 performed by the biotechnology company *Bioncotech Therapeutics* were considered as being among the *14 Most Relevant Scientific Hits in 2017* by the *SINC* agency (the *Spanish Information and Scientific News Service*). BO-112 is a derivative of the polyplex BO-110 generated at the CNIO by the Soengas laboratory. ■

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AWARDS AND RECOGNITION

- Estela Medrano Memorial Award for the most influential female melanoma researcher, the Society for Melanoma Research, USA.
- “Constantes y Vitales” Award for the Best Publication in Medicine, *Fundación AtresMedia*, Spain.
- “Premio Ejecutivas de Galicia 2017”, *Ejecutivas de Galicia*, Spain.
- Finalist “Premios MAS Mujeres a Seguir”, Spain.
- Olmeda *et al.*, *Nature* 2017; considered one of the Top 10 scientific publications in Spain in 2017, *Agencia EFE*.

MICROENVIRONMENT & METASTASIS JUNIOR GROUP

Héctor Peinado
Junior Group Leader

Staff Scientist
Susana García

Post-Doctoral Fellows
Marta Hergueta, Claudia Savini



OVERVIEW

Our laboratory is interested in analysing the main mechanisms involved in tumour-microenvironment communication during metastasis. Extracellular vesicles (EVs) and soluble factors are key players during this process. Tumour cells benefit from the secretion of these factors partly by influencing the behaviour of neighbouring cells in the tumour microenvironment. We are exploring the role of secreted factors and vesicles in metastasis using different approaches: 1) decoding the messages packaged in tumour-derived exosomes during melanoma metastasis, 2) defining novel factors involved in the crosstalk between adipose tissue and tumour, and 3) testing new therapies to prevent metastasis in rare diseases such as neurofibromatosis.

“We aim to block tumour-microenvironment communication as a novel way to control metastatic progression.”

Graduate Students
Ana I. Amor, Teresa
González, Alberto Hernández
(since September), Olwen Leaman
(March-October), Lucía Robado

Technicians
Marina Mazariegos, Cristina Merino,
Sara Sánchez-Redondo

Students in Practice
Ainhoa Almeida (February-June),
Alberto Hernández (January-June),
Alicia Teijeira (since October)

RESEARCH HIGHLIGHTS

Decoding tumour-microenvironment communication in metastasis

Tumour-secreted extracellular vesicles constitute a network of communication secreted by primary tumours favouring metastasis. In melanoma, tumour-adjacent lymph nodes (a.k.a. sentinel lymph nodes) are normally the first sites of metastasis. In this project, we are focused on unravelling the role of tumour-derived exosomes as entities promoting cellular and molecular alterations in the lymph node microenvironment, facilitating metastasis (FIGURE). In particular, we are investigating the effects of tumour exosomes in the lymphatic vasculature. In addition, we are developing nanoparticles (FIGURE B, left panel) as sensors of pre-metastatic niches mimicking tumour-derived exosomes (FIGURE B, right panel) that will help to identify future areas of metastasis. These studies will lead to the development of novel technologies and therapies to block metastatic disease.

Fatal triage: adipose tissue, coagulation and metastasis

Over the past few decades, the incidence of overweight and obesity has been increasing very rapidly in both developed and developing countries, making obesity one of the most serious health problems worldwide. Increasing evidences have revealed a link between obesity and the development of certain types of cancer; still the impact of obesity on metastasis is not well established. Recent data support a role for secreted factors [e.g. soluble factors and EVs] in the communication between tumour cells and adipose tissue during tumour metastasis. In this project, we are investigating the local crosstalk between the adipose tissue and tumour cells, analysing secreted factors and EVs as well as the role of platelets as systemic players in the metastatic process.

Testing new therapies to prevent metastasis

Neurofibromatosis type 1 (NF1) is a genetic disorder which can progress to Malignant Peripheral Nerve Sheath Tumour (MPNST), a highly aggressive sarcoma. In this project, we are analysing the main pathways involved in tumour-microenvironment crosstalk during metastatic progression. In order to find potential biomarkers and candidates to target the progression of the disease, we investigated the molecular

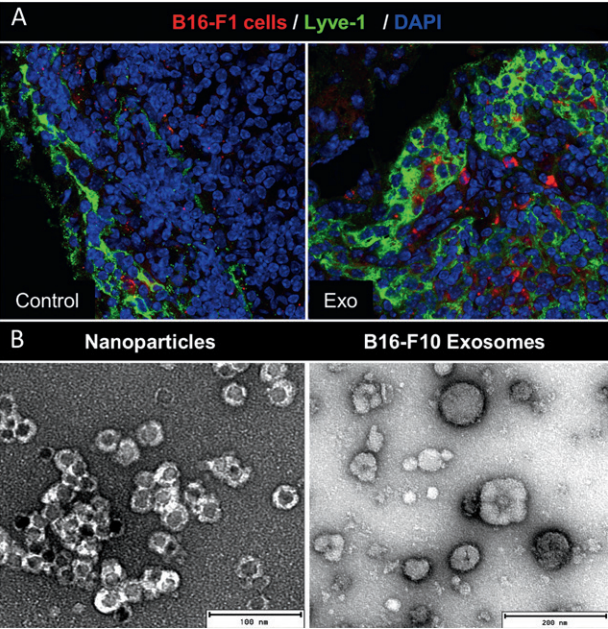


Figure (A) Exosome ‘education’ enhances lymph node metastasis. Low metastatic B16-F1-mCherry cells were injected intra-footpad after 10 days of exosome education (EXO) using exosomes derived from B16-F1-R2 cells (melanoma model derived from lymph node metastasis) and compared to PBS. LNs were analysed by confocal microscopy demonstrating the increase of metastasis (in red-mCherry) after exosome education. (B) Left panel - electron microscopy of iron oxide nanoparticles (IONPs) conjugated with specific peptides to detect pre-metastatic niches. Right - electron microscopy of B16-F10-derived exosomes.

signature of exosomes secreted by MPNST cell lines. We have identified several candidates and we are currently performing a multidrug screening in combination with these markers in order to find new therapies for the treatment of this disease. ■

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- A-Gonzalez N *et al.* (incl. García-Silva S, Mazariegos M, Peinado H) (2017). Phagocytosis imprints heterogeneity in tissue-resident macrophages. *J Exp Med* 214, 1281-1296.
- Santamaria PG *et al.* (incl. Peinado H). Lysyl oxidase-like 3 is required for melanoma cell survival by maintaining genomic stability. *Cell Death Differ.* PMID: 29229995.
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- Pathan M *et al.* (incl. Peinado H) (2017). A novel community driven software for functional enrichment analysis of extracellular vesicles data. *J Extracell Vesicles* 6, 1321455.

BRAIN METASTASIS JUNIOR GROUP

Manuel Valiente
Junior Group Leader

Post-doctoral Fellow
Neibla Priego



OVERVIEW

Brain metastasis is the most common neurological complication of cancer. When metastatic cells reach the brain, prognosis is poor given that available therapies (i.e. surgery and radiation) have limited benefits for patients and the disease inevitably progresses. The rise in the number of patients with brain metastasis is partially due to the increasing number of systemic therapies that work extra-cranial but not in the brain. In this scenario, cancer cells present at this highly demanding secondary site have additional time to evolve and develop into clinically detectable lesions. In the laboratory, we study why and how cells from different cancer types (breast cancer, lung cancer and melanoma) are able to access the brain, survive and colonise this vital organ. We dissect the biology of these processes *in vivo* using experimental models in order to challenge the current status of this unmet clinical need.

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

Graduate Students
Catia P. Domingues, Pedro García
(since October), Lucia Zhu

Technicians
Laura E. Doglio (until August),
Lourdes Osuna (since November),
Natalia Yebra

Student in Practice
Wendy E. Bindeman
(since September)

RESEARCH HIGHLIGHTS

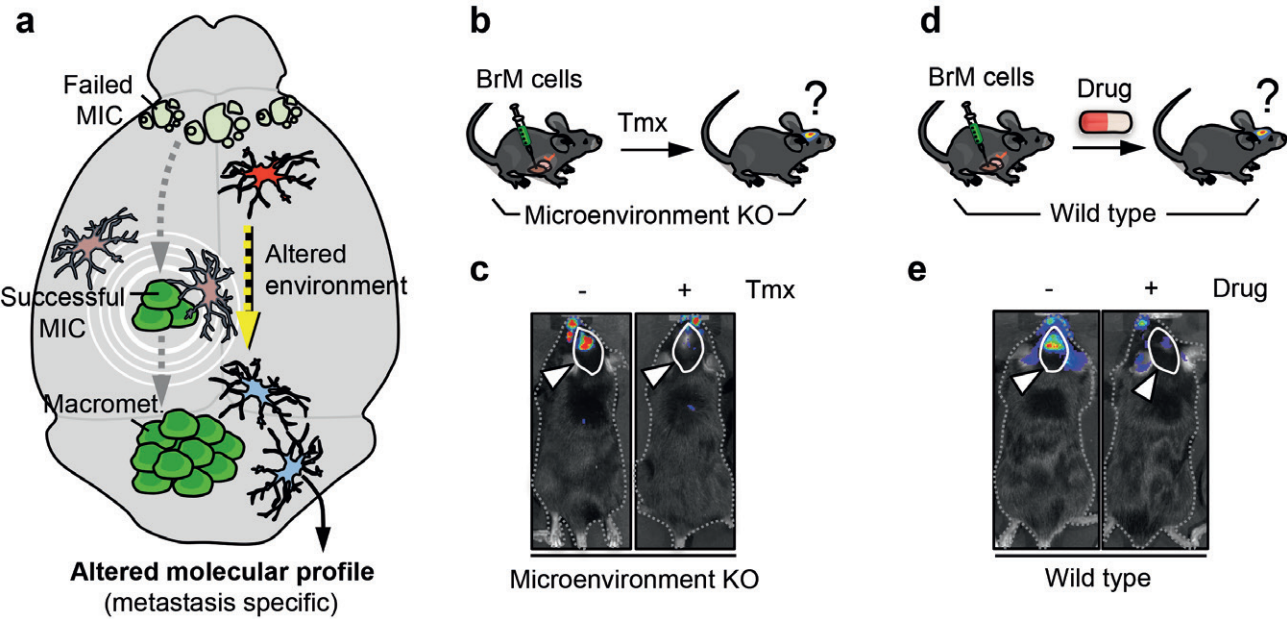


Figure (a) Metastasis initiating cells (MIC) not eliminated during the initial steps of colonisation grow in the brain and induce altered molecular profiles in the microenvironment. (b-c) Genetic and (d-e) pharmacologic approaches have validated important pro-metastatic components in the microenvironment.

We have identified a cell type-specific molecular marker that is present in the brain metastasis microenvironment and absent from the normal brain. This marker is present surrounding both experimental and human metastasis, independent of the source of the primary tumour.

By establishing a mouse model that is unable to activate this component of the microenvironment we have proven its pro-

metastatic role, since the development of brain metastasis is significantly reduced.

We have translated this finding into a novel therapeutic approach by which we can target brain metastasis by blocking discrete pro-tumorigenic populations within the heterogeneous microenvironment of brain metastasis. ■

PUBLICATIONS

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- ▶ Wasilewsky D, Priego N, Fustero-Torre C, Valiente M (2017). Reactive astrocytes in brain metastasis. *Frontiers in Oncology* 7, 298.
- ▶ PATENT
- ▶ Valiente Cortés M, Priego Bendeck N, Bosh Barrera J (2017). Methods for pre-

venting and treating brain metastasis. *EPI7382891.4*.

AWARDS AND RECOGNITION

- ▶ Beug Foundation's Prize for Metastasis Research.
- ▶ Bristol-Myers Squibb-Melanoma Research Alliance (MRA) Young Investi-

gator Award.

- ▶ Cátia Monteiro was awarded the *Best Poster Award*, CNIO Frontiers Meeting: 'Primary and Secondary Brain Tumours', Madrid, Spain.
- ▶ Wendy Bindeman was recipient of a Fulbright fellowship.
- ▶ Pedro García Gómez was awarded the La Caixa INPhINIT PhD Fellowship.

METABOLISM AND CELL SIGNALLING JUNIOR GROUP

Alejo Efeyan
Junior Group Leader

Staff Scientist
Ana Ortega



OVERVIEW

In the Metabolism and Cell Signalling Lab we study the interplay of nutrients, metabolism and cancer. Every cell in the organism receives signals emanating from the abundance of intracellular nutrients and from the nutritional state of the organism as a whole. Integration of cellular and systemic nutrient abundance cues is key for adequate cellular and organismal functions. Importantly, the components if these signalling cascades are functionally and genetically corrupted in disease states, such as cancer. Together with genetic mutations, environmental perturbations, such as those occurring in obesity, affect the cellular signalling cascades that control responses to nutrients and hormones. In the lab, we combine mouse genetics and cell biological tools to gain insight into the genetic and environmental corruptions of nutrient signalling cascades, aiming to conceive therapeutic interventions in the context of cancer, obesity and the process of ageing.

“Our mouse genetic approaches are beginning to reveal the intricacies of nutrient signalling in the fuelling of rapidly proliferative normal and malignant B lymphocytes.”

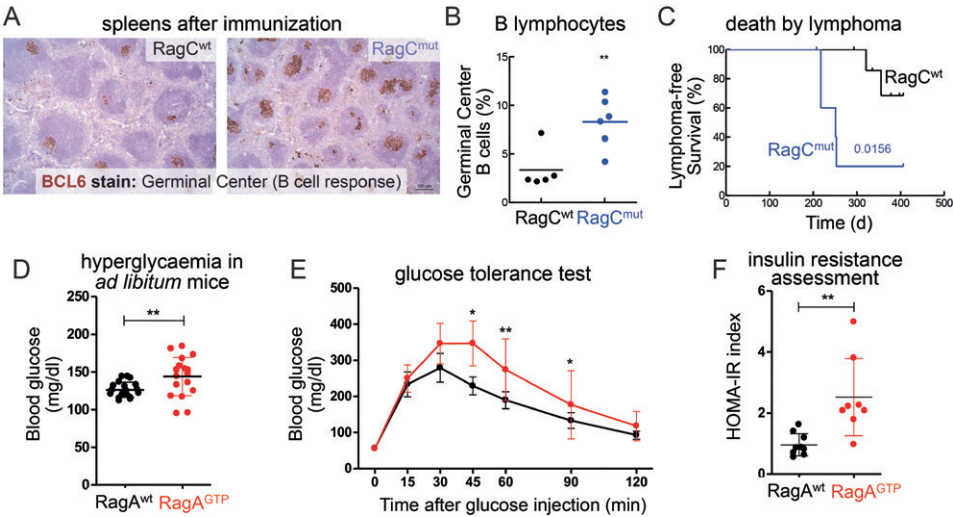
Graduate Students
Celia de la Calle, Nerea Deleyto, Ana Belén Plata (since September)

Technicians
Camino Menéndez (since March) (TS)*, Ana Sagrera (TS)*

*Titulado Superior (Advanced Degree)

Students in Practice
Álvaro Fernández (until June), Daniel de la Nava (June-August), Beatriz Fernández (February-May)

RESEARCH HIGHLIGHTS



Nutrient signalling in B lymphocytes

One of the most rapid proliferation bursts in mammalian cells is that of B lymphocytes upon encountering certain pathogens or antigens. This proliferation suddenly multiplies the energetic and metabolic demands of the activated cell, and accordingly, accurate nutrient signalling is key to successfully accomplish the energetically onerous rounds of growth and division. Recently, components of the Rag GTPase pathway, a key nutrient signalling pathway that enables the anabolic capacity of the cell for rapid proliferation, were found mutated in follicular lymphoma (FL), an incurable B lymphocyte tumour. We have generated novel strains of mice that express these mutations, explored their oncogenicity and studied the reasons for such oncogenicity. We found that these mutations both suppress cell death and enhance proliferation, leading to hyperactive B cell responses upon immunisation and accelerated lymphomagenesis (FIGURE). Our results open up a potential therapeutic avenue for FL.

Chronic signalling of elevated nutrients mimics a diabetic state

Does an excess in nutrient intake correlate with human disease states because of caloric value, or because of the signalling

cascades and cellular responses that overabundant nutrients activate? We sought to answer this question with genetically engineered mice to have a constitutively active nutrient-sensing cascade, but normal food intake. These mice express a modified form of the RagA GTPase (RagA^{GTP}) that activates the mechanistic target of rapamycin complex 1, the pathway responsible for the synthesis of most anabolic reactions (proteins, lipids and nucleotides). The sole activation of this nutrient-signalling cascade drove elevated glucose levels in circulation, caused glucose intolerance and elevated the HOMA-IR, a parameter used to diagnose insulin resistance in patients. We are currently in the process of dissecting the contribution of each organ in these metabolic perturbations. ■

PUBLICATIONS

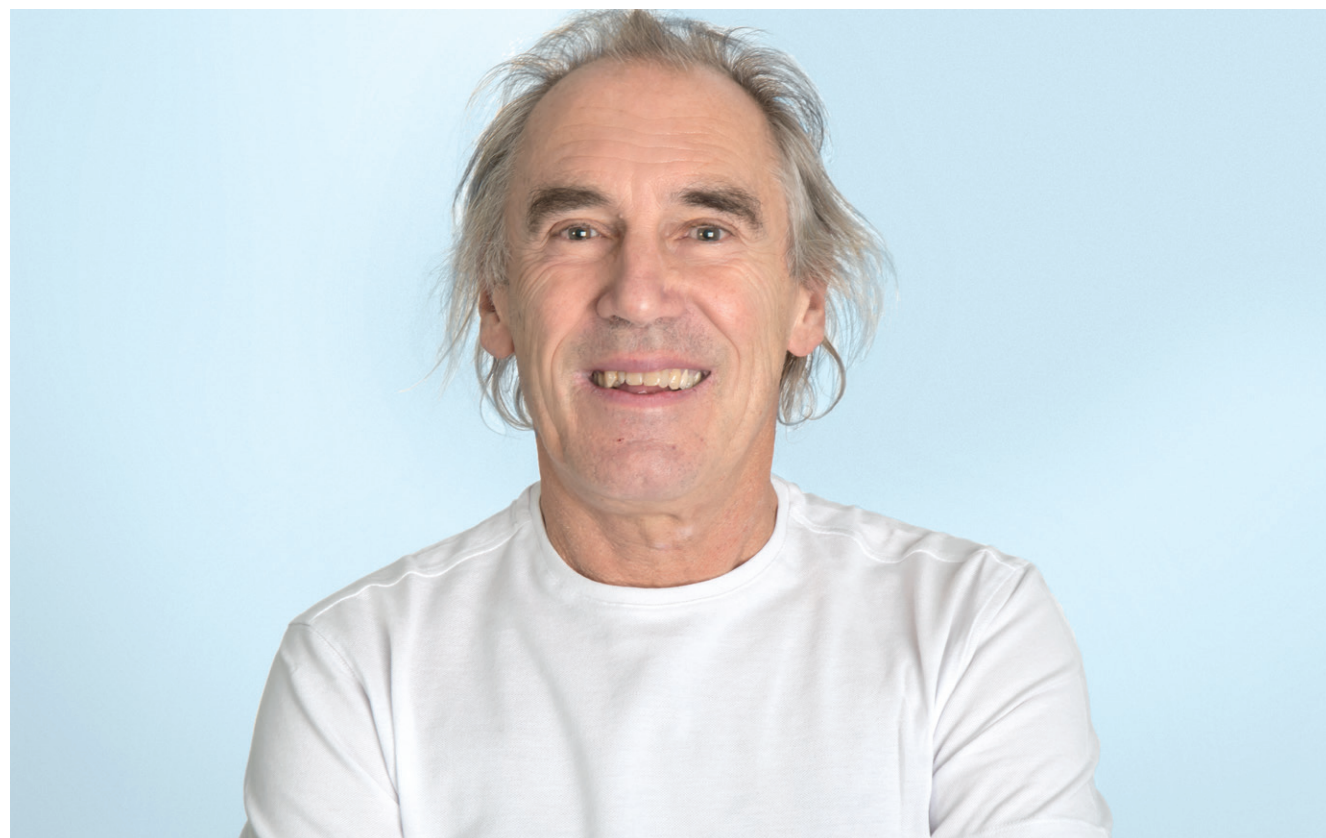
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CANCER CELL BIOLOGY PROGRAMME

ERWIN F. WAGNER Programme Director



The overall strategic goals of the Cancer Cell Biology Programme are to achieve a better understanding of the events leading to cancer development, progression and metastasis, and to discover molecular mechanisms that could provide a basis for novel therapies. The 5 Groups investigate how tumours grow as ‘extrinsic organs’; the spectrum of investigations ranges from epithelial cancers such as liver, skin and intestinal, to bone and brain tumours. The research covers aspects of tumour cell biology, ranging from tumour stem cells, tumour cell interactions with host cells/environment such as tumour-associated cells like macrophages and fibroblasts, to the role of inflammation, as well as cell adhesion, metabolism and metastasis. Powerful state-of-the-art mouse genetic models, human cellular systems, high-throughput genomic/proteomic and biochemical tools, as well as patient-derived materials, are employed. At present, these aspects are successfully covered and integrated in an interactive and collaborative spirit by the complementary research areas of the 2 Senior and 3 Junior Groups.

The Senior Group, led by Francisco X. Real, studies epithelial tumours focusing mainly on pancreatic and bladder cancer. The Group employs an integrative approach to understand the molecular patho-physiology of these tumours and applies this knowledge in the clinical setting. Mirna Pérez-Moreno’s Group investigates the role of cell adhesion, inflammation and cellular signalling in normal skin physiology and cancer. Nabil Djouder’s Group aims to dissect the contribution of nutrient and growth factor signalling pathways to cancer development, and in particular to gastro-intestinal cancers. Massimo Squatrito’s Group, which is partly supported by the Seve Ballesteros Foundation (F-SB), studies how brain tumours, mainly glioblastomas, develop and how they respond to therapy. Finally, my own Group focuses on understanding the role of the transcription factor complex AP-1 (Fos/Jun) in physiological and pathological processes, with a strong focus on aspects of inflammation and cancer, e.g. in the liver, lung, skin and bone. We are investigating the role of AP-1 in inflammatory skin diseases, such as psoriasis, but also aim to molecularly define the causes of lung fibrosis. We have continued our efforts to study how the whole organism responds to a locally growing tumour in the context of a complex metabolic impairment in cancer-associated-cachexia.

One of our Group Leaders Mirna Pérez-Moreno left the CNIO at the end of 2017 to accept a Professorship at the University of Copenhagen (Denmark). We are very thankful to Mirna for her important contributions to the Programme and we wish her the best of luck in her new position.

“Our main goal is to keep CNIO globally competitive and to ensure that CNIO remains an international institution. Members of seventeen different nationalities from 5 continents are represented in our Programme with the goal to perform top-level cancer cell biology, as well as to train students and postdocs to become the next-generation of promising scientists.”

GENES, DEVELOPMENT AND DISEASE GROUP

Erwin F. Wagner
Group Leader

Staff Scientists
Latifa Bakiri, Nuria Gago,
María Jiménez, Liliana Mellor,
Özge Uluçkan



Post-Doctoral Fellows
Kazuhiko Matsuoka, Álvaro Ucero

Visiting Graduate Student
Jennifer Cascino (Fulbright Fellow,
since September)

Technicians
Vanessa Bermeo, Ana Guío (TS)*

Graduate Student
Lucía T. Díez

Titulado Superior (Advanced Degree)

OVERVIEW

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

- Elucidating a causal link between inflammation, cancer and AP-1 (Fos/Jun) expression, using cell type-specific, switchable genetically engineered mouse models (GEMMs).
- Developing and characterising new GEMMs for cancer and human diseases, such as bone loss, arthritis, fibrosis and psoriasis, and applying these to preclinical studies.
- Using multiple approaches to compare mouse models of disease to human disease and to identify therapeutically relevant targets.

“Our goal is that CNIO remains an international and competitive institution. At present, 4 out of 5 Group Leaders in our department are foreigners, one of whom is partly funded by the Seve Ballesteros Foundation. Seventeen different nationalities from 5 continents are a testament to an international science culture, all focussing on unravelling the mysteries of inflammation, metabolism and cell differentiation in cancer.”

RESEARCH HIGHLIGHTS

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

Bone development, osteosarcomas and arthritis

We are studying the function of AP-1 proteins in bone development and disease using loss-(LOF) and gain-of-function (GOF) mouse models. In mice, transgenic c-Fos expression leads to osteosarcomas (OSs). Using an inducible bone-specific Wntless (Wls) LOF GEMM, we found that loss of Wnt signalling delays Fos-induced OS development. Our data also demonstrate that canonical Wnt signalling is not a determining factor, while increased Wnt7b and Wnt9a suggest the involvement of non-canonical Wnt signalling.

Rheumatoid (RA), Psoriatic (PsA) and Osteoarthritis (OA) are destructive joint pathologies linked to chronic inflammatory diseases. We are studying the function of AP-1 factors in the development of arthritis using GEMMs and experimental arthritis models. Using cell type-specific and inducible AP-1 LOF mouse models, we found that c-Fos and JunB are key regulators of surgery- and age-induced OA with distinct functions. We are also investigating how a cross-talk from skin to bone contributes to the development and progression of different types of arthritis, as well as whether inflammation generated from arthritic joints can influence disease development in adjacent and distant organs.

Liver disease—metabolism, fibrosis, inflammation and cancer

AP-1 proteins are important modulators of hepatic lipid metabolism as specific AP-1 dimers can either activate or repress PPAR γ transcription. Therefore, fatty liver disease and obesity most likely depend on AP-1 dimer composition. In addition, ectopic expression of Fra-2, but not Fra-1-containing AP-1 dimers in hepatocytes, leads to liver dysplasia. Mechanistically, molecular analyses point to the involvement of pathways connected to human hepatocellular carcinoma (HCC), such as the Wnt/ β -catenin and Myc pathways.

Deletion of c-Fos in hepatocytes protects from chemically-induced liver carcinogenesis, whereas additional inactivation in immune cells abrogates this effect. Ectopic c-Fos or expression of

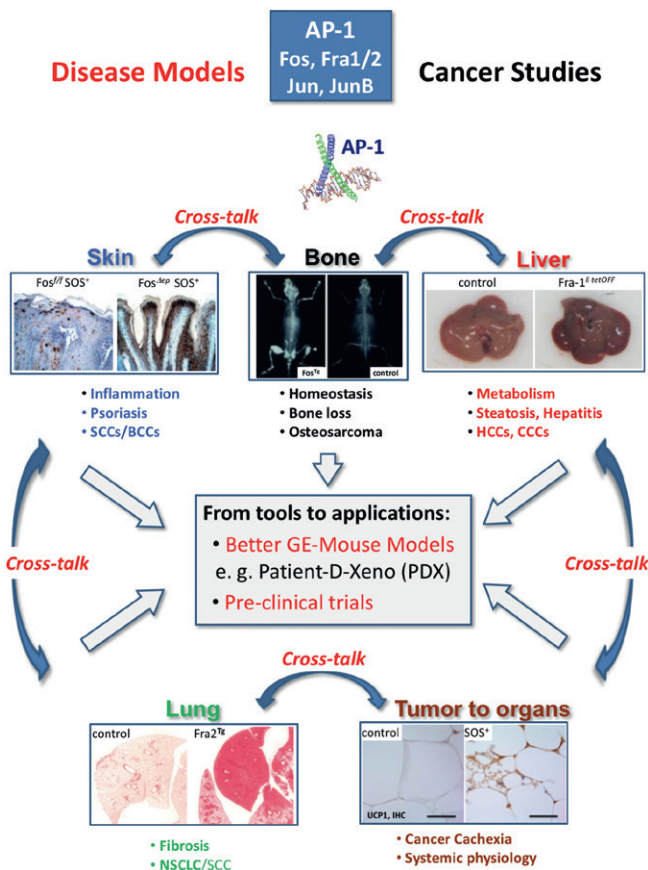


Figure Tet-switchable AP-1 transgenic mice were generated for ectopic expression of specific AP-1 monomers/dimers in skin, bone, liver and lung, which are complemented by loss-of-function mouse models. Proteomics, expression profiling, RNA-sequencing and ChIP-sequencing are employed to compare mouse models of disease to human disease as well as to identify

novel targets. Furthermore, we are investigating the systemic response of the mouse organism to a growing tumour in cancer cachexia. Preclinical studies are performed using different genetically engineered mouse models with compounds that target the identified molecules in order to determine the potential of translating our findings for the treatment of human disease.

Fos-dimers lead to altered cholesterol and bile acids metabolism, inflammation, fibrosis, hepatocyte/bile duct proliferation and tumours with HCC signatures. A robust connection between c-Fos expression and the activity of the LXR/RXR pathway, an important regulator of cholesterol homeostasis, was unravelled and most likely contributes to the oncogenic function of c-Fos in hepatocytes.

Cancer-associated cachexia (CAC)

We previously demonstrated that ‘browning’, a switch from white to brown fat, contributes to the wasting process in CAC and also documented the involvement of β -adrenergic signalling and IL-6 in this process. Using GEMMS and syngeneic transplantation models, we are dissecting the switch from a local inflammation-associated tumour to the systemic effects of CAC, with the ultimate aim to identify biomarkers and therapeutic targets. Our recent studies demonstrate a deregulation of the immune system with dramatic changes in lymphoid and myeloid populations. Ongoing studies in mice and in human samples aim to dissect the involvement of the central and peripheral nervous system, the Renin-Angiotensin-Aldosterone system, as well as the tissue-specific role of Ucp-1 during CAC development (in collaboration with Drs. R. Senarís, Spain, M. Petruzzelli, UK, H. Watzke, M. Poglitsch, and R. Zechner, Austria).

Defining a function for AP-1 in lung disease

Lung fibrotic diseases and non-small cell lung cancer (NSCLC) share some characteristics such as higher incidence in smokers, high morbidity and lack of effective treatments leading to high mortality. Using GEMMs we found that Fra/AP-1 proteins contribute to both diseases. While Fra-2 is associated with a fibrosis-specific innate immune response leading to disease progression, Fra-1 and Fra-2 promote tumour growth of K-Ras-induced NSCLC. Furthermore, Fra-2 expression is increased in lung fibrosis patient samples and correlates with poor survival in human NSCLC. Since AP-1 inhibition delayed lung fibrosis progression in preclinical models, our research focusses on deciphering the molecular mechanism and finding biomarkers and therapeutic targets downstream of AP-1. The lung fibrosis studies are conducted in collaboration with Daiichi Sankyo Company (Japan) and Genentech (USA), and the cancer studies with Mariano Barbacid’s Experimental Oncology Group and Luis Paz-Ares’ Lung Cancer Clinical Research Unit at the CNIO.

Skin cancer, inflammation and human disease

Characterisation of the epidermal inflammatory disease in mice lacking JunB suggests a skin to bone cross-talk. We reported that IL-17A production in skin causes bone loss by inhibiting

Wnt signalling in bone-forming osteoblasts, and showed that psoriasis patients suffer from bone loss that correlates with IL-17A levels. Epidermal-deficient JunB GEMMs also suffer from dysbiosis and chronic *S. aureus* colonisation, which is exacerbated in the absence of adaptive immunity. We are currently evaluating the role of the microbiota in skin inflammation using antibiotic treatments, high-throughput microbiota sequencing and are investigating the functional contribution of autophagy to controlling skin infections.

Comparative analyses in GEMMs and psoriatic patient samples unravelled novel molecules for targeted therapies, such as the antimicrobial proteins (AMPs) S100A8/A9, Lipocalin-2 and complement C3. We have generated several GEMMs to define the role of AMPs in inflammatory skin disease with a focus on the systemic effects beyond the skin in arthritis and bone loss. Although global deletion of S100A9 in the psoriasis-like mouse model alleviated skin inflammation and psoriatic arthritis, the cellular source of S100A9, with a crucial role in disease development and progression, is still unclear. Thus, we generated a new psoriasis-like GEMM with epidermal deletion of S100A9. Preliminary data show that keratinocyte-expressed S100A9 did not affect skin or joint inflammation, but reduced psoriatic-associated local bone loss. We are evaluating the role of keratinocyte-expressing S100A9 in these events using GEMMs and *in vitro* cultures.

Despite an important contribution in skin homeostasis and cancer, the role of epidermal stem cells (ESCs) in chronic inflammatory skin diseases is unclear. Using a lineage-tracking system in psoriasis-like GEMMs, we observed a differential behaviour of distinct subpopulations of epidermal cells, including keratinocytes and ESCs. RNA-sequencing revealed important changes in metabolism and extracellular matrix proteins in psoriatic-like ESCs. *In vitro* assays and human patient samples are being utilised to further dissect the contribution of these subpopulations to psoriasis.

Finally, using GEMMs for Squamous Cell Carcinomas (SCCs), we aim to identify therapeutic strategies for skin cancer prevention and to treat peri-neural invasion and metastasis. ■

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EPITHELIAL CARCINOGENESIS GROUP

Francisco X. Real
Group Leader

Staff Scientist
Victor J. Sánchez-Arevalo
(until June)



Post-Doctoral Fellows
Enrique Carrillo, Irene Felipe,
Eleonora Lapi, Miriam Marqués,
Sumit Paliwal (since November)

Graduate Students
Isidoro Cobo, Ana M. Maldonado
(since May), Irene Millán (since May),
Catarina Pereira, Mónica Pérez

Technicians
Itxaso Bellón (until November)
(TS)*, Miguel Deblas (since August),
Natalia Del Pozo, María Tania Lobato
(until June), Alba Ruiz (May-
September) (TS)*

**Titulado Superior (Advanced Degree)*

OVERVIEW

We focus on the molecular pathophysiology of pancreatic ductal adenocarcinoma (PDAC) and urothelial carcinoma (UC), with a disease-oriented approach. We use patient samples, cultured cells, and genetically modified mice, giving a similar weight to the 3 model systems. Primary observations made at either of these levels are then extended through additional work. To translate the findings, we bring this knowledge to a ‘population’ level, leveraging on information and samples from large patient cohorts.

In PDAC, a main hypothesis is that cell differentiation is a potent tumour suppressor mechanism acting early during carcinogenesis. We use the excellent genetic mouse models that are available because these processes cannot be readily studied using human samples. PDAC can originate both in pancreatic progenitors and in acinar cells. Understanding the contribution of early molecular events is crucial in order to design better strategies for early tumour detection and prevention in subjects at risk.

In UC, we focus on identifying new genes, using them for improved tumour taxonomy, characterising the mechanisms of action, and applying this knowledge for improved prediction of outcome and therapy.

“We exploit mouse models to assess the role of transcription factors as modulators of KRas-driven PDAC, thereby providing clues on early tumorigenesis. We use urothelial organoid cultures to study the mechanisms of action of new bladder cancer genes.”

RESEARCH HIGHLIGHTS

Pancreas cancer molecular pathophysiology

While the genetic/genomic changes associated with PDAC have been extensively described in the last few years by the genome consortia, the precursor lesions and molecular changes that precede tumour development are less well established. We have acquired extensive evidence indicating that incomplete acinar cell differentiation is associated with a scenario of pre-inflammation or inflammation and with predisposition to PDAC development using mutant *KRas*-driven genetic mouse models. These studies provide the basis for the pharmacological – or genetic - manipulation of acinar differentiation as a tumour preventative strategy.

One of the transcription factors we focused on is NR5A2, a member of the nuclear receptor family for which putative endogenous ligands as well as pharmacological agonists have recently been identified. *Nr5a2* germline heterozygosity leads to no overt pancreatic phenotype but it is associated with a pre-inflammatory state that sensitises mice to the oncogenic effects of mutant *KRas*. Interestingly, *Nr5a2* pancreas-specific heterozygosity – in conjunction with activation of mutant *KRas* in the pancreas (KPN^{+/-} mice) – leads to a progressive loss of the exocrine parenchyma, development of cystic tumours with variable degrees of mucinous differentiation, dysplasia, and to adenocarcinoma (FIGURE 1). In patients, cystic tumours have variable risks of undergoing malignant transformation and are becoming a very important clinical problem because they are being increasingly diagnosed in healthy adults thanks to improved imaging techniques. We plan to exploit this new mouse model of pancreatic cystic tumours in order to explore, in collaboration with B. Gauthier (CABIMER, Sevilla), whether pharmacological stimulation of Nr5a2 activity can prevent the Nr5a2 haploinsufficient phenotype in the context of both pancreatitis and cancer.

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

Our main goals are to refine current knowledge on the genomic landscape of UC and to apply this in the clinical setting.

Through exome sequencing we identified mutations in *STAG2*, coding for a cohesin subunit, and in *RBM10*, coding for a splicing regulator, as new UC genes that are more broadly involved in human cancer. *STAG2* inactivation in UC is not associated with aneuploidy, suggesting that regulation of chromatin architecture and gene expression mediate its tumour suppressor role. In collaboration with Ana Losada, we have developed a conditional *Stag2* knockout strain; the cooperation of *Stag2* inactivation with other genetic alterations occurring in UC (i.e. *Pik3ca*, *Hras*, and *Tp53*) is being analysed.

RBM10 is mutated in several epithelial tumours. Its inactivation in UC is not associated with stage or grade, but it occurs mainly in tumours with urothelial differentiation. In collaboration with J. Valcárcel (CRG, Barcelona), we have generated a conditional *Rbm10* knockout strain and are analysing the molecular mechanisms through which this gene contributes to UC development using a combination of cellular, molecular and bioinformatics approaches.

These studies are complemented with the use of normal murine urothelial organoids, for which we have established robust culture methods and have shown their ability to undergo urothelial differentiation. In addition, we are expanding these studies to human bladder cancers in order to develop precision medicine strategies.

Within the context of a project funded by the AECC, we have analysed whether UC molecular taxonomy is able to predict response to neoadjuvant chemotherapy (NAC). Patients with tumours having a Basal/Squamous (BASQ)-like phenotype

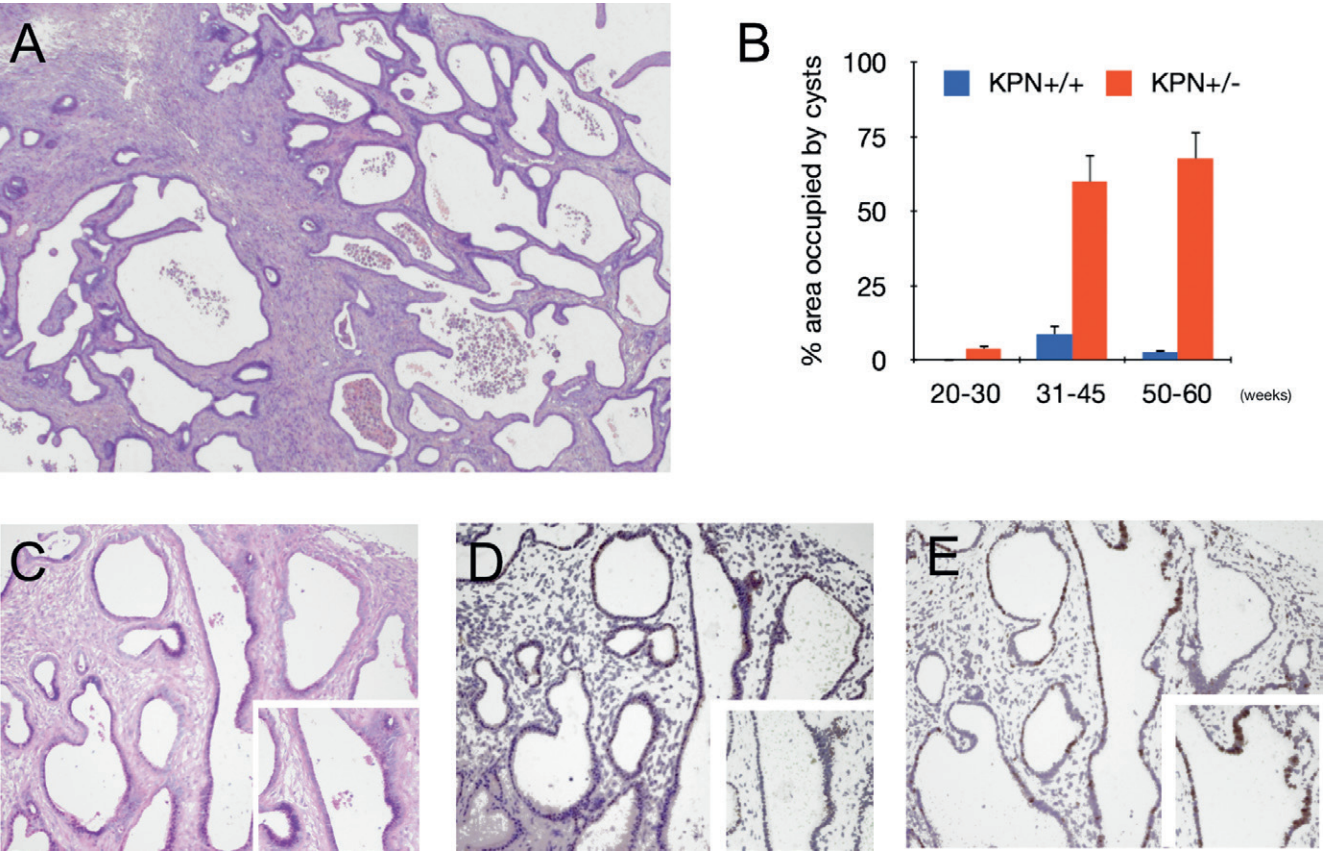


Figure 1 Nr5a2 pancreatic haploinsufficiency cooperates with mutant *Kras* in the development of pancreatic cystic lesions. (A) Histological aspect of the pancreas of KPN^{+/-} mice at 50 weeks of age, showing the replacement of the exocrine parenchyma by cystic lesions. (B) Quantification of the area occupied by cystic lesions in the pancreas of KPN^{+/-} and KPN^{+/+} mice in relationship with age. (C) PAS/Alcian blue staining showing the presence of mucin-producing cells. (D, E) Immunohistological analysis of Ki67 and p53 in the pancreas of 50 week-old KPN^{+/-} mice.

defined by immunohistochemistry are almost 4 times more likely to achieve a pathological complete response to platinum-based NAC. These studies will guide new clinical trials including molecular stratification criteria. This work is carried out in

collaboration with Núria Malats, A. Font, D. Castellano, and an extended group of Spanish uro-oncologists. ■

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morbidities with pancreatic cancer risk. *Ann Oncol* 28, 1618-1624.

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Book Chapter

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AWARDS AND RECOGNITION

Associate Editor, *Gut*.

Scientific Advisory Board Member, CIT Programme, *Ligue Nationale Contre le Cancer*, France.

Science Committee Member, Cancer Research UK, London, UK.

EPITHELIAL CELL BIOLOGY JUNIOR GROUP

Mirna Pérez-Moreno
(until December)
Junior Group Leader

Post-Doctoral Fellow
Silvia M. Janeiro (until December)

Graduate Student
Daniel Peña (until December)



OVERVIEW

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

“During 2017, we continued our efforts to uncover novel events controlling the behaviour of skin stem cells in order to open new insights into the mechanisms that control their regenerative characteristics, and how, when disrupted, they can lead to cancer.”

skin homeostasis, regeneration, and when perturbed lead to cancer. This information may provide insights for the future development of regenerative and anti-cancer therapies.

RESEARCH HIGHLIGHTS

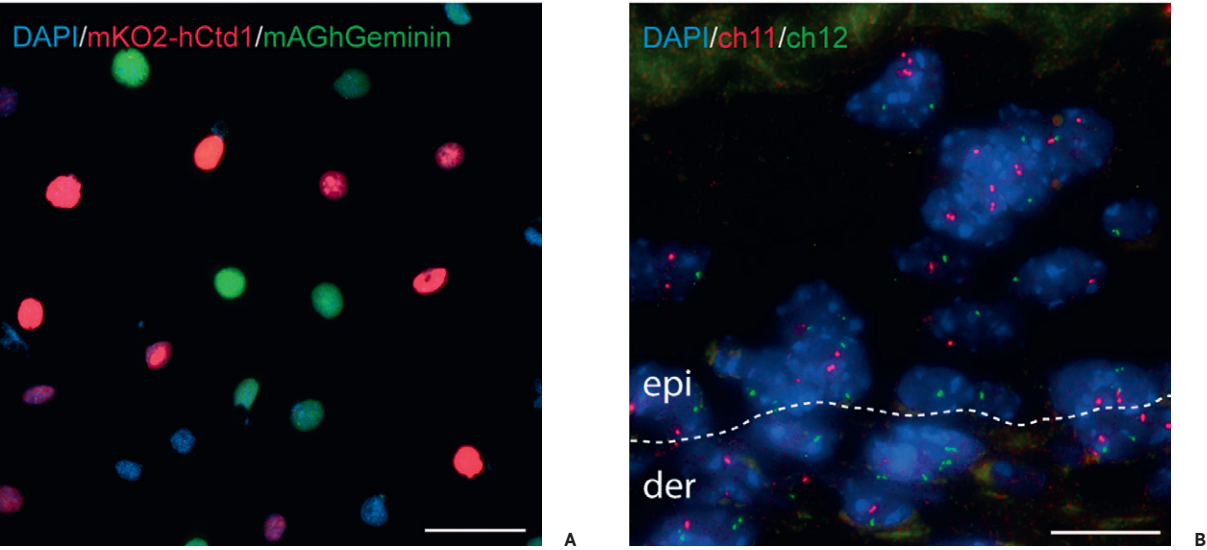


Figure (A) Mouse Keratinocytes expressing mKO2-hCtd1 (G1) and mAGhGeminin (S/G2/M phase). Scale bar 50 μ m. (B) FISH analysis for chromosomes 11 and 12 in newborn mouse skin. Scale bar 10 μ m.

Regulation of epidermal progenitor cells self-renewal and differentiation

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

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

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

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

The formation of tumours and their progression to malignancy undoubtedly involves the contributions of the tumour macroenvironment. Identifying the signalling mechanisms and cell types that contribute to tumour initiation and progression

to malignancy is instrumental for detecting potential targets for clinical applications in order to eradicate tumours.

The macroenvironment of many tumours is rich in cytokines, chemokines, and inflammatory enzymes. During 2017, we continued to explore the role of diverse cell-derived soluble mediators in modulating proliferation, migration and survival of skin cancer stem cells.

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

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► **AWARDS AND RECOGNITION**

► Ambassador of the American Society for Cell Biology, ASCB, USA.

GROWTH FACTORS, NUTRIENTS AND CANCER JUNIOR GROUP

Nabil Djouder
Junior Group Leader

Post-Doctoral Fellows
Hugo Bernard, Sebastián Thompson
(since February)



OVERVIEW

Research conducted over the last decade has focused mainly on understanding the functions and the role of newly discovered mutated genes in the development of various diseases, including cancer. Exposure to environmental factors through the regulation and expression of virulent eukaryotic proteins is an often ignored permanent challenge for an organism. Therefore, our laboratory devotes effort towards understanding the molecular mechanisms linking environmental stresses to disease pathogenesis.

Based on the integration of experimental mouse models combined with the use of human data and nanotechnology, we aim to provide a comprehensive study for a rational approach towards the development of novel mechanism-based diagnostics and therapeutics to detect, prevent and treat diseases.

“Our research directive is to understand mechanisms of diseases by using newly generated mouse models that recapitulate pathological features of human syndromes in order to guide early diagnosis and treatment.”

Graduate Students
Marta Brandt (until May),
Almudena Chaves, Sergio de
la Rosa (since March) (PEJ)*,
Amanda Garrido, Tatiana Grazioso,
Ana Teijeiro

Technician
Eva Martínez (PEJ)*

*Plan de Empleo Joven (Youth Employment
Plan)

RESEARCH HIGHLIGHTS

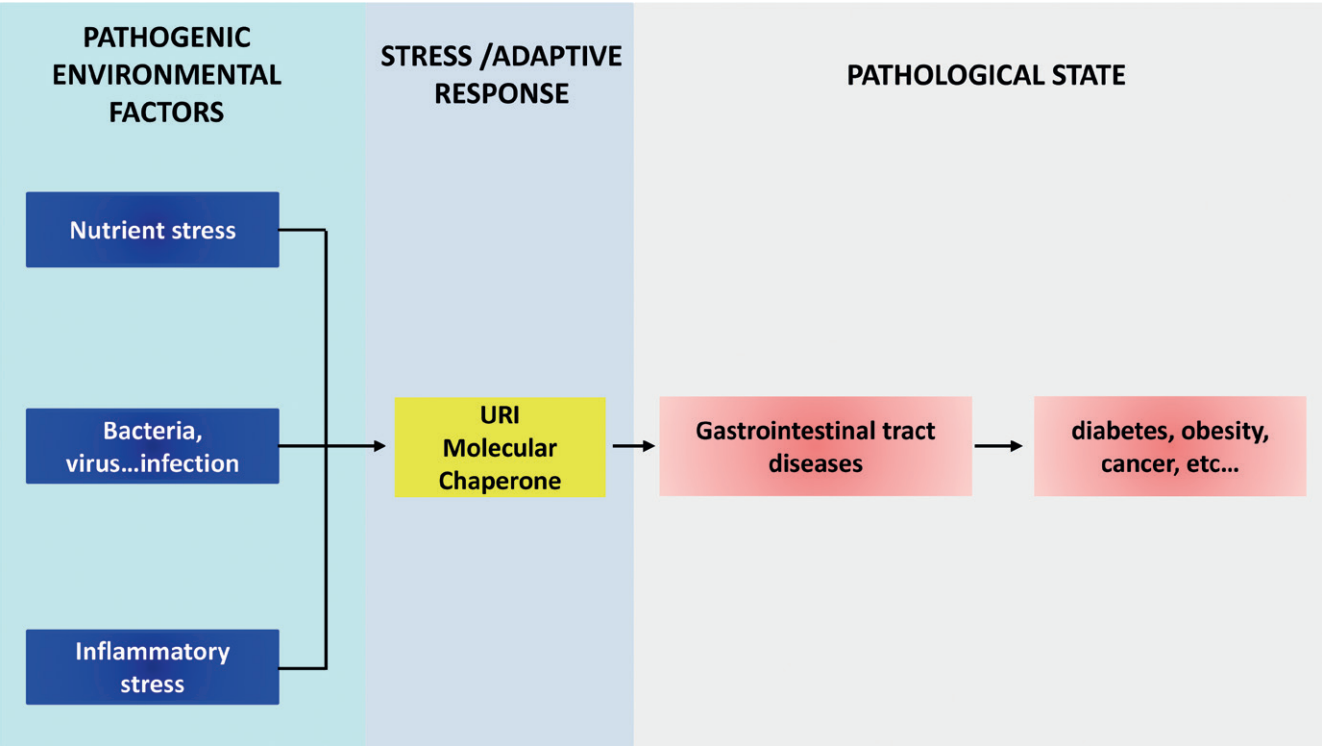


Figure Representation of our research directive. Our laboratory is mainly interested in understanding how pathogenic environmental factors lead to diseases associated with the gastrointestinal tract. Environmental stress modulates URI expression which may have buffering activities to maintain cellular homeostasis.

Pathogenic factors such as poor diets (under-nutrition or micronutrient deficiencies), nutrient overload, alcohol consumption, high-fat and low-fibre diets, ionising radiations, bacteria, virus infection, etc., are risk factors for diseases. How these environmental factors can alter the host's eukaryotic epithelial cells to cause various pathologies, potentially progressing to cancer, remains largely unknown. Finding new genes affected by environmental stressors, and understanding their functions and role in disease development, may pave the basis of future therapies. Therefore, we specifically focus on the identification of likely causal links between environmental stresses and pathologies, potentially progressing to cancer, and their diagnostics and treatment (theranostics) through the use of nanotechnology.

Molecular chaperones are essential to engage protective mechanisms to ensure cellular and protein homeostasis caused

by injurious environmental stimuli. Unconventional prefoldin RPB5 interactor (URI) is a co-chaperone whose expression is modulated by various pathogenic environmental factors. Using URI mouse models generated in our laboratory, combined with various genetic murine models and cutting-edge technologies, we are studying diseases predominantly associated with the gastrointestinal tract (FIGURE). ■

► **PUBLICATIONS**

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SEVE BALLESTEROS FOUNDATION-CNIO BRAIN TUMOUR JUNIOR GROUP

Massimo Squatrito
Junior Group Leader

Staff Scientists
Bárbara Oldrini, Alberto J.
Schuhmacher (until June)



OVERVIEW

A decade of studies has underlined the complexity of the genetic events that characterise the genomic landscapes of common forms of human cancer, including gliomas. While a few cancer genes are mutated at high frequencies (>20%), the greatest number of cancer genes in most patients appear at intermediate frequencies (2–20%) or lower. Strikingly, the functional significance of the vast majority of these alterations still remains elusive. A current high priority in glioma research is to functionally validate candidate genetic alterations in order to distinguish those that are significant for cancer progression and treatment response.

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

“The main focus of our Group is to uncover the genetic alterations present in glioma patients that are responsible for the aggressiveness and the poor treatment response of these tumours.”

Graduate Students
Carolina Almeida, Alvaro Curiel,
Veronica Matia

Technician
Claudia S. Troncone (until October)

RESEARCH HIGHLIGHTS

Precision glioma mouse models

It has gradually been established that the vast majority of human tumours are extraordinarily heterogeneous at a genetic level. To accurately recapitulate this complexity, it is now evident that *in vivo* animal models of cancer will need to recreate not just a handful of simple genetic alterations, but possibly dozens and increasingly intricate. In our laboratory, by combining the RCAS/TVA and CRISPR/Cas9 systems, we have developed novel mouse models for *in vivo* somatic genome editing that allow the targeting of specific cell types with definite genetic alterations in order to generate precision tumour models.

We have shown that somatic deletion in neural stem cells (NSCs) of a variety of known tumour suppressor genes (*Trp53*, *Cdkn2a* and *Pten*), in combination with the expression of an oncogene driver, leads to glioblastoma (GBM) formation. Using this approach, we are currently performing *in vivo* guide RNA (gRNA) screenings to identify novel tumour suppressor genes that contribute to gliomagenesis.

Gene fusions have been documented as cancer-drivers for more than three decades, providing valuable insights into the tumorigenesis process. The occurrence and importance of gene fusions in glioma has been appreciated only recently – largely due to high-throughput technologies – and gene fusions have been indicated as one of the major genomic abnormalities in GBM. By simultaneous delivery of pairs of gRNAs we generated different gene fusions, either by chromosomal deletion (*Bcan-Ntrk1*) or by chromosomal translocation (*Myb-Qk*), and we have shown that they have transforming potential *in vitro* and *in vivo*.

Lastly, using homology-directed-repair (HDR), we also produced tumours carrying the BRAF V600E mutation, frequently identified in a variety of subtypes of gliomas.

In summary, we have developed an extremely powerful and versatile mouse model for *in vivo* somatic genome editing, which will elicit the generation of more accurate cancer models particularly appropriate for pre-clinical testing. ■

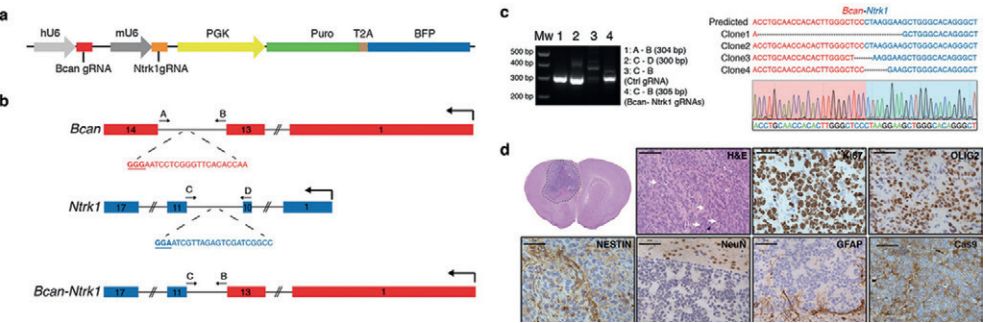


Figure *Bcan-Ntrk1* gene fusion drives high-grade glioma formation. (a) RCAS-gRNA-pair vector expressing the *Bcan* and *Ntrk1* gRNAs. (b) Schematic representation of the *Bcan* and *Ntrk1* gene loci and the *Bcan-Ntrk1* gene fusion. (c) PCRs were performed with the specified primers on genomic DNA extracted from the *Bcan-Ntrk1* expressing cells. (d) Characterisation of the *Bcan-Ntrk1*-induced tumours.

PUBLICATIONS

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E, Sauvé CG, Olar A, Lan ZD, Finocchiaro G, Phillips JJ, Berger MS, Gabrusiewicz KR, Wang G, Eskilsson E, Hu J, Mikkelsen T, DePinho RA, Muller F, Heimberger AB, Sulman EP, Nam DH, Verhaak RGW (2017). Tumor evolution of glioma intrinsic gene expression subtype associates with immunological changes in the microenvironment. *Cancer Cell* 32, 42-56.

- Oldrini B, Hsieh WY, Erdjument-Bromage H, Codega P, Carro MS, Curiel-Garcia A, Campos C, Pourmaleki M, Grommes C, Vivanco I, Rohle D, Bielski CM, Taylor BS, Hollmann TJ, Rosenblum M, Tempst

P, Blenis J, Squatrito M, Mellinghoff IK (2017). EGFR feedback-inhibition by Ran-binding protein 6 is disrupted in cancer. *Nat Communications* 8, 2035.

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STRUCTURAL BIOLOGY PROGRAMME

ÓSCAR LLORCA (since July) Programme Director



Programme's research areas and strategic goals

The main goal of the Structural Biology Programme is to use structural information to provide mechanistic understanding at the molecular level of how proteins and macromolecular complexes related to cancer function and, ultimately, to use the new mechanistic insights to help guide future drug design. Collaborations and synergies with other Programmes within the CNIO and the combination of different methodologies are essential to achieve these aims.

2017 has been a year of change for the Programme. The Groups headed by Alfonso Valencia and Santiago Ramón-Maiques have moved to different institutions, and the National Bioinformatics Institute Unit, headed by Salvador Capella, also left the CNIO during 2017. Alfonso Valencia, who led the Programme until February, was also responsible for the Structural Computational Biology Group, contributing, among other aspects, to large-scale genome projects. During Alfonso Valencia's directorship of the Programme several organisational changes took place; in 2016, he managed the selection of candidates for two Junior Group Leader positions, the reorganisation of the Bioinformatics Unit, and the installation of a new cluster, acquired in 2016 but installed during 2017. During their stay at CNIO, Santiago Ramon's Group (Structural Bases of Genome Integrity Group) helped to advance our understanding of genome integrity; they focused mainly on the structure and function of CAD, a key component in the metabolism of pyrimidines.

We are very thankful to all the above mentioned Groups for their dedication and their contribution to the high-level science conducted at the CNIO. We wish them all the best in their new ventures.

Currently, the Programme has several Core Units: Spectroscopy and NMR, Electron Microscopy (EM), Bioinformatics, Crystallography and Protein Engineering, and Biological Text Mining. These Units provide access, maintenance and expertise for technologies in Structural Biology and Bioinformatics. Units are an essential element for the Programme and their activities are vital to facilitate the access to Structural methods for non-experts.

During 2017, three new groups joined the Structural Biology Programme; it now counts three Junior and one Senior Research Groups. The Junior Groups headed by Daniel Lietha and Iván Plaza Menacho, work on structural and mechanistic aspects in cancer cell signalling and protein kinases, with emphasis on the search for new inhibitors. The Junior Group headed by Rafael Fernández-Leiro, and the Senior Group, led by Óscar Llorca, work on genome instability and DNA repair pathways.

“In 2017, we solved several structures of proteins involved in cancer. The implementation of Cryo-EM will place us at the forefront of structural studies and drug discovery to fight against cancer.”

Frontier Structural Biology in cancer requires a strong technical component in cryo-electron microscopy (cryo-EM). Incorporating the so-called ‘resolution revolution’ in cryo-EM – now capable of observing macromolecules at high resolution – is the Programme's main strategic goal for 2017 and 2018. The Programme has recruited two experts in cryo-EM, Rafael Fernández-Leiro and Óscar Llorca, who are working together with the EM Unit to set up an upgraded facility for high-resolution cryo-EM. A new electron microscope with a direct electron detector suitable for high-resolution studies of proteins and complexes is now being acquired. The synergy of cryo-EM with other Structural Biology technologies already in place in the Programme will facilitate the exploitation of the molecular and structural understanding of biological mechanisms for its application in drug design/discovery.

Summary of milestones & major achievements of the Programme during 2017

During 2017, Moreno-Morcillo and colleagues from Santiago Ramón's Group provided clues to the assembly of the multifunctional protein CAD in the July issue of the journal *Structure*. Le Coq and colleagues from Daniel Lietha's Group published in the August issue of *eLife*, new crystal structures of SH2-containing-inositol-5-phosphatases, proteins that play important roles in regulating the PI3K/Akt pathway in physiology and disease.

Iván Plaza Menacho, Rafael Fernández-Leiro and Óscar Llorca joined the Programme in 2017. We live in exciting times at the Structural Biology Programme; new groups have been recruited and the cryo-electron microscopy equipment is being upgraded. The prospect of using images of single molecules to obtain high-resolution information of complexes will become a reality at CNIO.

MACROMOLECULAR COMPLEXES IN DNA DAMAGE RESPONSE GROUP

Óscar Llorca (since July)
Group Leader

Staff Scientists
Javier Coloma (since December),
Andrés López (since December),
Ángel Rivera (since December),
Marina Serna (since November)



Visiting Post-Doctoral Fellow
Hugo Muñoz (since August)

Visiting Master Student
Adrián Del Rincón (since September)

Visiting Graduate Student
Carlos Fernández (since September)

OVERVIEW

Avoiding cancer requires active DNA repair mechanisms. In addition, tumour progression needs down-regulation of the cell response to DNA damage. Large and dynamic macromolecular complexes perform many of the activities of the DNA damage response. We cannot fully understand these biological reactions without understanding the structure of the participating molecules and how they interact. Our aim is to study the structural architecture of these large complexes and understand how they function. We are interested in several processes of relevance in cancer, including DNA repair, DNA damage signalling, DNA replication, and RNA quality surveillance mechanisms. We use cryo-Electron Microscopy (cryo-EM) as our main structural technique. Cryo-EM methods are revolutionising Biology and they are at the frontier of drug discovery. We can now explore the structure of complexes at close-to-atomic resolution by observing individual molecules in the electron microscope.

“We use cryo-EM to provide new mechanistic insights of how Hsp90 assists in the maturation of the phosphatidylinositol-3-kinase-like kinases (PIKKs), relevant for targeted cancer therapy.”

RESEARCH HIGHLIGHTS

Hsp90-dependent maturation of phosphatidylinositol-3-kinase-like kinases (PIKKs)

The family of phosphatidylinositol-3-kinase-like kinases (PIKKs) comprises several proteins, including ATM, ATR, DNA-PKcs and mTOR, considered important molecular targets for cancer therapy. It has recently been appreciated that the assembly and maturation of active and functional PIKK complexes requires Hsp90 and a dedicated co-chaperone, the R2TP complex. Interestingly, the R2TP and Hsp90-mediated maturation of PIKKs is a regulated process in the cell, opening up an opportunity to inhibit PIKKs by interfering with their maturation. With that final goal in mind, we have started to address the structural and mechanistic understanding of human R2TP. The comparison between human and yeast R2TP complexes offers some relevant clues about the function of this complex, and in 2017, we reported the structural architecture of R2TP from yeast (Rivera-Calzada *et al.*, *Structure* 2017). This work was performed in collaboration with Laurence H. Pearl (Genome Damage and Stability Centre, University of Sussex, UK).

R2TP is one of the most complex of the various Hsp90 co-chaperones identified so far: being a multi-protein complex itself, and possessing components (RUVBL1/Rvb1 and RUVBL2/Rvb2 in humans/yeast) that like Hsp90 possess inherent ATPase activity of their own. We have described the architecture and catalytic properties of the yeast R2TP complex using a combination of cryo-electron microscopy, structural mass spectrometry, and biochemistry. Our data provide structural and mechanistic insights of how R2TP couples an Hsp90 dimer to a PIKK kinase (FIGURE). The structural and functional characterisation of the human R2TP complex is now underway, revealing an unsuspected structural and functional complexity compared to the simpler yeast model.

Electron microscopy as a tool to help understanding human diseases as well as contributing to the development of new potential therapies

As part of our collaboration with the Santiago Rodriguez de Córdoba's group at the *Centro de Investigaciones Biológicas* (CIB), we used electron microscopy (EM) methods to contribute to the understanding of how several mutations identified in patients can cause diseases linked to the complement system, a component of innate immunity. For this, we analysed the structure of components of the complement system using EM. In addition, electron microscopy was used as part of an extensive characterisation of several monoclonal antibodies with potential therapeutic and diagnostic applications in diseases related to mutations and polymorphisms in proteins of the complement system (Subias Hidalgo *et al.*, *Eur J Immunol* 2017). The EM structures of each antibody bound to its target protein allowed the identification of the epitopes in the 3D structure of the target. Also, these structures provided models to explain the functional properties of the antibodies as defined by Rodriguez de Córdoba's group. These works highlight the potential of synergising methods of structural biology with genetic, functional and clinical studies, in order to understand and cure diseases. ■

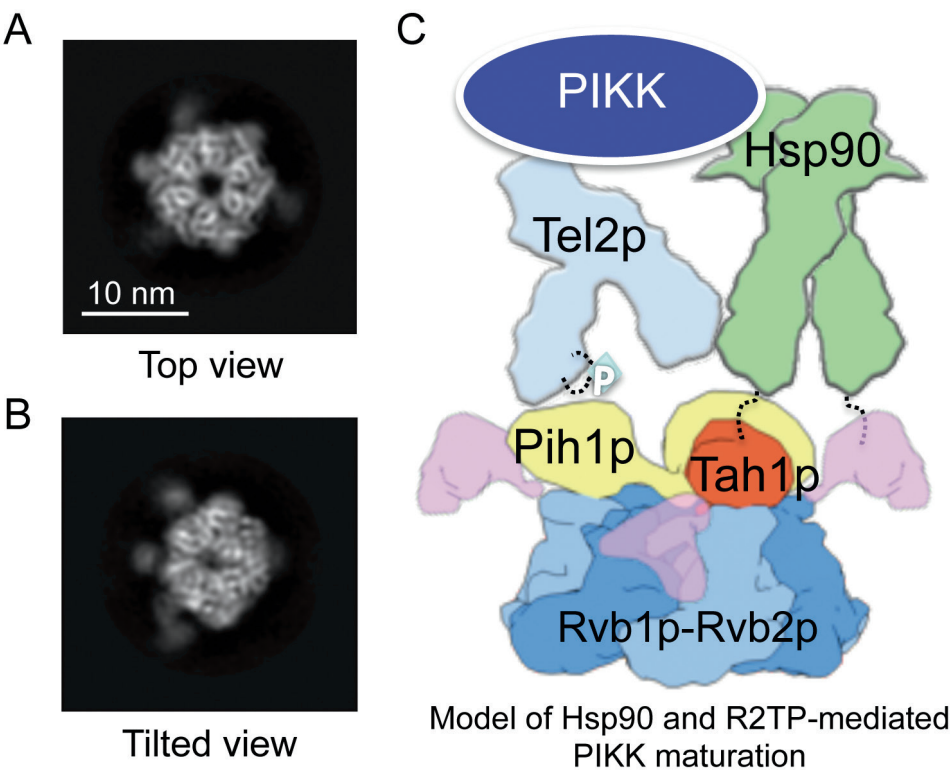


Figure Structure of the yeast R2TP complex. Top (**A**) and tilted (**B**) views of the R2TP complex obtained using cryo-EM. Scale bar represents 10 nm. (**C**) Model of the Hsp90 and R2TP-mediated assembly of the PIKK family of kinases, based on the findings by Rivera-Calzada *et al.* (2017). R2TP functions as a

platform that brings Hsp90 and the client proteins in proximity, allowing Hsp90 action on the PIKKs. The role of ATP hydrolysis by Hsp90 and the Rvb1-Rvb2 ATPases is not yet understood, but we find that R2TP assembly activates ATP hydrolysis by Rvb1-Rvb2.

PUBLICATIONS

- ▶ Huecas S, Ramirez-Aportela E, Vergoños A, Nuñez-Ramírez R, Llorca O, Diaz JF, Juan-Rodríguez J, Oliva MA, Castellén P, Andreu JM (2017). Self-Organization of FtsZ Polymers in Solution Reveals Spacer Role of the Disordered C-Terminal Tail. *Biophysical J* 113, 1831-1844.

PUBLICATIONS AT OTHER INSTITUTIONS

- ▶ Rivera-Calzada A, Mohinder P, Muñoz-Hernandez H, Luque-Ortega JR, Gil-Carton

D, Degliesposti G, Skehel JM, Prodromou C, Pearl LH and Llorca O (2017). The structure of the R2TP complex defines a platform for recruiting diverse client proteins to the HSP90 molecular chaperone system. *Structure* 25, 1145-1152.

- ▶ Subías Hidalgo M, Yébenes H, Rodríguez-Gallego C, Martín-Ambrosio A, Domínguez M, Tortajada A, Rodríguez de Córdoba S, Llorca O (2017). Functional and structural characterization of four mouse monoclonal antibodies to complement C3 with potential therapeutic and diagnostic applications. *Eur J Immunol* 47, 504-515.

- ▶ Fuentes-Pérez ME, Núñez-Ramírez R, Martín-González A, Juan-Rodríguez D, Llorca O, Moreno-Herrero F, Oliva MA (2017). TubZ filament assembly dynamics requires the flexible C-terminal tail. *Sci Rep* 7, 43342.
- ▶ López-Perrote A, Harrison RE, Subías M, Alcorlo M, Rodríguez de Córdoba S, Morikis D, Llorca O (2017). Ionic tethering contributes to the conformational stability and function of complement C3b. *Mol Immunol* 85, 137-147.

Book Chapter

- ▶ Fernandez-Rodriguez C, Llorca O, Martino F (2017). *Transmission Cryo-electron Microscopy in Drug Discovery: In: Biophysical Techniques in Drug Discovery*. Publisher: Royal Society of Biochemistry. DOI: 10.1039/9781788100016.

CELL SIGNALLING AND ADHESION JUNIOR GROUP

Daniel Lietha
Junior Group Leader

Post-Doctoral Fellows
Iván Acebrón, Johanne Le Coq

Graduate Students
Marta Acebrón, José Vicente
Velázquez

Technician
Pilar Redondo



OVERVIEW

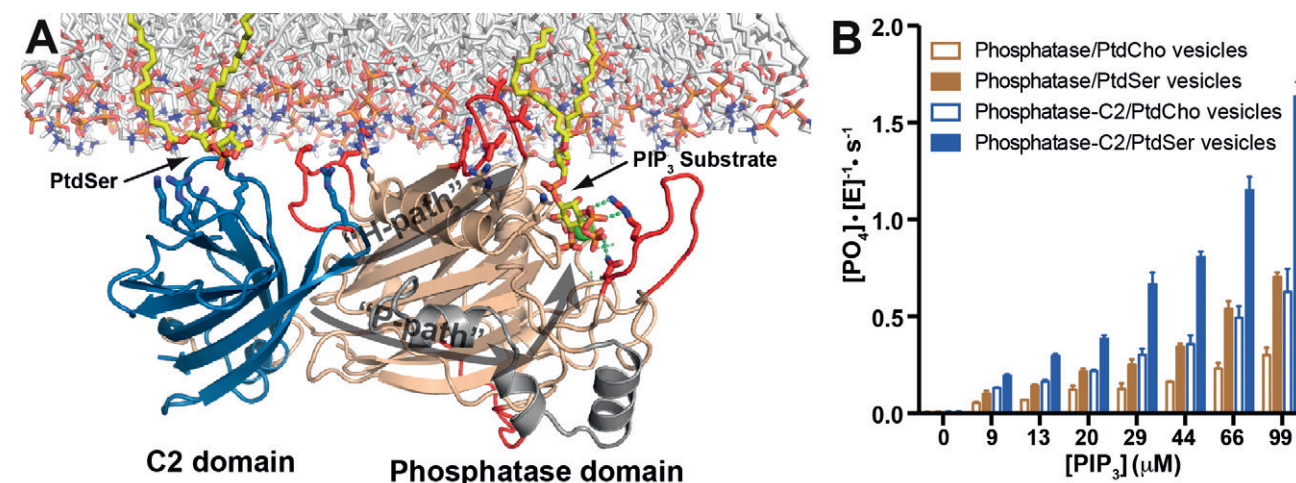
Our Group studies regulatory mechanisms of key signalling switches controlling growth and adhesion signals. Such signals regulate important cellular processes such as proliferation, adhesion and survival. We use structural techniques, such as X-ray crystallography and electron microscopy, in combination with biochemical and functional studies to understand these mechanisms at atomic detail and to rationalise how oncogenic events result in their deregulation. The structural understanding allows us to design potential anti-cancer therapeutics that interfere with oncogenic deregulation.

We focus on growth and adhesion signalling systems that interact and are regulated by specific lipids in the plasma membrane. Specifically, we pursue two main questions: (i) how are adhesion signals in focal adhesion complexes triggered by membrane

“We identify detailed structural and mechanistic insights in order to understand how growth and adhesion signals are triggered to cause tumour invasion, and we use this information for high-specificity drug design.”

interactions of Focal Adhesion Kinase? and (ii) how are cellular growth signals regulated by changes in phosphatidylinositol (3,4,5)-trisphosphate (PIP₃) levels affecting signalling of the Akt pathway?

RESEARCH HIGHLIGHTS



We discovered that Focal Adhesion Kinase (FAK) interacts with PIP₂ lipids at cell adhesion sites and that this induces formation of large FAK oligomers on the membrane. This triggers conformational changes in FAK resulting in FAK autophosphorylation, Src recruitment and, in turn, full FAK activation via phosphorylation by Src. Currently, we are studying the atomic architecture of FAK oligomers bound to lipid membranes by electron microscopy, and are investigating how force, induced at focal adhesion sites by actomyosin contraction, induces changes to these structures to activate focal adhesion signalling. We utilise these mechanistic insights to discover highly specific allosteric FAK inhibitors. We employ experimental and virtual screening using fragment based approaches to identify allosteric ligands, and then use a structure based approach to develop these fragments into inhibitory lead compounds.

SH2-domain-containing inositol 5-phosphatases (SHIP) remove the 5-phosphate from PIP₃ and thereby, like PTEN, negatively regulate PIP₃ levels in the plasma membrane. Despite their importance, little is known about the mechanisms of SHIP regulation. We solved a crystal structure containing the catalytic and C2 domains of SHIP2, showing an extensive interface between

the two domains. We have shown that the C2 domain of SHIP2 binds phosphatidylserine (PtdSer), which positions the active site towards its membrane substrate for increased catalytic efficiency (FIGURE). In addition, although the C2 domain interacts far from the active site, we have found that the C2 allosterically induces strong catalytic activation of SHIP2. We employed molecular dynamics simulations to guide a mutagenesis study that has identified distinct allosteric signalling pathways emanating from hydrophobic or polar interdomain interactions, which differentially affect lipid chain or head group regions of the substrate. In addition, through cell biology experiments, we have confirmed that mutations at the domain interface affect downstream signalling to Akt. ■

PUBLICATIONS

- Le Coq J, Camacho-Artacho M, Velázquez JV, Santiveri CM, Gallego LH, Campos-Olivas R, Dölker N, Lietha D (2017). Structural basis for interdomain communication in SHIP2 providing high phosphatase activity. *Elife* 6, e26640.
- Dao P, Lietha D, Etheve-Quelquejeu M, Garbay C, Chen H (2017). Synthesis of novel 1,2,4-triazine scaffold as FAK inhibitors with antitumor activity. *Bioorg Med Chem Lett* 27, 1727-1730.

STRUCTURAL BASES OF GENOME INTEGRITY JUNIOR GROUP

Santiago Ramón-Maiques
(until February)
Junior Group Leader

Post-Doctoral Fellow
Maria Dolores Moreno



OVERVIEW

Safeguarding genome integrity is essential for correct cell functioning and to prevent cancer. Our Group is interested in having a good understanding of central cellular processes affecting the integrity of the genome, such as the metabolism of nucleotides, replication, recombination and repair of DNA, and the maintenance and recognition of chromatin architecture. These tasks are performed by proteins and other macromolecular components that associate to form complex and fascinating cellular machines. We combine protein engineering, X-ray crystallography, single-particle electron microscopy, together with biochemical and functional studies, in order to understand the structure and function of these macromolecular complexes at the atomic level. This knowledge should guide us in the design of compounds to modulate the activity of these machines, providing new opportunities and strategies for fighting tumours.

“We provided new insight into the architecture of CAD, the antitumoural target initiating and controlling *de novo* biosynthesis of pyrimidines. We proved that CAD is a dimer of trimers and that the DHO and ATC domains form the central supporting framework of the 1.5 MDa CAD particles.”

Graduate Student
Francisco Del Caño

Technician
Araceli Grande (until August) (TS)*

**Titulado Superior* (Advanced Degree)

RESEARCH HIGHLIGHTS

The metabolism of cancer cells is characterised by an increased rate of *de novo* synthesis of pyrimidines, which fuel DNA and RNA synthesis during rapid cell growth. In animals, the *de novo* pathway is initiated and controlled by CAD, a multi-functional protein with four enzymatic domains: glutaminase (GLN), carbamoyl phosphate synthetase (CPS), aspartate transcarbamoylase (ATC) and dihydroorotase (DHO). Recent reports that CAD up-regulation is modulated by phosphorylation during cell growth and proliferation, and the finding of the first CAD-deficits in children with glycosylation problems, developmental delays or epilepsy, have raised the interest in this protein for therapeutic intervention.

Over the past few years, we determined the structures of the DHO and ATC domains of human CAD, revealing the first atomic glimpse of a complex machinery. However, CAD forms ~1.5 MDa hexamers and to understand the functioning of this mega-enzyme we need to explain how the DHO and ATC domains assemble together and interact with the other enzymatic domains.

Now, we show that a region of CAD, spanning the DHO and ATC domains including the long linker holding the mTORC1 phosphorylation site, self-associates into a ~500 kDa complex. We prove that this sub-complex is a dimer of trimers and that it is conserved in the CAD-like from fungi, which has an inactive DHO-like domain. We solved the crystal structures of the ATC and inactive DHO-like domains of the fungus *Chaetomium thermophilum*, thereby showing the similarity with the human CAD homologues. Based on the conserved structural features, we proposed a model for the architecture of CAD, in which the DHO-ATC complex is the central element of the particle. The model helps to explain the intricate functioning of CAD, such as the channelling of metabolites and the communication of conformational changes between domains. This knowledge has enabled us to map clinical mutations to the structural domains, in turn helping us to understand the molecular bases of the first diagnosed CAD diseases. ■

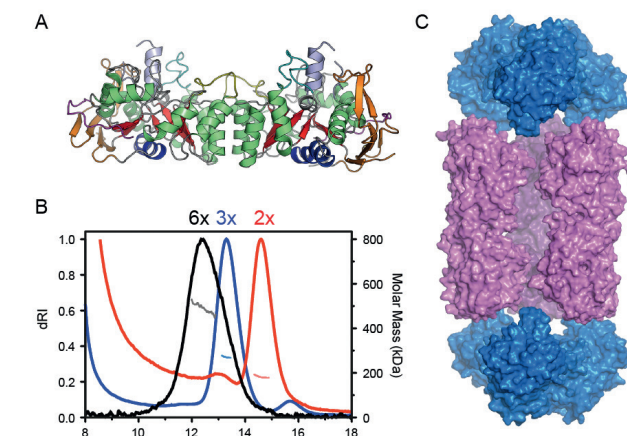


Figure (A) Crystal structure of the dimeric inactive DHO domain of *Chaetomium thermophilum* CAD-like protein. (B) SEC-MALS analysis of DHO-ATC mutants proves that CAD assembles as a dimer of trimers. (C) Model of the central core of the CAD particle, with two ATC trimers (in blue) joined by three DHO dimers (in pink).

PUBLICATION

- Moreno-Morcillo M, Grande-García A, Ruiz-Ramos A, Del Caño-Ochoa F, Boskovic J, Ramón-Maiques S (2017). Structural insight into the core of CAD, the multifunctional protein leading *de novo* pyrimidine biosynthesis. *Structure* 25, 912-923.

KINASES, PROTEIN PHOSPHORYLATION AND CANCER JUNIOR GROUP

Iván Plaza Menacho (since May)
Junior Group Leader

Post-Doctoral Fellow
Pablo Soriano (since December)



OVERVIEW

Our research focuses on the structural and molecular understanding of protein kinase function. How protein kinases are activated and regulated by postranslational modifications and allosteric inputs, and how they assemble into macromolecular protein complexes to transmit signals inside the cell. With a special emphasis on how these mechanisms are corrupted in cancer due to oncogenic mutations and other oncogenic insults (inflammation, DNA-damage, acquired drug resistance). Crucially, such atomic and molecular information can be translated into the design and development of more potent and specific protein kinase inhibitors leading eventually to more effective drugs for the treatment of cancer patients.

“Going beyond the boundaries of the current understanding of protein kinase function: from structure to targeted therapies in cancer.”

Students in Practice
Paula Casas (since January, *Universidad de Alcalá de Henares*),
Nicolás Cuesta (since September, *Universidad de Salamanca*), Alba Morán (since October, *Universidad Autónoma*)

RESEARCH HIGHLIGHTS

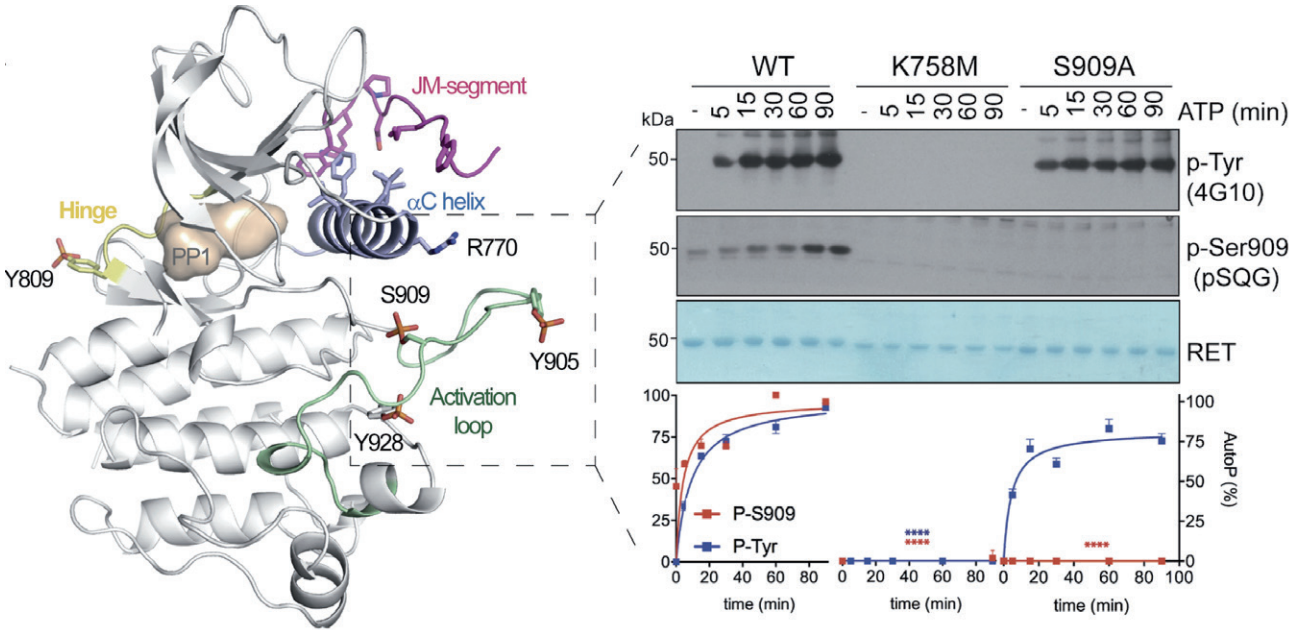


Figure Crystallographic identification of an unexpected activation-loop phospho-S909 reveals Intrinsic RET dual-specificity activity. (Left) Cartoon representation of phosphorylated RET JM-KD crystal structure. (Right) WB analyses using a specific antibody against RET phospho-S909 epitope (pSQG).

Our main scientific goal is to understand the mechanisms of protein kinase function, regulation and signalling at the atomic and molecular level, and how these mechanisms are corrupted in cancer and disease.

In summary, our achievements during 2017 relate to:

→ RET functions as a dual-specificity kinase acting as a multi-phospho site substrate-signalling platform. We have showed,

for the first time, the intrinsic RET dual-specificity kinase activity.

→ The allosteric regulation of RET catalytic domain activity by juxtamembrane elements and structure-function identification of a PIF-like pocket on RET. This is the first study revealing mechanisms of allosteric control by flanking elements on RET kinase activation and signalling with important drug- discovery implications. ■

PUBLICATIONS

- ▶ Radaelli S, Plaza-Menacho I, Mologni L (2017). Novel targeted Therapeutics for MEN2. *Endocr Relat Cancer* 25, T53-T68.
- ▶ Plaza-Menacho I (2017). Structure and function of RET in multiple endocrine neoplasia type 2. *Endocr Relat Cancer* 25, T79-T90.

PUBLICATIONS AT OTHER INSTITUTIONS

- ▶ Plaza-Menacho I,*, Barnouin K, Barry R, Borg A, Orme M, Chauhan R, Mouilleron S, Martinez-Torres J, Meier P, McDonald N* (2016). RET functions as a dual-specificity kinase that requires allosteric inputs from juxtamembrane elements. *Cell Reports*

17, 3319-3332. *Corresponding author

- ▶ Dubey BN, Lori C, Ozaki S, Fucile G, Plaza-Menacho I, Jenal U, Schirmer T (2016). Cyclic di-GMP mediates a histidine kinase/phosphatase switch by noncovalent domain cross-linking. *Science Adv* 2, e1600823.

AWARDS AND RECOGNITION

- ▶ Marie Curie Fellow and Experienced Researcher.
- ▶ Ramon y Cajal Fellow.

GENOME INTEGRITY AND STRUCTURAL BIOLOGY JUNIOR GROUP

Rafael Fernández Leiro
(since September)
Junior Group Leader

Technician
Araceli Grande (since September)
(TS)*

*Titulado Superior (Advanced Degree)



OVERVIEW

Safeguarding the genetic information is essential to all forms of life. Two key cellular processes keep it free from errors: DNA replication and repair. Importantly, when either of these processes don't work properly genetic information may be damaged or lost. This has deleterious effects, ultimately leading to disease. Deregulation and malfunction of the protein machinery that safeguards our genome are a hallmark of cancer, but it still remains unclear how this happens at the molecular level. "The devil is in the detail", and our aim is to understand to the highest level of detail exactly what goes wrong with these molecular machines, and when, so that we can act on it to correct it as well as to prevent it from happening.

These macromolecules are like real-life machines with intricate mechanisms that enable them to perform their activities. To

"Our aim is to understand to the highest level of detail what goes wrong with the protein machinery that protects our genome, and when, so that we can act on it to correct it as well as to prevent it from happening."

understand how they work, we use cryo-electron microscopy and biochemistry in an integrative approach. Beyond fundamental research, this structural information provides the necessary details for drug development.

RESEARCH HIGHLIGHTS

Mismatch repair

DNA mismatch repair (MMR) is critical for genome stability. The DNA mismatch repair machinery loads onto newly synthesised DNA and searches for mismatches. The recognition of an error in DNA by the MutS protein leads to an ATP-dependent conformational change that transfers MutS into a sliding clamp state. Only this MutS state is able to activate the MutL ATPase that in turn promotes the cleavage of the DNA for repair. These protein complexes are extremely dynamic and flexible and many steps of the cycle have remained elusive to structural analysis. Using cryo-EM we capture multiple functional steps and we can now study the conformational changes that these proteins undergo in order to recognise the mismatch and license downstream events that lead to repair. These studies started during my postdoctoral research and will be continued at the CNIO in collaboration with Titia Sixma (Netherlands Cancer Research Institute) and Meindert Lamers (Leiden University).

DNA replication and repair—focus on mitochondria

Eukaryotic cells have two genomes: nuclear and mitochondrial. However, how the integrity of the mitochondrial genome is maintained through the equilibrium between DNA replication, repair and degradation, and organelle dynamics still remains unclear. We are interested in understanding these pathways because of their implications on ageing and disease, and in particular their relationship to cancer.

Cryo-electron microscopy (cryo-EM)

Important recent technological developments in microscopes, detectors and image processing tools have significantly improved the resolution of cryo-EM, enabling the structural analysis of many elusive macromolecules to an unprecedented level of detail. We are upgrading our cryo-EM facility and will have state-of-the-art equipment. Combined with many other approaches already established at CNIO, we use cryo-EM to study diverse macromolecular complexes involved in cancer. ■

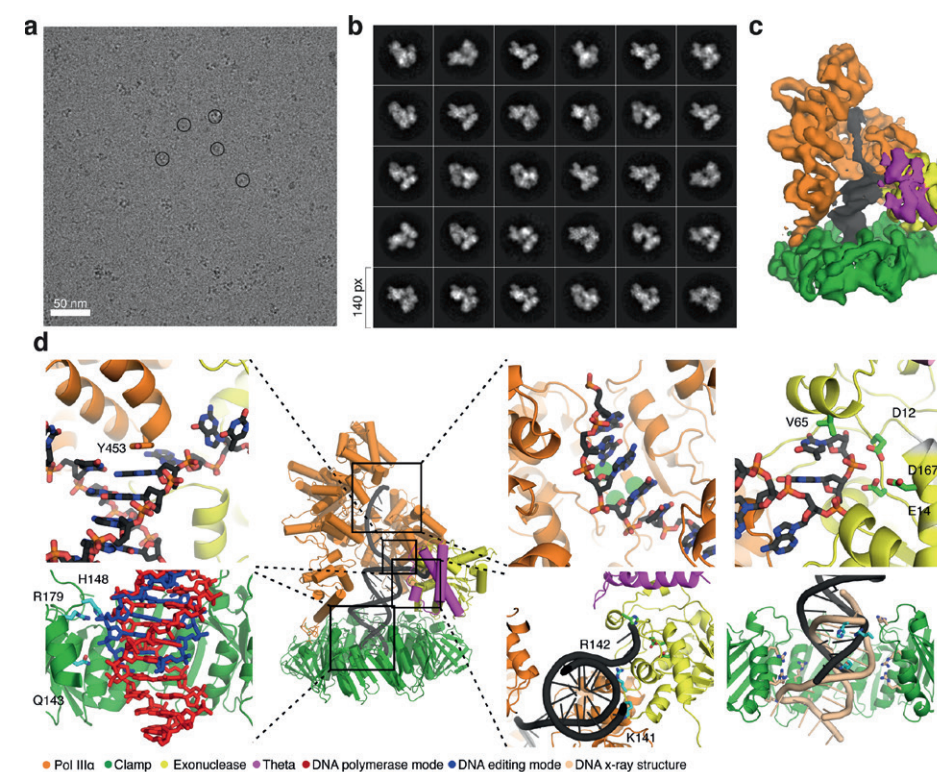


Figure Adapted from work "Self-correcting mismatches during high-fidelity DNA replication" published in *Nat Struct Mol Biol* in 2017, studying the mechanism of DNA proofreading

to illustrate our workflow: (a) cryo-EM micrograph, (b) 2D averages, (c) 3D reconstruction and analysis combined with (d) biochemistry.

PUBLICATIONS AT OTHER INSTITUTIONS

- Fernández-Leiro R, Conrad J, Yang JC, Freund SM, Scheres SH, Lamers MH (2017). Self-correcting mismatches during high-fidelity DNA replication. *Nat Struct Mol Biol* 24, 140–143.
- Vizoso-Vázquez A, Lamas-Maceiras M, Fernández-Leiro R, Rico-Díaz A, Becerra M, Cerdán ME (2017). Dual function of Ixr1 in transcriptional regulation and recognition of cisplatin-DNA adducts is caused by differential binding through its two HMG-boxes. *Biochim Biophys Acta - Gene Regul Mech* 1860, 256–269.
- Fernandez-Leiro R, Scheres SHW (2017). A pipeline approach to single-particle processing in RELION. *Acta Crystallogr D Struct Biol* 73, 496–502.

SPECTROSCOPY AND NUCLEAR MAGNETIC RESONANCE UNIT

Ramón Campos-Olivas
Unit Head

Technician
Clara M. Santiveri (TS)*

**Titulado Superior* (Advanced Degree)



OVERVIEW

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

“In 2017, we quantified interactions of proteins with lipids and small molecule inhibitors, thus contributing to the understanding of the molecular bases of allosteric activation of enzymes, as well as structure-activity relationships for synthetic enzyme inhibitors.”

RESEARCH HIGHLIGHTS

Our Core Unit incorporates a broad range of instrumentation for the biophysical characterisation of biomolecules and their interactions, including spectrophotometers, a fluorimeter, isothermal titration and differential scanning calorimeters, a circular dichrograph, dynamic and multi-angle static light scattering apparatuses, and a surface plasmon resonance (SPR) instrument. Research groups mostly from, but not limited to, the Structural Biology Programme extensively used these technologies throughout 2017. For example, in collaboration with the Cell Signalling and Adhesion Group, we conducted quantitative binding measurements using SPR (FIGURE) to establish that both the phosphatase and C2 domains of the human SHIP2 protein bind phosphatidylserine (PS) lipids, thereby most likely helping to position the active site towards its substrate. SH2-containing-inositol-5-phosphatases (SHIPs) dephosphorylate the 5-phosphate of phosphatidylinositol-3,4,5-trisphosphate (PI(3,4,5)P₃) and play important roles in regulating the PI3K/Akt pathway in physiology and disease.

The Unit hosts a 700 MHz NMR spectrometer that is well equipped with probes, and a sample changer for running up to 120 samples automatically. This provides the required throughput for the screening of small molecule protein binders (together with the CNIO's Structural Biology and Experimental Therapeutics -ETP- Programmes), as well as for metabolomics measurements that, this year, were performed in collaboration with the CNIO-Lilly Cell Signalling Therapies Section (from the ETP), as well as the Genes, Development and Disease and the Growth Factors, Nutrients and Cancer Groups (from the Cancer Cell Biology Programme). Collectively, with these and other groups, we implemented sample preparation protocols and developed spectroscopic and analytical tools to characterise the metabolites present in different biological samples. ■

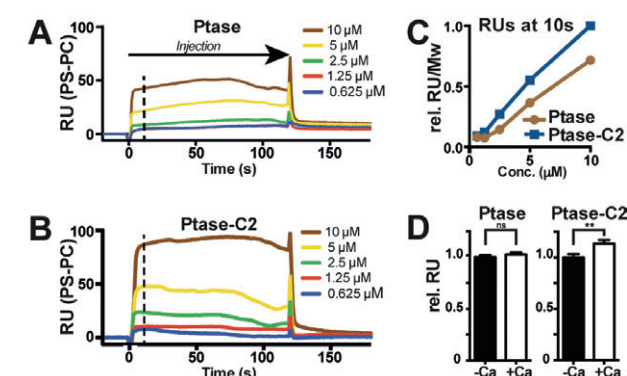


Figure SHIP2 Ptase domain (A) and Ptase-C2 domains (B) binding to PS by SPR. Sensorgrams show the difference in response between the active cell coated with 30% PS vesicles and the reference cell containing Pcholine (PC) vesicles. (C) SPR response units (RU) at 10 s (dashed line in A and B) as a function of concentration indicate stronger binding for Ptase-C2. (D) Calcium dependency of the SHIP2-PS interaction. Relative SPR responses at 5 μM protein were recorded as in C, but in presence or absence of 0.5 mM CaCl₂.

PUBLICATIONS

- Bakiri L, Hamacher R, Graña O, Guio-Carrión A, Campos-Olivas R, Martínez L, Dienes HP, Thomsen MK, Hasenfuss SC, Wagner EF (2017). Liver carcinogenesis by FOS-dependent inflammation and cholesterol dysregulation. *J Exp Med* 214, 1387-1409.
- San José-Enériz E, Agirre X, Rabal O, Vilas-Zornoza A, Sanchez-Arias JA, Miranda E, Ugarte A, Roa S, Paiva B, Estella-Hermoso de Mendoza A, Alvarez RM, Casares N, Segura V, Martín-Subero JL, Ogi FX, Soule P, Santiveri CM, Campos-Olivas R, Castellano G, de Barrena MGF, Rodríguez-Madoz JR, García-Barchino MJ, Lasarte JJ, Avila MA, Martínez-Climent JA, Oyarzabal J, Prosper F (2017). Discovery of first-in-class reversible dual small molecule inhibitors against G9a and DNMTs in hematological malignancies. *Nat Commun* 8, 15424.
- Franco-Echevarría E, González-Polo N, Zorrilla S, Martínez-Lumbreras S, Santiveri CM, Campos-Olivas R, Sánchez M, Calvo O,

González B, Pérez-Cañadillas JM (2017). The structure of transcription termination factor Nrd1 reveals an original mode for GUAA recognition. *Nucleic Acids Res* 45, 10293-10305.

Le Coq J, Camacho-Artacho M, Velázquez JV, Santiveri CM, Gallego LH, Campos-Olivas R, Dölker N, Lietha D (2017). Structural basis for interdomain communication in SHIP2 providing high phosphatase activity. *Elife* 6, e26640.

Book Chapter

- Santiveri CM, López-Méndez B, Huecas S, Alfonso C, Luque-Ortega, JR, Campos-Olivas R (2017). A biophysical toolkit for molecular interactions. In: eLS. John Wiley & Sons Ltd, Chichester. DOI: 10.1002/9780470015902.a0027015.

BIOINFORMATICS UNIT

Fátima Al-Shahrour
Unit Head

Post-Doctoral Fellow
Hector Tejero



OVERVIEW

Bioinformatics is a key discipline for understanding the cancer genome and for the future of cancer therapeutics. Bioinformatics-based approaches have the ability to transform the huge amount of biological data into comprehensive models that provide an in-depth understanding of cancer disease and the complex relationships among genotype and phenotype that are needed to identify cancer driver molecular alterations and new therapeutic targets.

The CNIO Bioinformatics Unit (BU) has several goals:

- To develop new computational methodologies and bioinformatics tools to enable the integration of biological and clinical data.
- To achieve genome analysis in cancer patients’ data to identify new biomarkers and mechanisms of drug response.

“PanDrugs is a feasible method to identify actionable molecular alterations and to prioritise drugs that facilitate the interpretation of the genomic landscape and clinical decision-making in cancer patients.”

- To provide bioinformatics support with data analysis and interpretation using computational and statistical methods.
- To maintain the scientific computing facilities at the CNIO and to provide training in bioinformatics tools and methods.

Graduate Students
Guillermo Martín-Serrano
(since February), Javier Perales,
Kevin Troulé

Technicians
Andrés Cañada (TS)*, Ángel Carro
(until January) (TS)*, Coral Fustero
(PEJ-L)**, Gonzalo Gómez (TS)*,
Osvaldo Graña (TS)*, Elena Piñeiro
(TS)*, Miriam Rubio (until April) (TS)*

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

RESEARCH HIGHLIGHTS

In 2017, the CNIO Bioinformatics Unit published 24 peer-reviewed articles (the full list is available on our web site <http://bioinformatics.cnio.es/>) as a result of our ongoing research projects and scientific collaborations with CNIO Research Groups and other national and international research institutions.

During this year, we developed several bioinformatics tools for the analysis of next-generation sequencing data in collaboration with the SING group (University of Vigo): **RubioSeq+** for DNA-Seq analysis (Rubio-Camarillo *et al.* 2017), **nextpresso** for RNA-Seq analysis (Graña *et al.* 2017), and **bicycle** for bisulfite-seq analysis (Graña *et al.* 2017). Remarkably, the RUBioSeq+ (<http://rubioseq.bioinfo.cnio.es/>) tool supports an interactive graphical user interface (GUI) that facilitates its usage for biomedical translational researchers who lack computational or bioinformatics skills.

Also, our Group focuses on gaining a better understanding of the impact of cancer genomics on the making of clinical decisions. To this aim, we have developed two methodologies to guide the selection of therapies, propose sequential treatments and drug repositioning: **PanDrugs** (<http://pandrugs.bioinfo.cnio.es>) and **SATIE** (<http://satie.bioinfo.cnio.es>).

All our tools are freely available and have been applied in different genomic studies undertaken in numerous scientific collaborations, such as: the study of the pre-metastatic activity of midkine in cancer (Olmeda D *et al.*, 2017), the identification of a novel oncogenic Braf kinase-inactive mutation (Nieto, P *et al.*, 2017), and also the prediction of drug response in pancreatic cancer patient-derived xenograft mouse models using transcriptomics data (Rajeshkuma NV *et al.*, 2017).

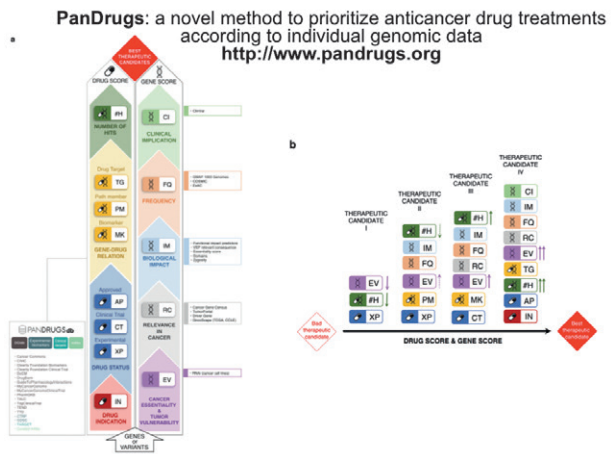


Figure PanDrugs score calculation. (a) Gene Score (GScore) uses the biological and clinical impact of mutations, their frequency, relevance and vulnerability in cancer. Drug indication, gene-drug association and number are used to calculate Drug score (DScore). (b) ‘Best therapeutic candidates’ are based on the accumulated and weighted scoring of GScore and DScore.

Many more bioinformatics analyses were performed together with other CNIO groups, such as: the development of a targeted sequencing assay for Pheochromocytoma and Paraganglioma diagnostics (Currás-Freixes *et al.*, 2017) and the study of NUP98-HOXA9 fusion in leukaemia (Rio-Machin A *et al.*, 2017), among others. ■

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*please see BU’s web site for a list of all publications.

ELECTRON MICROSCOPY UNIT

Jasminka Boskovic
Unit Head

Technician
Carlos Rodríguez (PEJ-L)*

*Plan de Empleo Joven-Licenciado
(Youth Employment Plan-Graduate)
(until December)



OVERVIEW

The Electron Microscopy (EM) Unit is a research laboratory as well as a central core facility. It is available to CNIO researchers and the wider research community, providing access to Transmission Electron Microscopy and supplying expertise in EM image analysis. Our Unit offers standard specimen preparation techniques for proteins, protein complexes and vesicles, data collection and data processing services that are tailored to meet the particular needs of the users. We also give training to regular users on the use of our equipment and provide further guidance regarding specimen preparation.

“We have made a step forward in our understanding of *de novo* synthesis of pyrimidines by providing fundamental information about the internal structural organisation of the multifunctional protein CAD.”

RESEARCH HIGHLIGHTS

The Electron Microscopy Unit is a research facility that assists with different biological scientific projects ranging from the macromolecular to the cellular level. The EM Unit's scope of work includes sample preparation protocols, negative staining, cryo-EM, and data collection methods, as well as 2D and 3D data processing.

In collaboration with CNIO's Structural Bases of Genome Integrity Group (Structural Biology Programme), we carried out negative staining electron microscopy to gain insight into the molecular architecture of DHO-ATC hexamers. The EM analysis clearly indicated that despite crosslinking, the particle presents a structural flexibility that prevents the determination of a complete 3D model. A combination of structural and biophysical studies enabled us to propose a model that sets the DHO and ATC complex as the central element in the architecture of multifunctional protein CAD. Our model suggests that CAD is more than the sum of its parts, and that the detailed study of the pieces will give limited information in comparison with the complexity that would be revealed by the structural determination of the full-length particle.

We continued our collaboration with Dr. Iván Ventoso, from the *Centro de Biología Molecular ‘Severo Ochoa’* (CSIC-UAM) and the *Departamento de Biología Molecular, Universidad Autónoma de Madrid* (UAM). The EM Unit participated in the study of the topology and dynamics of the scanning ribosomal 43S pre-initiation complex (PIC). Our data supports a model where the eIF4F complex works at the leading edge of the scanning PIC, rather than acting as a simple clamp to prevent backsliding as previously suggested.

We have advanced in our understanding of PI(4,5)P2-mediated induction of Focal Adhesion Kinase (FAK) clustering at the cell membrane by applying 2D electron crystallography. This work is the continuation of our collaboration with CNIO's Cell Signalling and Adhesion Group (Structural Biology Programme). ■

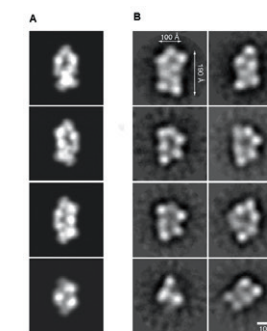


Figure (A) Selected 2D projections filtered to 25 Å of a DHO-ATC model formed by the association of two ATC trimers and three DHO dimers. (B) Representative class averages of crosslinked and negatively stained ctDHOlike-ATC particles.

• PUBLICATION

Moreno-Morcillo M, Grande-García A, Ruiz-Ramos A, Del Caño-Ochoa F, Boskovic J, Ramón-Maiques S (2017).

Structural insight into the core of CAD, the multifunctional protein leading *de novo* pyrimidine biosynthesis. *Structure* 25, 912-923.

CRYSTALLOGRAPHY AND PROTEIN ENGINEERING UNIT

Inés Muñoz
Unit Head

Staff Scientist
Jorge L. Martínez



OVERVIEW

The Crystallography and Protein Engineering Unit (XTPEUnit) is a central core facility as well as a research laboratory; its main goal is to fulfil the requests of research groups at the CNIO and outside our Centre, by providing on-demand services at different levels. These services range from the production of recombinant proteins for different types of biochemical/biophysical assays and monoclonal antibody production, to macromolecular structural determination at low resolution by small-angle X-ray scattering (SAXS), or at high resolution by X-ray crystallography. The latter includes co-crystallisation in the presence of inhibitors, during the guided drug design process.

“Our Unit is fully committed to spreading the use of the advanced technologies used in Structural Biology, and to show how beneficial the information provided by protein images given at high-resolution is in understanding the biology of cancer.”

Graduate Student
Sílvia L. Gomes

Technicians
Daniel Calvo (PEJ-L)*, Miguel A. Navarro (since February) (PEJ-L)*, Álvaro Otero (until November) (PEJ-L)*, Federico Ruiz (since June) (TS)**, Alicia Virseda (until November) (PEJ-L)*

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

RESEARCH HIGHLIGHTS

This year, we worked closely with the Experimental Therapeutics Programme on several projects based on the production of proteins such as full-length human MASTL that enable new biochemical experiments. Other projects were directly focused on structural characterisation by X-ray crystallography in support of drug discovery projects, as was the case of the human proteins MASTL kinase-domain, HASPIN and CDK8/CyclinC complex in which the proteins were crystallised in the presence of compounds developed at the Medicinal Chemistry Section (FIGURE). Especially relevant was our continuous work on the production of proteins for the generation of antibodies by the Monoclonal Antibody Unit (Biotechnology Programme). During 2017, this smooth collaboration has led to the production of several cancer-involved proteins such as mouse PD1, PD-L2 and HAS1, and human IL11 and NOMO1, among others.

The Unit also undertakes several internal collaborations with other CNIO Groups. Especially noteworthy are the ones established with the Telomeres and Telomerase Group, the Gastrointestinal Cancer Clinical Research Unit, the Melanoma Group, and the Experimental Oncology Group. Additionally, the Unit maintains external collaborations with groups at the Physical Chemistry Department (University of Granada), the Environmental Biology Department (CIB-CSIC), the Department of Biomedicine (University of Bergen, Norway), the Department of Crystallography and Structural Biology (*Instituto de Química-Física Rocasolano*, CSIC), and the Department of Molecular Engineering (Århus University, Denmark).

Along 2017, the Unit also sustained its own scientific projects. We continued to characterise the role of ephrinB2 in different pathologies by carrying out a further structural characterisation

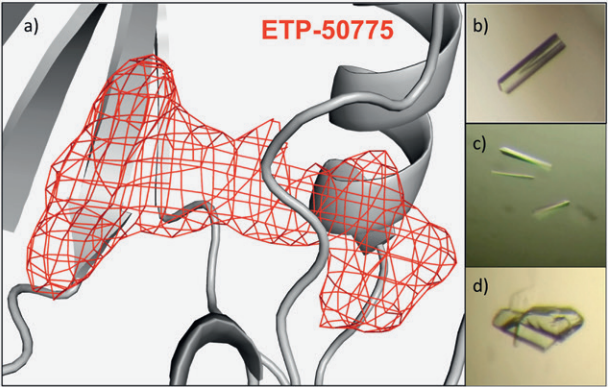


Figure (a) Ribbon representation of the X-ray structure of CDK8/CyclinC in complex with the drug ETP-50775. The coloured omit map belongs to the electron density of the bound compound (synthesised at CNIO's Medicinal Chemistry Section). (b, c, and d) Protein crystals corresponding to the single-chain antibodies B11 and 2B1, and the complex of the human extracellular domain of ephrinB2 with 2B1.

of the blocking single-chain antibodies, which we developed at our Unit by using a combination of X-ray crystallography and SAXS (FIGURE). We have also initiated a drug-discovery project targeting the function of the Mdm2-MdmX E3 complex, in collaboration with the Pharmacology and Therapeutics Department at the Roswell Park Cancer Institute (Buffalo, USA). ■

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AWARDS AND RECOGNITION

Member of the Board of Directors, *Asociación de Usuarios de Sincrotrón de España*.

BIOLOGICAL TEXT MINING UNIT

Martin Krallinger
Unit Head

Staff Scientists
José Antonio López (since August),
Marta Villegas (since July)



OVERVIEW

CNIO and the State Secretariat for Telecommunications and the Information Society (SETSI) are collaborating on the identification and development of Text and Data mining resources to transform the growing amount of key health information hidden in electronic health records, scholarly communications, patents or social media into structured data of practical utility in the clinic, as well as for basic biomedical research. The integration of results from clinical and biomedical text mining systems with bioinformatics infrastructures is critical to empower precision medicine approaches that enable a more comprehensive and systematic understanding of the molecular characteristics of cancer patients. Our Unit is funded through an ‘encomienda’ between the SETSI and CNIO as part of the 90 million Euro Strategic National Plan for the Advancement of Language Technologies. The mission of the Unit is to characterise the

“Clinical text and data mining is a key technology to understand health-related big data and to empower precision medicine approaches for cancer treatment.”

state-of-the-art of clinical text mining systems, provide assistance to adapt and apply clinical text mining components to various use cases and to generate key resources including components, annotated data and evaluation infrastructures.

Technicians
Diana De La Iglesia, Aitor
González (TS)* (since July), Ander
Intxaurreondo (TS)* (since February),
Jesús Santamaría (TS)*
(since March), Santiago de la Peña
(TS)* (until June)

**Titulado Superior* (Advanced Degree)

RESEARCH HIGHLIGHTS

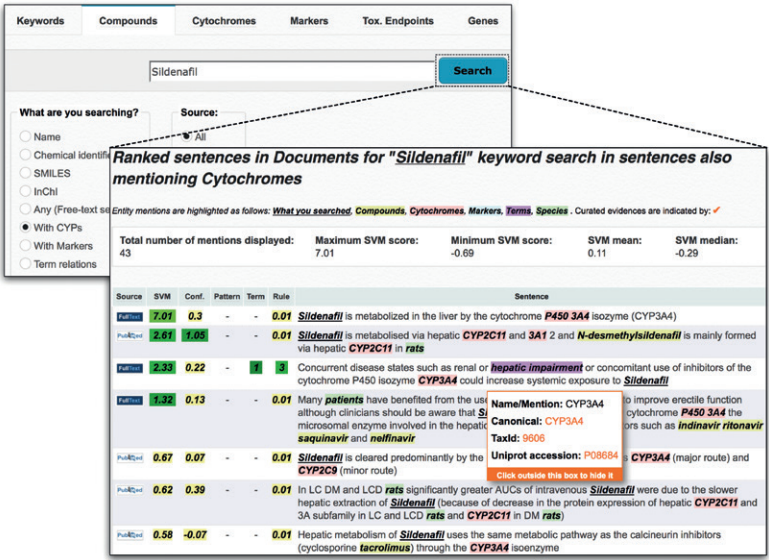


Figure Text mining search engine LimTox for chemical entities and genes.

The Biological Text Mining Unit began its journey in January 2017, with the aim of providing consultancy, guidance and technical assistance for cognitive computing and text-mining technologies applied to clinical and biomedical documents of relevance, particularly to precision medicine approaches. The Unit has been focusing on fostering collaborative efforts to address the needs faced by healthcare providers (*Hospital Virgen del Rocío* or *Hospital XII de Octubre*), Spanish national health-related agencies (the Spanish Medical Agency, the Spanish National Library of Health or the Spanish Royal Medical Academy) and artificial intelligence and language technologies academic research groups.

Clinical text mining represents a strategic innovative research area with considerable potential to leverage uptake of cognitive computing and big data text analytics in health.

To cover integration and discoverability aspects of existing resources, we have examined the design requirements, annotation formats and standards for interoperability of biomedical language technology infrastructures, and have constructed a registry of language processing components and a medical document repository. The Unit has coordinated community assessment evaluation challenges (BioCreative and IberEval) with the aim to benchmark existing tools, to determine cutting edge computational strategies and to

enable the development of statistical machine learning models by providing access to Gold Standard annotated training datasets through an evaluation platform. These community assessments included tasks related to the technical evaluation of systems for the automatic recognition of mentions of biomedical named entities in running text (genes, proteins, chemical compounds/drugs, cell lines, diseases, anatomic terms and mutations) or the automatic extraction of drug-target associations. Moreover, we have applied text mining methodologies to concrete use cases, including the implementation of semantic search engines for toxicology and for cancer or the use for disease and gene concepts recognition to assist in the discovery of inverse comorbidities between cancer and neurodegenerative diseases. ■

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STRUCTURAL COMPUTATIONAL BIOLOGY GROUP

Alfonso Valencia (until February) Group Leader	Graduate Students Maria Rigau (until July), Juan Rodríguez (until June), Jon Sánchez
Staff Scientists Andrea Nicole Dölker (until June), Vera Pancaldi (until May), Michael Tress	Technicians Miguel Madrid (until June) (TS)*, Filipe N. Were (until March) (TS)* <i>*Titulado Superior (Advanced Degree)</i>

OVERVIEW

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

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

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

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

NATIONAL BIOINFORMATICS INSTITUTE UNIT

Salvador J. Capella Gutierrez (until June) Unit Head	Technicians Andrés Cañada (until June) (TS)*, José M. Fernández (until June) (TS)*, José M. Rodríguez (until June) (TS)* <i>*Titulado Superior (Advanced Degree)</i>
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OVERVIEW

The Spanish National Bioinformatics Institute (*Instituto Nacional de Bioinformática, INB*) is a component of the National Infrastructure of Biomolecular and Bioinformatics Resources Platform (*Plataforma en Red de Recursos Biomoleculares y Bioinformáticos, PRB2*) of the Spanish National Institute of Health Carlos III (*Instituto de Salud Carlos III, ISCIII*). The INB is the Spanish Node of ELIXIR, the permanent European Infrastructure for Life Sciences.

The INB is composed of 10 working nodes distributed across 9 different research centres. The INB Unit at the CNIO undertakes the coordination of the institute. As the coordination node, the goals of the INB Unit are to:

- Design, promote and ensure the execution of the INB’s scientific-technical and training programmes, undertaken with the support of all nodes.
- Promote the collaboration between INB nodes and third parties, including research consortia, other research infrastructures, small and medium enterprises (SMEs), and the industry.

The main difference between the INB Unit and other Programme Units is that its activities are not restricted to the CNIO Groups alone. Moreover, the INB Unit is entirely funded by external agencies; namely, the ISCIII at the national level and ELIXIR at the European level. ■

- Coordinate the Spanish participation in ELIXIR. Promote the implementation and adoption of ELIXIR guidelines among the Spanish bioinformatics community.

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► PUBLICATIONS

- Cañada A, Capella-Gutierrez S, Rabal O, Oyarzabal J, Valencia A, Krallinger M (2017). LimTox: a web tool for applied text mining of adverse event and toxicity

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HUMAN CANCER GENETICS PROGRAMME

JAVIER BENÍTEZ Director



The Human Cancer Genetics Programme (HCGP) is a translational research programme working on areas related to genetics, genomics, pharmacogenetics, molecular cytogenetics and the environmental bases of human cancer. The HCGP works in close collaboration with the clinical community.

Three Research Groups and three Units currently form the HCGP. The Human Genetics Group, led by Javier Benítez, focuses on contributing to the understanding of the genetic bases of some hereditary tumours, mainly breast cancer. Mercedes Robledo leads the Hereditary Endocrine Cancer Group that aims to identify new major susceptibility genes related to hereditary endocrine tumours as well as to define markers associated with differences in anticancer drug response and toxicity. Both Groups are also involved in the search for low susceptibility alleles that explain sporadic cancers. The Genetics and Molecular Epidemiology Group, led by Núria Malats, works not only from a genetic but also from a non-genetic point of view. She analyses exogenous factors that contribute to explain, together with genetic factors (low susceptibility alleles), the susceptibility to pancreatic and bladder cancer. The Genotyping Unit, headed by Anna González-Neira, supports our three research groups from a technical point of view, and provides support to other groups from the CNIO as well as to external users. They also work in pharmacogenetics in the framework of their own line of research. The new Molecular Cytogenetics and Genome Editing Unit, coordinated by Sandra Rodríguez-Perales, contributes to this provision of support with classical and molecular cytogenetics techniques and with new genome editing technologies. In addition, her research is focused on the design of human stem cell models carrying cytogenetic alterations. Finally, the Familial Cancer Unit coordinates the clinical part of the Programme through the CNIO Familial Cancer Consultancy, which is located at the *Hospital de Fuenlabrada*. Miguel Urioste is responsible for these activities and leads a research line focused on hereditary colorectal cancer.

The Programme collaborates closely with the clinical community, not only to foster cooperation in genetic diagnosis but also to promote training and education. This year the Familial Cancer Consultancy attended around 500 consultancies, performed 1,350 genetic diagnosis and carried out more than 1100 cytogenetic studies. In addition, the Programme's Groups have hosted 9 resident physicians from different Spanish hospitals for 3-month periods. We also offer professionals from different international research centres the opportunity to join us, either as visitors or for training visits consisting of short-term stays of 1-3 months (a total of 6 international and 2 national visitors were hosted in 2017). In terms of education, 1 foreign and 9 national Master's

“Once again, the challenge of summarising our research in a few sentences arises. We continue to conduct translational research with the overarching goal of improving the diagnostics, prevention and treatment of cancer. And yes, we are able to achieve this goal despite the multiple economic and administrative obstacles that we face.”

students and 10 national and 2 international PhD students have worked on their research projects, 6 of whom have already successfully defended their thesis.

Finally, one of the main objectives of the Programme is to establish research collaborations with national and international groups; this is well demonstrated by our publication record as well as the key roles held by several of the Programme's members in consortia and international projects. Currently, we collaborate with 13 international Consortia that are representative of the main types of tumours that we focus on. In addition, we participate in 4 international projects from Europe and North America.

Milestones and major achievements of the Programme in 2017 include: (1) Mercedes Robledo: the identification of the high susceptibility gene *GOT2* as being responsible for a percentage of familial pheochromocytoma; (2) Javier Benítez: the identification of the high susceptibility gene *PTHR1* as being responsible for familial neuroendocrine tumours; (3) Anna González-Neira: the identification of *GPR35* as a new susceptibility gene for anthracycline -induced cardiotoxicity; (4) Sandra Rodríguez: new Head of the Molecular Cytogenetics and Genome Editing Unit; (5) Núria Malats, Board Member of the International Pancreatic Cancer Case-Control Consortium (PanC4); (6) Mercedes Robledo, Member of the Organiser Committee of the 'International Symposium on Pheochromocytoma and Paraganglioma', Sydney, Australia, (7) Núria Malats, Co-organiser of 'The Barcelona Debates on the Human Microbiome: From Microbes to Medicines', CosmoCaixa Barcelona, Spain.

HUMAN GENETICS GROUP

Javier Benítez
Group Leader

Staff Scientist
Ana Osorio



Post-Doctoral Fellow
Oriol Calvete

Graduate Students
Juan Miguel Baquero, Paloma Martín,
Beatriz Paumard (until October),
Alejandra Tavera

Technicians
Alicia Barroso, Carlos Benítez-Buelga
(until July) (TS)*, Victoria Fernández (TS) *

*Titulado Superior (Advanced Degree)

OVERVIEW

For several years, the Human Genetics Group has been working on the understanding of the genetic bases of familial cancer, especially breast cancer. We continue to focus on this activity but have now extended it to include the discovery of new high susceptibility genes that explain families with rare tumours, as well as low susceptibility alleles that can help us to understand the genetic bases of cancer. In addition, we want to define the role of glycosylase genes involved in the base excision DNA repair pathway (BER) across the different phases of the cell cycle, and to explore new alternative treatments based on this pathway.

“During 2017, we set up our studies and established collaborations with urologists and oncologists working in testicular cancer; we discovered a new gene, *PTHR1*, that in combination with *ATP4A* (digenic model) is responsible for a new form of gastric neuroendocrine tumour. We also confirmed that up to 2% of Spanish breast cancer families are due to mutations in the *ATM* gene.”

RESEARCH HIGHLIGHTS

Deciphering the role of rare variants in breast cancer

We are participating in an H2020 project funded by the EU with the main objective of clarifying the role of some rare variants and genes in breast cancer development. The study includes, in a first step, the Next Generation Sequencing (NGS) of 35 genes already known as candidates in a set of 40,000 breast cancer cases and 40,000 controls from more than 50 countries. In a second step, we will sequence, in the same group of samples, a new set of > 30 genes. The third step will include the selection of the best and already confirmed genes in order to build a new diagnostic gene panel ready to be used in clinical practice. Our Group is coordinating the WP2 that is comprised of Cambridge University, Lund University and our Centre as partners. We are responsible for gene selection, sample management from the different groups, library preparation, sequencing and variant calling.

Breast and ovarian cancer susceptibility genes

By performing whole exome sequencing (WES) in 4 BRCA families, we have found a set of new candidate breast cancer

genes. We have identified a deleterious mutation in the known breast cancer susceptibility gene *ATM* in 1 family and, by extending the study to a larger series, we have established that 2% of Spanish breast cancer families are explained by germline mutations in this gene (Tavera *et al.*, *Br Cancer Res Treat* 2017). In a second family, we have found a deleterious mutation in an excellent candidate gene from a family of DNA helicases that have a role in the Homologous Recombination (HR) DNA repair pathway. The high interest of this finding has prompted us to start a screening of the gene by targeted NGS in a series of 700 BRCA families and 700 controls. We have found at least 3 additional deleterious mutations among the cases, suggesting that the gene could actually explain a small percentage of the BRCA families. Other candidate genes, some of them direct interactors of *BRCA1* and *BRCA2*, have been selected for further analysis in larger series of cases and controls in order to establish their role in the disease.

Ovarian cancer families are rare. We performed WES in 5 families and identified novel missense variants in the known ovarian cancer susceptibility gene *RAD51C*, among other candidates. Through functional studies, we were able to determine its pathogeny and its possible involvement in ovarian cancer

risk. Because there are many variants in *RAD51C* with no clear implication in the disease, we have developed an algorithm based on different predictors that permit a good classification of RAD51C variants and their implication in ovarian families (Gayarre *et al.*, *Br J Cancer* 2017).

Familial cancer exome project

In 2015, we published the identification by NGS of a new gene, *POT1*, as being responsible for families with multiple tumours including cardiac angiosarcomas (CAS). We extended our study to a large series of families with angiosarcomas, sporadic CAS and sarcomas in order to elucidate the role of *POT1* in cancer development. Our results have led us to the indication of testing for *POT1* mutations in families with angiosarcomas with or without CAS (25% with mutations), sporadic CAS, and cardiac sarcomas (10% with mutations). (Calvete *et al.*, *Eur J Hum Genet* 2017).

In 2015, we also published the identification of a gene, *ATP4A*, responsible for a recessive family with gastric neuroendocrine tumours (Calvete *et al.*, *Hum Mol Genet* 2015). By analysing additional families, we have identified a second gene *PTHRI* that, combined with *ATP4A*, explains a second family based on a digenic model. Carriers of *ATP4A* present megaloblastic anaemia and low ferritin levels, *PTHRI* carriers hypothyroidism and rheumatoid arthritis, while only those members carrying mutations in both genes develop gastric carcinoid (FIGURE). It also suggests the genetic heterogeneity of this disease (Calvete *et al.*, *Gastric Cancer* 2017).

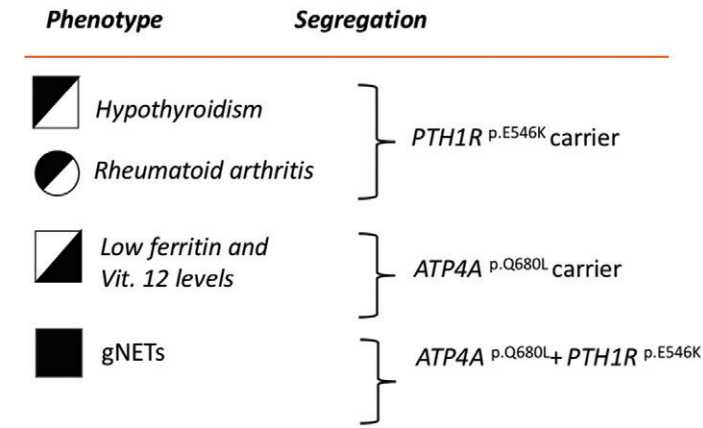
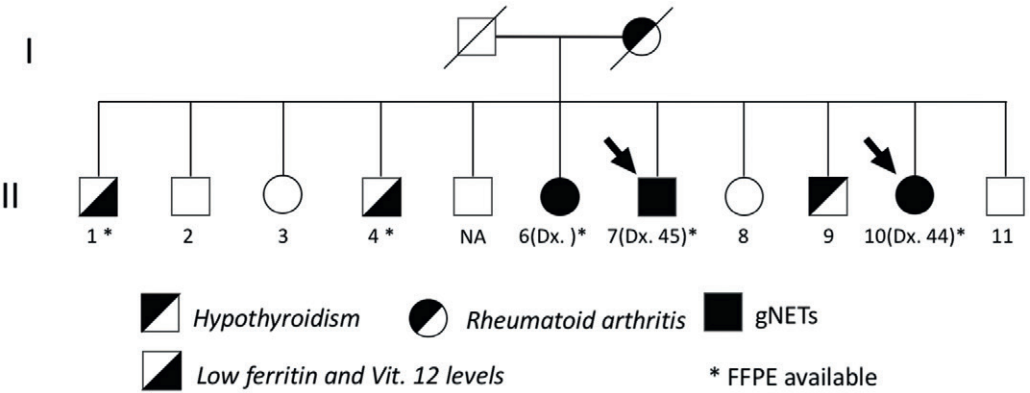
We have identified the *NHEJ1* gene as being responsible for a family with a child that developed severe pancytopenia and bone marrow aplasia correlated with the presence of short telomeres (Carrillo *et al.*, *Hum Mol Genet* 2017). We downregulated *NHEJ1* expression in 293T and CD34⁺ cells and we found increased p21 expression, reduced telomerase activity and decreased expression of several telomerase/shelterin genes. Because the decrease in

expression of telomerase/shelterin genes did not occur when we inhibited expression of other *NHEJ* genes mutated in SCID patients – *DNA-PK*, *Artemis* or *LigaseIV* – we propose that NHEJ1 is responsible for the inhibition of telomerase activity.

Over the last 3 years, we have started to work with families with testicular cancer thanks to a collaboration that we established with the Spanish Germ-Cell Cancer Group and several institutions that are dedicated to the follow-up and treatment of patients with testicular cancer, for which the genetic bases are poorly known. We have already collected 21 families with at least 2 first-degree affected members who have been sequenced and analysed using bioinformatics tools (a total of 71 exomes). We are using different statistical and bioinformatics approaches (family based association tests, based on Kernel and burden tests) to identify candidate genes and variants for validation.

SNPs and the BER pathway

We have studied 3 genes from the BER pathway (*OGG1*, *NEIL2* and *UNG*) containing SNPs that modify cancer risk in *BRCA1/2* mutation carriers and are modifiers of Telomere Length (TL) (Benítez-Buelga *et al.*, *Oncotarget*, second review; Baquero JM *et al.*, *Int J Cancer*, submitted). We want to evaluate the global effect of endogenous factors (represented by the 3 genes) on TL. On the other hand, this pathway seems to be an excellent model for new treatments based in OGG1 and NEIL2 inhibitors. Our collaboration with Helleday’s group (Karolinska Institute) will permit us to explore new drugs in specific situations (BRCA carriers) and in different types of tumours (breast and ovarian). ■



Segregation is in accordance with the phenotype and suggests a digenic model

Figure Pedigree of the family with gastric carcinoid with mutations in *ATP4A* and *PTHRI*. Only members carrying mutations in both genes develop gastric carcinoid (II-6, II-7; II-8), while members with mutations in *ATP4A* or *PTHRI* genes present different symptoms.

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OVERVIEW

Our Group is mainly interested in identifying genetic risk factors involved in endocrine tumour susceptibility. Through a comprehensive analysis of tumour genomic features we have been able to propose diagnostic and prognostic markers to identify altered pathways that could be therapeutically targeted, and to identify new major susceptibility genes.

We are also interested in defining markers associated with differences in anticancer drug response and toxicity. We are applying targeted and whole-exome next-generation sequencing to a large series of clinically well-characterised patients. The aim is to identify new therapeutic approaches to personalise cancer treatment. These efforts will collectively improve the diagnosis, prognosis and treatment of patients.

“We identified new susceptibility genes for paraganglioma, established clinical features related to MDH2 disruption, and discovered predictive biomarkers of mTOR inhibitor response.”

RESEARCH HIGHLIGHTS

New mutation in a Krebs cycle-related gene in pheochromocytoma (PCC) or paraganglioma (PGL)

Mutations in Krebs cycle genes are frequently found in patients with PCC/PGL. Disruption of SDH, FH or MDH2 enzymatic activities leads to the accumulation of specific metabolites, which give rise to epigenetic changes in the genome that cause a characteristic hypermethylated phenotype. Tumours showing this phenotype, but no alterations in the known predisposing genes, could harbour mutations in other Krebs cycle genes. We used downregulation and methylation of RBP1, as a marker of a hypermethylation phenotype, to select PCCs and PGLs for targeted exome sequencing of a panel of Krebs cycle-related genes. Methylation profiling, metabolite assessment and additional analyses were also performed in selected cases. Following this rationale, a germline *GOT2* variant, c.357A>T, was found in a patient with multiple tumours and metastasis. The presence of this variant was associated with higher tumour mRNA and protein expression levels, increased GOT2 enzymatic activity in lymphoblastic cells, and altered metabolite ratios both in tumours and in GOT2 knockdown HeLa cells transfected with the variant. Thus, we propose *GOT2* as a new susceptibility gene related to metastatic PGL. This study further attests to the relevance of the Krebs cycle in the development of these tumours, and points to this central metabolic pathway as being targetable in metastatic PCC/PGL patients.

MDH2 mutations in pheochromocytoma and paraganglioma

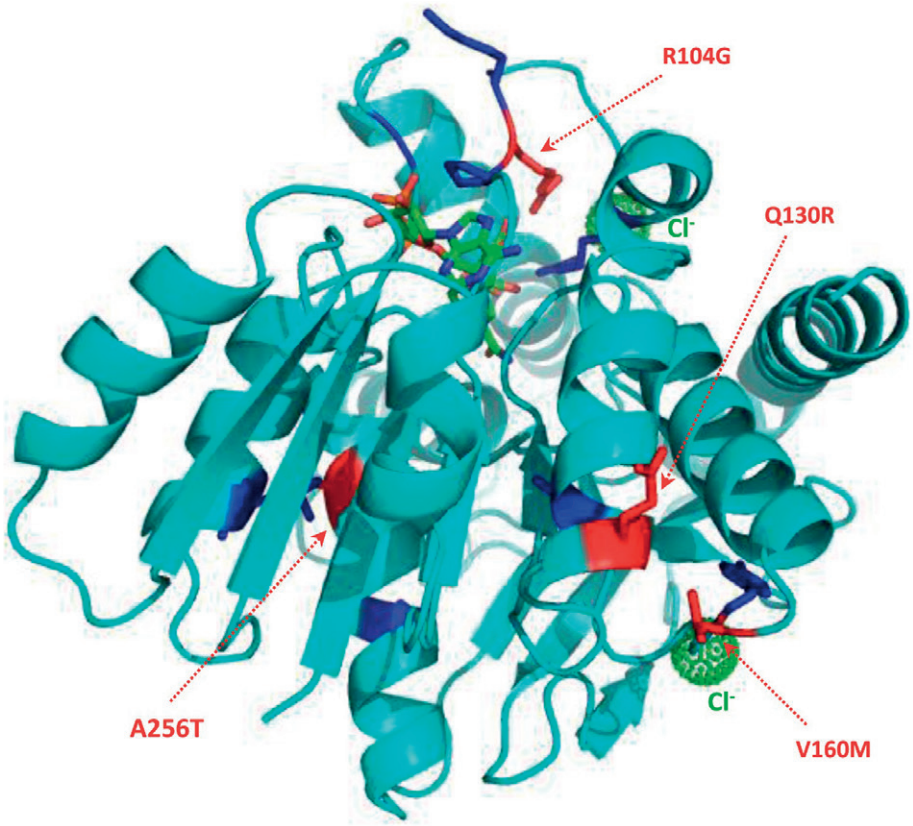
MDH2 has recently been proposed by our Group as a novel PCC/PGL susceptibility gene. We aimed to determine the prevalence

of MDH2 mutations among PPGL patients and to establish the associated phenotype. Through a worldwide network we recruited close to 800 PCC/PGL patients. In this study we needed to implement a multidisciplinary approach with up to five functional assays and twenty *in silico* predictions (FIGURE), including MDH2 enzymatic activity and affinity, an immunofluorescence assay to evaluate MDH2 localisation, and a molecular dynamics (MD) simulation approach to examine the potential protein structure changes of the most controversial variants. With this initiative we were able to ascertain the prevalence of *MDH2* mutations in PPGL in less than 1% and to highlight the importance of including this gene in the routine genetic screening of the disease, especially in metastatic, noradrenergic-secreting, multiple tumours and in young patients.

Novel CNVs in pharmacogenes and mTOR pathway mutations to predict outcomes in cancer therapies

Personalised cancer treatment is of enormous clinical and social relevance since it can lead to safer and more efficient therapies. This year we focused on: i) understanding the effect of copy number variants (CNVs) on adverse drug reactions (ADRs); and ii) discovering biomarkers predictive of mTOR inhibitor response. ADRs cause around 6.5% of admissions to hospitals, accounting for 5-10% of the annual hospital costs. Genetic factors are responsible for many ADRs and these could be prevented by genetic tests. We performed the first systematic assessment of the CNV landscape in pharmacogenes by integrating data from 2,504 whole genomes and 59,898 exomes. We described novel exonic deletions and duplications in 97% of the genes analysed. Novel deletion frequencies ranged from singletons

Figure Mapping of *MDH2* mutations onto the crystal structure (PDB ID: 2DFD). In red, missense variants found in our study; in blue, amino-acid changes in neighbouring positions (dE5A) in other types of cancers reported in COSMIC v80.



up to 1%, and accounted for >5% of all loss-of-function alleles in 42% of studied genes. Thus, CNVs are an additional source of pharmacogenetic variability with important implications for drug response and personalised therapy. Regarding mTOR inhibitors, by next generation sequencing (NGS) on tumour tissues, we found that those patients with somatic mutations

directly activating the pathway (in *MTOR*, *TSC1*, *TSC2*) had improved responses. Furthermore, multiregion NGS allowed us to determine that when these mutations were acquired early on during tumour development they resulted in extraordinary responses to these drugs. ■

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OVERVIEW

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

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

The strategic goals of the Group are to:

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

“We have contributed to the characterisation of pancreatic cancer aetiology by identifying both risk and protective factors that mainly point to the role of the immunological status in pancreatic carcinogenesis.”

RESEARCH HIGHLIGHTS

Research findings

During 2017, the Group centred its research on pancreatic and bladder cancers. Regarding **pancreatic cancer** (PC), we have completed several analyses using the resources from the PanGenEU Study. In this regard, we reported that specific multimorbidities aggregate and associate with PC risk in a time-dependent manner. A common genetic basis between these conditions and PC was observed through a bioinformatics analysis (FIGURE 1). The common genetic basis between these conditions and PC pointed to a mechanistic link between these diseases. Using the same population and applying both a case-control and a reconstructed cohort approach, we confirmed that family history of any cancer, of PC or diabetes, conferred a higher risk of PC and also that smoking notably increased the PC risk associated with family history of PC. Furthermore, we actively participated in an international large-scale investigation that identified important disparities on PC resection practice within and between countries. It also evidenced that most PC patients remain untreated through resection, although resection was associated with better survival, highlighting pivotal potentially modifiable areas in PC clinical practice. Regarding **bladder cancer** (BC), GMEG assessed the complex interrelationships between asthma and BC in the Spanish Bladder Cancer/EPICURO Study population. This study corroborated the notion that asthma was associated with a reduced risk of cancer, especially among aggressive tumours pointing to the relationship between immune response mechanisms and bladder carcinogenesis. The Group also participated in the discovery and validation of both urine and tumour prognostic marker combination in a large European study of non-muscle invasive BC. Whole transcriptome expression profiling of 476 tumours from the same European-based study enabled the further characterisation of bladder cancer subtypes. GMEG also performed a review of the genetic susceptibility to BC risk and progression based on GWAS. Most of the variants were common and conferred small risk and, therefore, they were not clinically actionable at the individual level.

Methodological contributions

Integrative analytic approaches considering different regulatory levels (i.e., host and tumour) are still scarce. To model this multilevel structure, we proposed to apply our previously described Global-LASSO method and compared it with other approaches to integrate genomic, epigenomic, and transcriptomic data from tumour tissue with blood germline genotypes from 181 individuals with bladder cancer included in the TCGA Consortium (FIGURE 2). Global-LASSO performed better than the other methods. GMEG also developed the ‘DoriTool’ that, to our knowledge, is the most complete bioinformatics tool offering

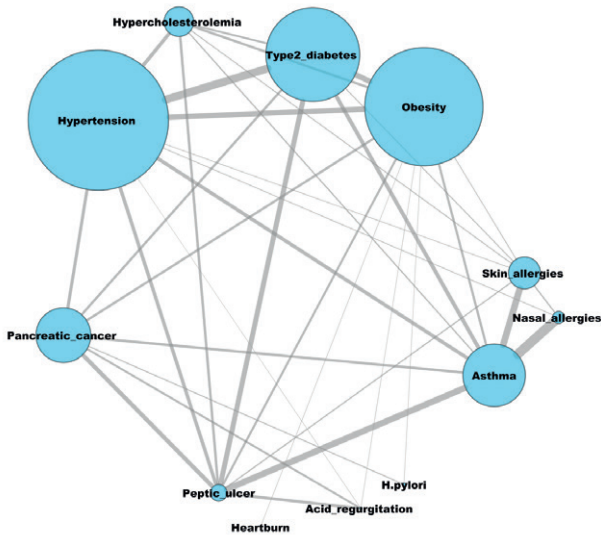


Figure 1 Genetic system for multimorbidity patterns and pancreatic cancer. Association between pancreatic cancer and medical conditions of the detected multimorbidity patterns based on the number of shared genes.

functional ‘in silico’ annotation of variants previously associated with a trait of interest, shedding light on the underlying biology and thereby helping the researchers in the interpretation and discussion of the results.

Translational activities

A critical review of both PC host- and tumour-based markers evidenced the poorly transferred knowledge into the patient management domain. International and multidisciplinary strategies to identify new markers and properly validate the promising ones are urgently needed in order to implement cost-efficient primary and secondary prevention interventions in PC. During 2017, we organised a Multistakeholder Brainstorming Meeting on European PC research in Brussels under the auspices of the EUPancreas COST Action (BM1204) and the Pancreatic Cancer Europe (EPC) multistakeholder platform. A report pinpointing the gaps and challenges identified by the researchers and stakeholders attending the meeting were distributed, and recommendations were made for future European activity on PC research. ■

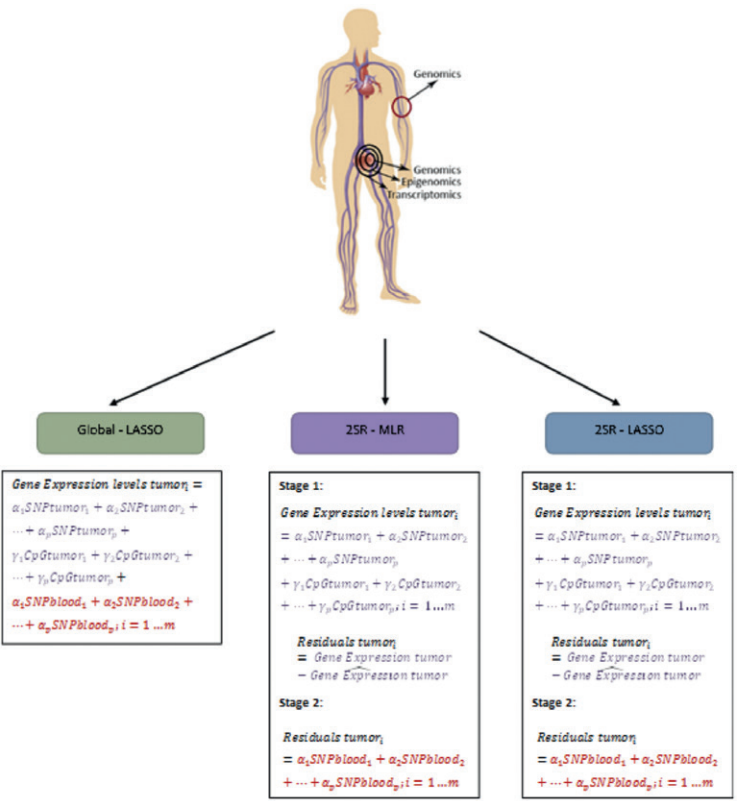


Figure 2 Global-LASSO, 2SR-MLR, and 2SR-LASSO models applied to assess the association between the germline SNPs (host omics) and gene expression levels (tumour omics) adjusting for SNPs and CpGs.

Pineda et al. *Genet Epidemiol* 2017

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Book Chapter

López de Maturana E, Malats N (2017). Genetic testing, genetic variation and genetic susceptibility. In: Hyeon Ku J, ed. *Bladder Cancer*, Elsevier.

AWARDS AND RECOGNITION

Board Member, Pancreas Cancer Case-Control Consortium (PanC4) International Consortium.

Chair, Research Work Stream, Pancreatic Cancer Europe (<http://www.pancreatic-cancereurope.eu/work-streams/research>).

FAMILIAL CANCER CLINICAL UNIT (FCCU)

Miguel Urioste
Clinical Unit Head

Graduate Student
Laura Pena

Technicians
Maika González, Fátima Mercadillo



OVERVIEW

Approximately 1 to 2% of the U.S. population harbours a genetic mutation in well-known genes that confer predisposition to cancer. Carriers of such genetic mutations could benefit from established preventive strategies. For example, more than half a million individuals in the U.S. harbour a pathogenic mutation in a Lynch syndrome gene that confers a high risk of colorectal cancer, a tumour readily preventable through annual colonoscopy. Like Lynch syndrome-related genes, there exist a few dozen of such cancer predisposition genes that could be candidates for carefully focused targeted sequencing, including those responsible for hereditary breast and ovarian cancer syndrome, familial adenomatous polyposis, and hereditary endocrinopathies. Identifying the individuals who have a high risk of developing cancer over time and implementing prevention or early intervention strategies could improve population health.

“It is more important to know the person who has a disease, than the disease the person has” (Hippocrates’ aphorism).

Advances in genetic and genomic tools are transforming medical practice and are contributing to the elucidation of the genetic basis of cancer predisposition. It is now easier to identify individuals with a moderate to high risk of developing cancer. However, the use of these new genetic technologies in a clinical setting requires the expertise of genetically educated professionals. The FCCU is committed to spreading the role of genetics in medicine; it takes part in educational initiatives that aid the implementation of genetic and genomic technologies in the clinical setting.

EDUCATIONAL, CLINICAL, DIAGNOSTIC AND RESEARCH HIGHLIGHTS

The FCCU maintains its engagement to enhance the comprehensive understanding of the principles of genetics in a clinical scenario. We maintain a narrow relationship with almost all of the professionals involved in hereditary cancer care in our country, as well as with *SEOM* and *AEGH*. We actively participate in general genetics courses, cancer susceptibility classes, seminars targeted at different clinical disciplines, etc., in hospitals from all over Spain, medical schools and other centres. The Cancer Genetics Consultancy in the *Fuenlabrada University Hospital (HUF)* enables us to offer a more integrated model of instruction, training future health-care providers through rotations in the Consultancy, and inviting them to participate in clinical sessions provided by the *CNIO Human Genetics Programme* and conducted by *HUF’s Hereditary Cancer Clinical Committee*. We have also continued the relationship with certain patient associations in order to circulate basic knowledge regarding cancer predisposition. We regularly participate in the annual meetings of different associations and have also initiated specific research collaborations with some of them.

Our clinical and diagnostic activities this year can be summarised as follows: 506 patients visited our consultancy at *HUF* (24.02% increase over 2016), and 428 genetic diagnostic studies were performed in the FCCU laboratory (21.59% increase). We are also currently working with a recently reviewed multigene panel that includes more than 90 cancer predisposition genes.

The FCCU actively contributes to the research on less frequent cancer predisposition syndromes. The PTEN hamartoma tumour syndrome (PHTS) collectively refers to several rare diseases with overlapping clinical features, a germline cause and an increased predisposition to various cancer types. Our study presents a series of 144 individuals of Spanish origin with PHTS in whom we interrogate the mutational and clinical spectra. Comparisons with respect to other population studies are discussed and guidelines for PHTS patient management are suggested. This is the largest study in Spanish PHTS patients. Our results are in agreement with previously published works in other populations, with a few exceptions such as a higher frequency of mutations in exon 1 (FIGURE). Finally, we discuss the usefulness of the diagnostic

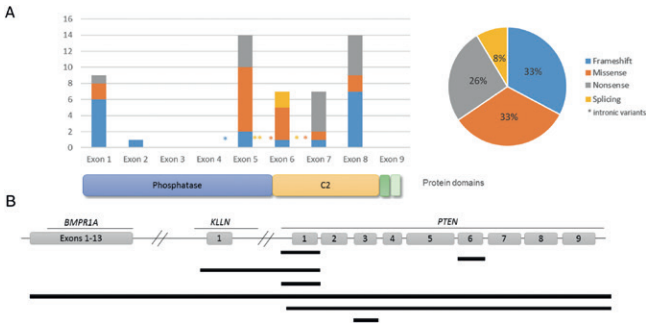


Figure Spectrum of *PTEN* mutations in our PHTS cohort. (A) Distribution and molecular consequence of point mutations. (B) Genetic regions affected in the 7 large deletions.

criteria established for this disease, based on our findings in the *PTEN+* vs the *PTEN-* cohorts. A manuscript reporting all this information in detail is ready for submission. We are also working with the PTEN Research Foundation (UK) in order to increase awareness and diagnosis of PHTS and to build professional networks that facilitate advances in the understanding of the disease.

Our research in colorectal cancer (CRC) has continued to focus on early-onset forms of CRC (EOCRC). We have identified a recurrent deletion of 16p13.12-p13.11 chromosomal region in EOCRC. This deletion was associated with a better prognosis. The *NOMO1* gene is located in this chromosomal region, and we also observed homozygous deletion of this gene mainly associated with EOCRC, and particularly with microsatellite stable subtypes. Our findings may serve as a starting point for further studies to confirm the potential carcinogenic value of this 16p13.12-p13.11 deletion, which would place *NOMO1* in a suitable position as a potential therapeutic target for EOCRC treatment. ■

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MOLECULAR
CYTOGENETICS AND
GENOME EDITING UNIT

Sandra Rodríguez
Unit Head

Post-Doctoral Fellow
Raúl Torres (until November)



OVERVIEW

Chromosomal rearrangements are very common events involved in the initiation and development of several solid and many haematological neoplasias. The research activity of the Molecular Cytogenetics and Genome Editing Unit covers the main topics related to human cancer cytogenetics and genome engineering: from classical cytogenetics techniques to new genome engineering tools, including the CRISPR-Cas9 system. We are focusing on the implementation and development of new technologies to enhance the knowledge about the (cyto)genetics of tumours and to discover new potential therapeutic targets. With the combined usage of CRISPR-Cas9 genome editing and cellular technologies, we are creating human *in vitro* models that recapitulate chromosomal, genetic and epigenetic cancer alterations. The members of the Unit also participate in collaborative projects with clinical and basic science investigators across the CNIO and other Institutes.

“We have applied genome engineering approaches for cancer modelling, reproducing chromosome rearrangements and gene alterations. We provide access to the latest Cytogenetic and CRISPR technologies.”

Technicians
M. Carmen Carralero (until May),
M. Carmen Martín, Marta Martínez-
Lage (TS)*, Francisco J. Moya
(until October) (TS) *

*Titulado Superior (Advanced Degree)

RESEARCH HIGHLIGHTS

Optimising CRISPR-Cas9 to model cancer aberrations
in human stem cells

Efficient methodologies for recreating cancer-associated chromosome translocations are in high demand as tools for investigating how such events initiate cancer. The CRISPR-Cas9 system has been used to recreate the genetics of these complex rearrangements at native loci while maintaining the architecture and regulatory elements. However, the CRISPR system remains inefficient for gene editing in human stem cells. We have optimised new strategies to enhance the efficiency of CRISPR-mediated translocation induction in human stem cells, including mesenchymal and induced pluripotent stem cells. We found that the generation of targeted translocation is significantly increased when using a combination of ribonucleoprotein complexes (Cas9 protein+sgRNA) and ssODNs. The CRISPR-Cas9-mediated generation of targeted translocations in human stem cells opens up new avenues to model cancer.

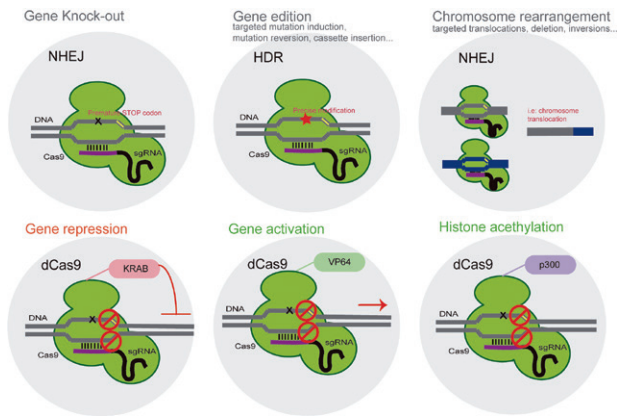


Figure Overview of CRISPR-Cas9 applications. CRISPR/Cas9 technology can be used for gene and chromosome editing, transcriptional regulation and epigenetic regulation, among others. Abbreviation: NHEJ, non-homologous end joining; sgRNA, single guide RNA; HDR, homologous directed repair; dCas9, dead Cas9.

Technological and translational activities

We provide state-of-the-art Molecular Cytogenetic and Genome Editing services. The Unit makes available various techniques, which may provide more sensitive and accurate tools to analyse cancer cells, to research groups; these techniques include RNA-FISH, chromosome stability studies based on a combined array CGH-FISH approach, or the use of CRISPR libraries to perform high-throughput functional analysis. For gene editing experiments, we have set up a specific PCR-based

FISH analysis to detect the genome integration site of small constructs including LV particles. In 2017, we carried out over 1,500 assays for experimental and clinically-oriented projects. ■

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HUMAN GENOTYPING-CEGEN UNIT

Anna González Neira
Unit Head

Graduate Student
Sara Ruiz (until April)



OVERVIEW

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

“Our goal is to advance in our understanding of patient responses to treatment in order to personalise cancer patient care.”

Technicians
Charo Alonso, Nùria Álvarez,
Belén Herráez, Rocío Nuñez (TS)*,
Guillermo Pita (TS) *

*Titulado Superior (Advanced Degree)

Student in Practice
María Rodrigo (since July,
Universidad Complutense de Madrid)

RESEARCH HIGHLIGHTS

Two novel genes associated with anthracycline-induced cardiotoxicity

Anthracycline chemotherapeutic agents are widely used in the treatment of breast cancer; however, chronic anthracycline-induced cardiotoxicity (AIC) is a serious long-term complication leading to substantial morbidity. Our aim was to identify new genes influencing the susceptibility to AIC. We studied the association of variants on the Illumina HumanExome BeadChip array in cancer anthracycline-treated patients. Through gene-based tests (SKAT-O), which have greater statistical power to detect association with rare variation and that can evaluate the cumulative effect of multiple genetic variants, we identified novel significant associations for 2 genes: the first one, ETFB (electron transfer flavoprotein beta subunit) is involved in mitochondrial β -oxidation and ATP production (Ruiz Pinto *et al.*, *Breast Cancer Res Treat* 2017), and the second one, GPR35 (G protein-coupled receptor 35), is a gene with potential roles in cardiac physiology and pathology (Ruiz Pinto *et al.*, *Pharmacogenet Genomics* 2017). Further functional studies are currently being undertaken.

Genetic Factors underlying the risk of alopecia in patients treated with chemotherapy

Alopecia is a common toxicity of anticancer drugs and is considered by patients as being the second worst physical side-effect of chemotherapy, after nausea and vomiting. Alopecia associated with conventional doses of chemotherapy has traditionally been considered to be reversible in all cases upon cessation of therapy. However, the observation of some cases of alopecia that persists several years after the end of adjuvant chemotherapy has prompted us to identify genetic factors associated with the appearance of this toxicity in a cohort of cancer patients. We

performed a GWAS (Genome Wide Association Study) involving patients suffering from this adverse drug reaction and patients without the toxicity; both were treated with the same drug and dose. We found several significant loci (P value $<10^{-6}$) associated with the risk of developing alopecia. Currently we are replicating the most significant hits in an independent cohort of patients.

Identification of genetic variants associated with docetaxel and anthracycline efficacy

Docetaxel and anthracycline are widely used in the treatment of breast cancer, although the benefit is limited to only a small proportion of patients, and preoperative biomarkers predictive of clinical outcome remain lacking. We carried out a pharmacogenetic study in 181 patients with locally advanced breast cancer who were previously enrolled in a phase 2 randomised clinical trial (NCT00123929); a trial in which patients were randomly assigned to receive doxorubicin (anthracycline) or docetaxel (taxane) in neoadjuvant therapy. We assessed whether genetic variants in 15 key transport or metabolism genes relevant to doxorubicin and docetaxel drugs could play a role as predictive biomarkers. We identified a genetic variant located in the promoter of ABCC2, as the strongest association with tumour response observed in patients treated with doxorubicin (P=0.009). We also identified a significant association for an intronic variant located in CYP1B1 associated with docetaxel tumour response (P=2.15x10⁻⁴). Our integrated pathway-based approach enables the revealing of promising genetic biomarkers for treatment outcome in breast cancer patients (submitted for publication). ■

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CLINICAL RESEARCH PROGRAMME

MIGUEL QUINTELA-FANDINO Acting Programme Director



The Clinical Research Programme has 2 main aims: 1) to translate preclinical research into novel clinical care standards; and 2) to address novel clinical oncology challenges with preclinical research. The specific areas of work include: 1) development of novel agents; 2) study of the mechanisms of action of novel compounds and tackling drug resistance; and 3), moving forward in the field of biomarkers, functional taxonomy and precision medicine.

Currently, the Programme is composed of 4 Clinical Research Units, 1 supporting Unit and clinical trials Management. The Breast Cancer Clinical Research Unit, headed by Miguel Quintela-Fandino, has successfully translated its preclinical research in angiogenesis into two novel independent clinical trials. The effects of targeting immune reprogramming in response to hypoxia-inducing antiangiogenics and mitochondrial metabolism in response to hypoxia-correcting antiangiogenics are being tested prospectively in 2 ongoing trials launched in 10 hospitals of the Spanish National Healthcare System. The Prostate Cancer Clinical Research Unit, under the supervision of David Olmos, has completed its biobanking collection of >17000 samples that will allow the defining of biomarkers of activity and resistance against the main agents used in the management of advanced prostate cancer. They have also gathered a large patient cohort that will determine, for the first time, novel genetic markers associated to inherited prostate cancer in the European population. The Lung Cancer Clinical Research Unit, led by Luis Paz-Ares, has significantly contributed to the discovery of biomarkers that will impact the selection tools for targeted therapies in advanced lung cancer. They have also led several practice-changing international clinical trials. Finally, the Haematological Malignancies Clinical Research Unit, headed by Joaquín Martínez-López, has developed novel tools for the diagnosis and surveillance of the clinical course of different haematological malignancies. Regarding drug development, a novel role for the MEK pathway during drug resistance in acute myeloid leukaemia has been elucidated. An exciting novel line of research based on the *ex vivo* expansion of natural killer cells is currently ongoing.

“The Clinical Research Programme aims to improve cancer care by developing novel agents and personalising therapeutic approaches on the basis of biomarkers.”

The Molecular Diagnostics Unit and Clinical Trials Management, provide support to the Programme in different areas. The Molecular Diagnostics Unit, led by Luis Lombardía, executes a variety of molecular tests that serve the purpose of screening patient candidates for CNIO clinical trials. The latter, led by Antonio López, takes care of the operational procedures of multi-centric clinical trials, including tasks such as site selection, protocol development or regulatory and legal affairs oversight.

During 2017, the Programme established novel alliances with ‘big pharma’ including companies such as AstraZeneca or Bayer AG that support our current research; this reflects the level of interest that our activities raise in the drug development field. The number of CNIO-sponsored/led trials has increased to 9 and the collaborating hospitals to over 60.

BREAST CANCER JUNIOR CLINICAL RESEARCH UNIT

Miguel Quintela-Fandino
Clinical Research Unit Head

Staff Scientists
María José Bueno, Silvana A. Mouron



OVERVIEW

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

- Discovery of new targets for breast cancer prevention: role of fatty acid synthase (FASN).
- Breast cancer functional taxonomy: by using a systems biology approach, we are clustering the disease into subtypes defined by biologic features that constitute therapeutic targets.

“In 2017, the Breast Cancer Group has confirmed the role of FASN as a therapeutic target for the prevention of epithelial cancer development and has launched 2 clinical trials aimed at assessing the adaptive mechanisms of escape from antiangiogenic therapy.”

- Study of the mechanisms of resistance against targeted therapies.
- Conduct investigator- initiated clinical trials.

Clinical Research Fellow
Juan V. Apala

Graduate Students
Sara Fernández, Gonzalo Pérez
(until November)

Technicians
Verónica Jiménez, José Francisco López (until June) (TS)*, Esperanza Martín (until April)

*Titulado Superior (Advanced Degree)

RESEARCH HIGHLIGHTS

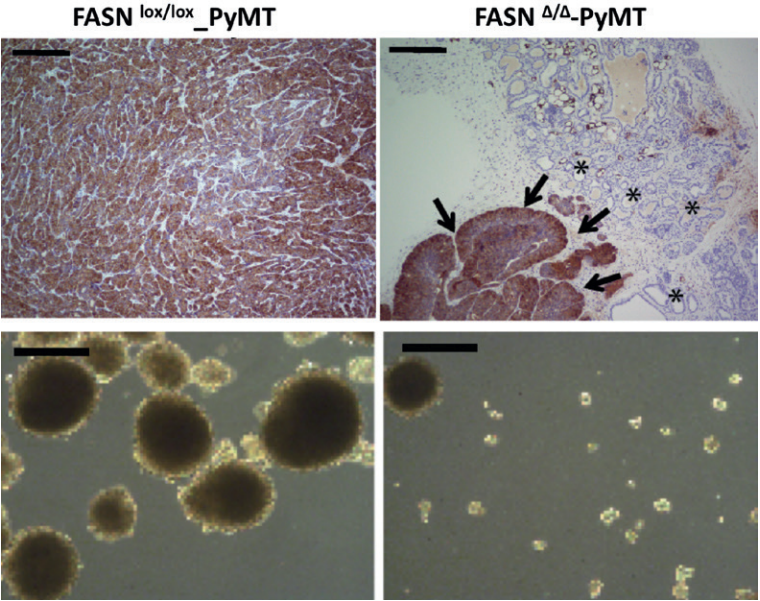
Our studies about fatty acid synthase (FASN) have finally revealed a role for this molecule as a therapeutic target for preventing the development of epithelial cancers. FASN, an enzyme expressed at almost undetectable levels in adult tissues, elicits the metabolic impulse that cells undergoing the transformation process require to complete the transition from organised 2D-growth to de-organised, anchorage-independent 3D-growth. FASN inhibitors are well tolerated and have proven to abrogate the development of breast and lung cancers driven by oncogenes such as Pi3K, K-RAS or HER2. These findings set the grounds for future clinical interventions.

On the clinical side of our activities, in 2017, we launched 2 independent clinical trials addressing the adaptive mechanisms of escape against chronic exposure to antiangiogenics that our laboratory tackled between 2012 and 2016; the results were

published last year. In one of the trials, CNIO-BR-008, we are exploring the concept of hypoxia-induced immunodepression. In a number of patients, instead of vascular normalisation, antiangiogenics deteriorate the vascular network, an occurrence associated with increased PD-L1 and kynurenine signalling; these patients can be detected by 18F-Misonidazole PET. In this trial we are exploring the therapeutic effect of adding MEDI-4736 (an anti-PD-L1 monoclonal antibody) to bevacizumab in breast cancer patients who experience disease progression while in treatment with bevacizumab monotherapy, stratified by Miso-PET response. In the second trial, CNIO-BR-009, we are exploring the synergy between the mitochondrial inhibitor ME-344 and bevacizumab in patients who experience the opposite adaptive mechanism – vascular normalisation followed by mito-switch, detected by Miso-PET. ■

Figure Impaired ability to transform in absence of FASN: the upper-left panel shows a tumour arising in the breast epithelium of a wild-type PyMT mouse, stained for FASN. The upper-right corresponds to a FASN KO animal, where the KO is incomplete. It can be appreciated how only the

FASN-positive areas are able to form tumours, whereas only minor proliferations that are unable to break the basal membrane are observed in the FASN-negative areas. Below, examples of anchorage-independent 3D growth of PyMT cells, positive (left) or negative for FASN.



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PROSTATE CANCER
JUNIOR CLINICAL
RESEARCH UNIT

David Olmos
Junior Clinical Research Unit Head

Clinical Investigator
Elena Castro

Clinical Research Fellow
Nuria Romero, Rebeca Lozano

Graduate Students
Ylenia Cendón, Lorena Magraner, Paz Nombela



OVERVIEW

Prostate cancer (PrCa) is one of the most heritable human cancers, as almost 60% of the PrCa risk is attributable to genetic factors. Inherited mutations in several genes involved in DNA damage response and repair (DDR) have been reported to predispose men to prostate cancer; this includes mutations in *BRCA2*, the genetic event known to confer the greatest risk of the disease. Recent studies have revealed that germline deleterious mutations in DDR genes are present in 8-12% of patients with metastatic PrCa, mutations in *BRCA2* being the most prevalent ones. Our Group has previously established that these mutations are an independent prognostic factor for the disease. These observations have come full-circle with the development of Poly (ADP-ribose) polymerase inhibitors to treat advanced prostate cancer. Due to the high prevalence of DDR germline mutations and the implications for the patients and their families, clinical

guidelines have recently been updated to recommend genetic screening to all patients with metastatic PrCa. In consequence, the number of mutation carriers is likely to grow over the next few years, whilst the most appropriate treatment and management still remains unknown. This year our Unit's research has focussed on gaining a better understanding of the clinical implications of *BRCA2* and other DDR defects; the molecular characterisation of these tumours and the identification of biomarkers can enable us to predict the benefit derived from currently available therapies.

Technician
Vanessa Cañadilla

Student in practice
Isabel Santos (July-November)

Visiting scientists
Teresa Garcés (*Instituto de Investigación Biomédica de Málaga*), Gala Grau (*Instituto de Investigación Biomédica de Málaga*), Ana M. Gutiérrez (*Hospital Universitario*

de Móstoles, Madrid), Fernando López (*Hospital Ramón y Cajal, Madrid*), Maria I. Pacheco (*Instituto de Investigación Biomédica de Málaga*), Leticia Rivera (*Instituto de Investigación Biomédica de Málaga*),

Benjamin Olmos (since October) (*Hospital Universitario Virgen de la Victoria, Málaga*)

RESEARCH HIGHLIGHTS

PROREPAIR study. PROREPAIR-B (Castro *et al.*, Abstract LBA32, ESMO 2017) is the first prospective study to address the prevalence of germline DNA damage response genes, deleterious mutations and their impact on clinical outcomes following conventional treatments for metastatic prostate cancer. The study included 419 mCRPC patients. *BRCA2* (3.3%), *ATM* (1.9%) and *BRCA1* (0.9%) were the most commonly mutated genes. Pathogenic variants in all of the 107 analysed genes were identified in 15% of patients. Carriers and non-carriers presented similar characteristics at baseline, but carriers, particularly *BRCA2* carriers, progressed earlier and lived shorter lives than non-carriers despite the administered treatments. This is a prospective multicentre cohort study involving 38 Spanish centres within the PROCURE network (see below).

SWITCH Phase II study. In 2017, we also presented the final results of the SWITCH study; a multicentre, single arm, open label, single-stage, phase II study. Clinically stable mCRPC patients who had PSA and/or limited radiographic progression after at least 12-weeks on Abiraterone plus prednisone, switched to Abiraterone plus dexamethasone. PSA50 response rate was 34.6%, Median time to biochemical and radiological progression were 5.3 and 11.8 months, respectively. Patients with AR gain detected in plasma ctDNA did not respond to the switch, while patients with AR normal status benefitted the most. The change of prednisone to dexamethasone in clinically stable patients progressing to Abiraterone plus prednisone could revert some resistances and induce durable responses in advanced prostate cancer.

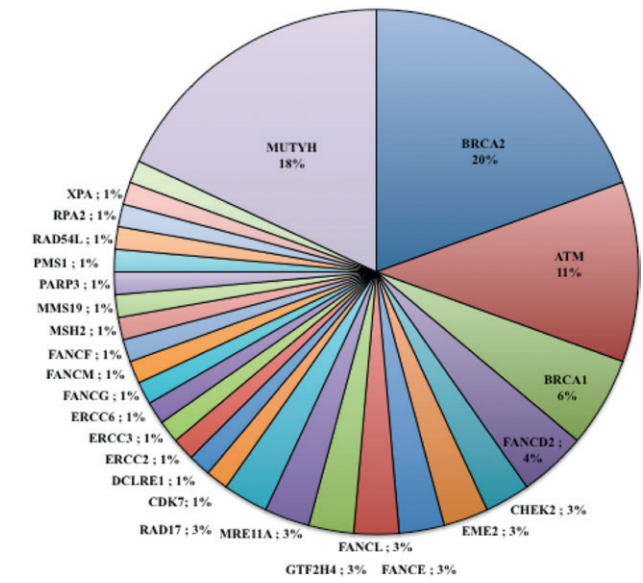


Figure The pie chart illustrates the gene distribution of the 72 pathogenic variants in DNA Damage Repair genes found in 68 mCRPC patients out of 419 included in the PROREPAIR-B study.

PROCURE biomarkers platform. This network, started by our Group in 2013, involves 63 participating oncology departments across Spain. Over 900 patients have been enrolled in the 5 currently active prospective studies (PROREPAIR, PROSTAC, PROSABI, PROSENZA, PRORADIUM). This network has attracted international attention and partners from Italy (MEET-URO group) and Australia (Peter McCallum Cancer Centre) have joined us this year. ■

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AWARDS AND RECOGNITION

Scientific Committee Member, 2017 ESMO Congress, Madrid, Spain.

Faculty Board Member, 2017 EORTC-EC-ACR-ESMO Methods in Clinical Cancer Research Workshop, Netherlands.

Elena Castro: awarded the 2017 Stewart-Rahr Young Investigator Award (Prostate Cancer Foundation, USA); Faculty, ESMO Preceptorships in Prostate Cancer.

Nuria Romero received an "ESMO 2017 Merit Award", ESMO Congress, Spain.

Rebeca Lozano was awarded: 'Best Communication' Award, 4th Androgen Project Meeting in Prostate Cancer; 'Best Poster' Award, SEOM annual Meeting, Spain.

MOLECULAR DIAGNOSTICS UNIT

Luis Lombardía
Unit Head

Technician
Diana Romero



OVERVIEW

The activity of the Molecular Diagnostics Unit (MDU) is primarily focused on providing an abundant assortment of molecular assays to clinicians working in hospitals of the Spanish National Health System; our aim is to support the management of their patients with different cancers. Occasionally, MDU also provides support, with its technical expertise and logistics, to CNIO's Clinical Research Units and Research Groups. Furthermore, in order to keep our catalogue updated, our efforts are persistently geared towards strengthening, upgrading and reducing the turnaround times and costs of the assays that are currently available. Also, with the aim of further expanding our end users portfolio, we are tasked with implementing and offering new molecular tests based on the latest discoveries in the field and/or the needs of clinicians or researchers. Finally, a crucial part of our mission is to host professionals and students who aspire to be instructed in this area.

“In comparison to 2016, MDU has seen a substantial increase (around 30%) of the number of tests required by hospitals, thus contributing to a better individualised care of the patients.”

RESEARCH HIGHLIGHTS

During 2017, we completed the standardisation and implementation of a new assay, launched in 2016, which will enable us to detect mutations in exons 3, 6, 7, 9, 10 and 11 of the tumour suppressor gene TET2 and, therefore, enable a better prediction the prognosis of patients with myeloproliferative neoplasms.

We have also expanded our catalogue with the addition of 2 new molecular diagnostic tests based on bi-directional Sanger sequencing. The first one will enable the detection of activating mutations in exons 13, 14, and 22 to 28 of the proto-oncogene *ERBB2* (Erb-B2 Receptor Tyrosine Kinase 2, aka *HER2*) in patients with breast, ovarian, colorectal, gastroesophageal and lung cancers. These oncogenic alterations can cause resistance to treatments with reversible tyrosine kinase inhibitors and, thus, promote a more aggressive and metastatic disease. Therefore, the detection of these mutations will significantly expand the range of patients that could benefit from useful targeted therapies (FIGURE).

The other assay will enable us to detect mutations in the *POLE* (Polymerase Epsilon) gene. This gene codes for a DNA polymerase

with a proofreading exonuclease activity, which thereby allows high-fidelity DNA replication to occur. Quite recently, a group of endometrial carcinomas (EC) – not sufficiently distinctive to allow accurate diagnosis based on routine histological staining – has been identified with a high rate of somatic missense point mutations, commonly reported at 3 hotspots in exons 9, 13 and 14 of *POLE*. These tumours are associated with improved progression-free-survival, which is not derived from a higher sensitivity to chemotherapy but are more likely linked to enhanced immunogenicity. Since the clinical praxis is to give adjuvant chemotherapy to most patients with EC, this test will enable us to avoid unnecessary treatments in *POLE*-mutated cancer patients.

Additionally, as in previous years, we continue to interact with national and international standardisation groups in order to maintain a high level of quality standards in the molecular diagnostic tests provided by us. Finally, we uphold our policy of welcoming clinicians and technical specialists in order to mentor and train them in the field. ■

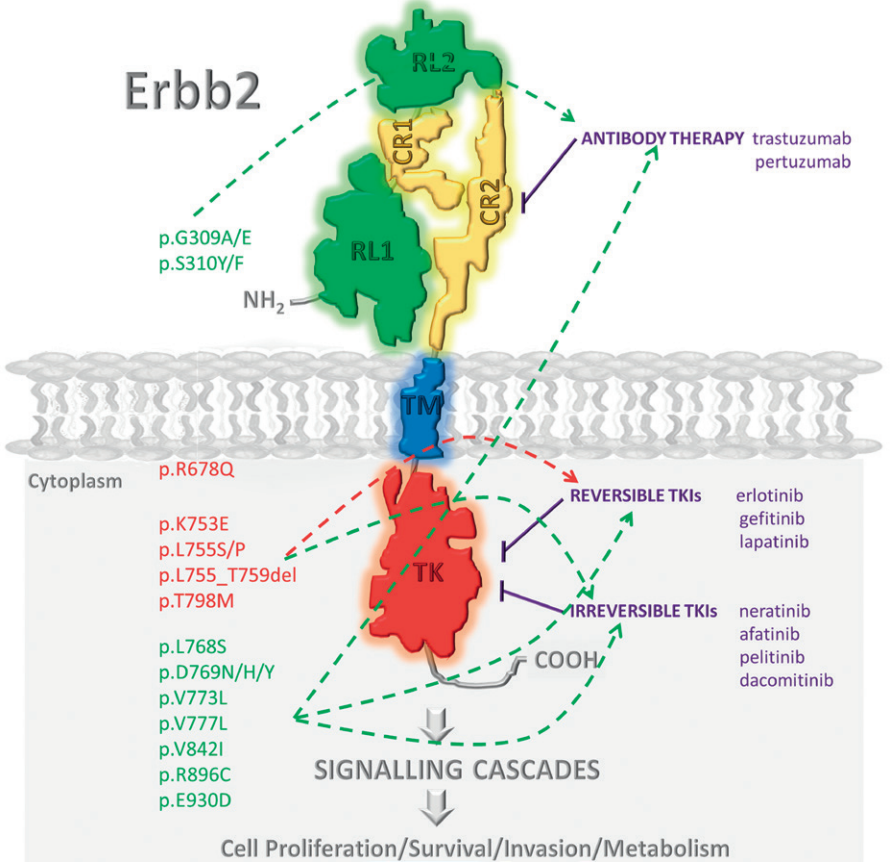


Figure Mutations in *ERBB2* can produce either sensitivity (green dashed arrows) or resistance (red) in the response of patients with breast cancer to different therapies. (RL: Receptor L; CR: Cysteine Rich; TM: Transmembrane; TK: Tyrosine Kinase; TKI: TK inhibitors).

H12O-CNIO HAEMATOLOGICAL MALIGNANCIES CLINICAL RESEARCH UNIT

Joaquín Martínez-López
Clinical Research Unit Head



Staff Scientists
Rosa Ayala, Teresa Cedena, Miguel Gallardo, Inmaculada Rapado, Beatriz Sánchez-Vega

Post-Doctoral Fellows
Lucía Fernández, Almudena García (since November), María Linares, Ricardo Sánchez, Antonio Valeri

Graduate Students
Alicia Arenas (until November), Isabel Cuenca, Alejandra Leivas, M. Luz Morales, Alejandra Ortiz, Yanira Ruíz, Laura Sánchez (since October)

Technicians
Alba García (until November), Esther Onecha

Student in Practice
Sara Rodríguez Collado (August)

OVERVIEW

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

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

“During this year, the Group has developed new markers and tools to provide a better diagnosis, surveillance and treatment of haematological diseases such as multiple myeloma, leukaemia and lymphoma.”

RESEARCH HIGHLIGHTS

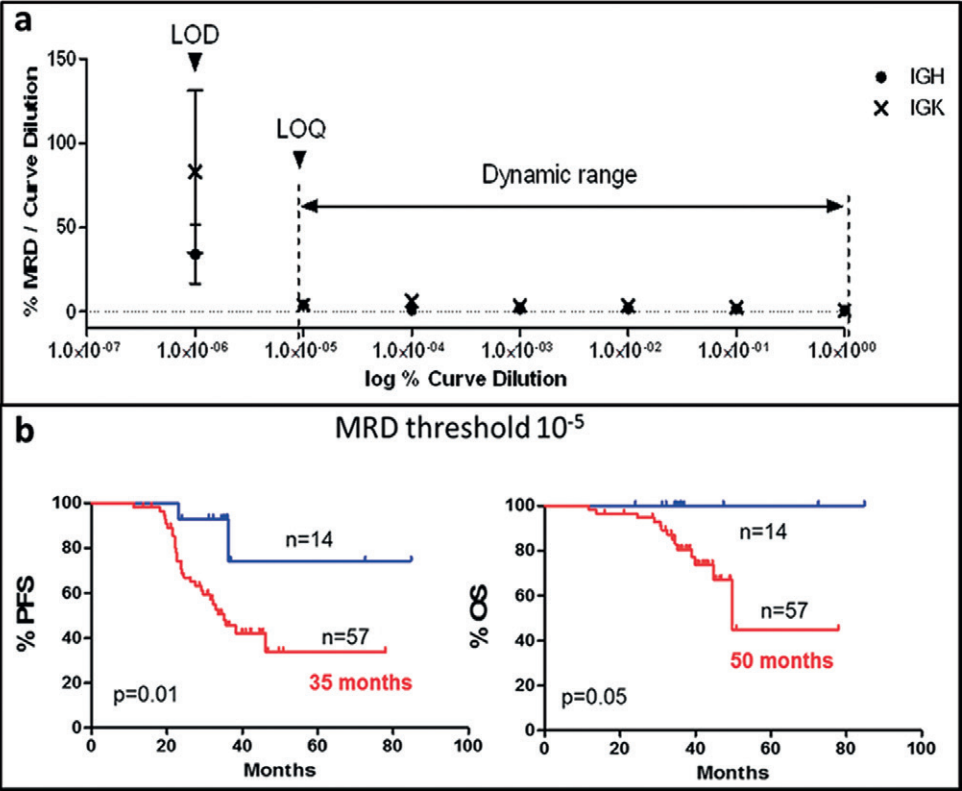
During this year, our Group has developed new tools for the diagnosis and surveillance of different haematological diseases using Next Generation Sequencing (NGS):

- Targeted RNASeq for the identification of Ph-like B-acute lymphoblastic leukaemia (ALL).
- A novel NGS method for studying BCR-ABL1 protein variants in cDNA from bone marrow and cerebrospinal fluid blast cells from ALL patients.
- A simplified in-house deep-sequencing method to identify and quantify Minimal Residual Disease in multiple myeloma

(MM) patients using NGS of immunoglobulin rearranged genes (FIGURE 1).

- A specific NGS panel of different genes (*NPM1*, *IDH1*, *IDH2* or *DNMT*) for evaluating relapse, drug response and Minimal Residual Disease in myeloid diseases.

Figure 1 Minimal Residual Disease detection in multiple myeloma patients using NGS. (A) Plot of the dynamic range, limit of quantification (LOQ) and limit of detection (LOD). (B) Progression-free survival (PFS) and overall survival (OS) plots according to MRD levels (blue, negative; red, positive).



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On the other hand, new molecular targets have been explored:

- The new role of PAK 4 in multiple myeloma.
- The control that hnRNP K exerts on proliferation and differentiation programmes in haematological malignancies such as lymphoma.
- The activation of the MEK/ERK1/2 pathway during drug resistance in acute myeloid leukaemia.
- The value of PTCH1 for predicting imatinib response in chronic myeloid leukaemia patients in chronic phase.

effective *in vitro*, *ex vivo* and in patients with relapsed or refractory myeloma (FIGURE 2).

Moreover, we have demonstrated that the NKG2D receptor, expressed in both natural killer cells and CD8+ T cells, NKG2D-NKG2DL could be used as an immunotherapeutic strategy against cancer; NKG2D-4-1BB-CD3z CAR-redirected memory T cells efficiently targeted NKG2DL-expressing osteosarcoma cells *in vivo* and *in vitro*. ■

Finally, a novel treatment strategy using autologous activated and expanded natural killer cells plus anti-myeloma drugs has been developed for multiple myeloma; the strategy has been

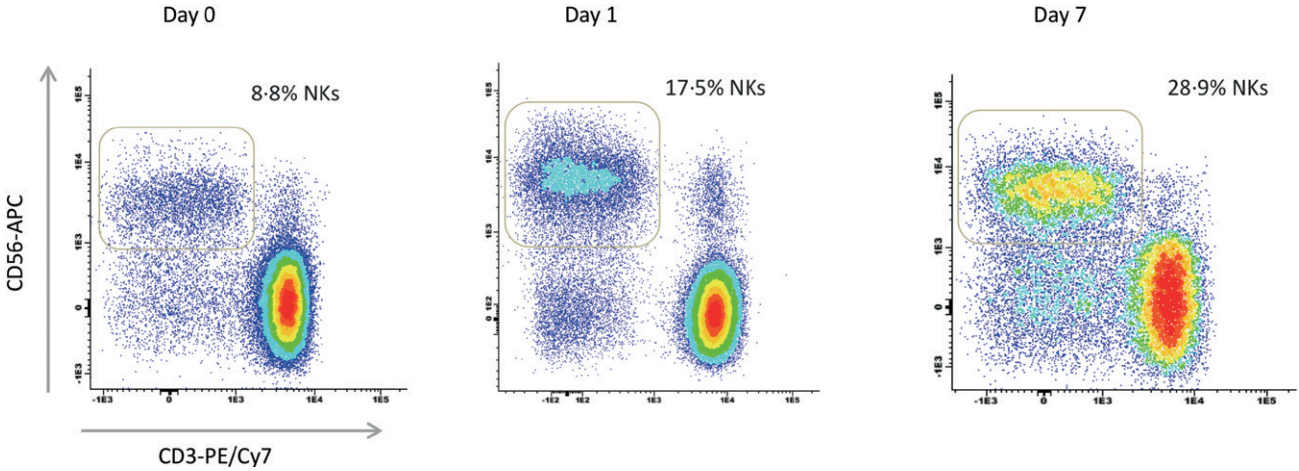


Figure 2 Representative flow cytometry data of lymphocyte populations from a single patient during the first cycle using autologous activated and expanded natural killer cells plus anti-myeloma drugs.

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H12O-CNIO LUNG CANCER CLINICAL RESEARCH UNIT

Luis G. Paz-Ares
Clinical Research Unit Head

Staff Scientists
Teresa Argullo, Irene Ferrer, Rocio García, Eva M. Garrido (since March), Sonia Molina, Santiago Ponce, M. Carmen Riesco (since February), Jon Zugazagoitia (until September)



Post-Doctoral Fellows
Pablo Gella (since March), María Pedraza (since February), Beatriz Soldevilla

Graduate Students
Carlos Carretero (since April), Santiago García (since November), Ángela Marrugal, Cristina Ojeda (since February), Laura Ojeda,

Álvaro Quintanal, Javier Ramos (since March), Patricia Yagüe

Technicians
M. José Durán, Laura García, Beatriz Gil, Rocío Suárez

OVERVIEW

Lung cancer is the most frequent cause of cancer-related deaths worldwide. Our Unit is dedicated to the study of lung cancer, combining basic research studies with other more clinically oriented research studies, closer to solving the problems of lung cancer patients. The two main research areas of our Unit include: the identification of new molecular biomarkers that can be used in the clinic for diagnostic, prognostic and predictive purposes; and the development of novel treatment strategies that include targeted therapies and immunotherapeutics. For example, we have elucidated the molecular determinants of the oncogenicity of FGFRs, and have discovered biomarkers to monitor the efficacy of FGFR inhibitors in lung cancer. On the other hand, we have developed a patient-derived xenograft (PDX) platform of non-small-cell lung cancers to test new therapeutic strategies. Finally, our Unit has extensive experience in bringing new drugs to the clinic (phase I trials), as well as in conducting practice-changing phase II/III trials in the fields of precision oncology and immuno-oncology.

“We have significantly contributed to the discovery of biomarkers that will impact the current selection for targeted therapies (e.g. FGFR inhibitors). We have led randomised clinical trials with biological therapies and immunotherapy in lung cancer that have resulted in treatment changes in clinical practice (e.g. Durvalumab in stage III NSCLC or Afatinib in EGFR mutated NSCLC).”

RESEARCH HIGHLIGHTS

Biomarker discovery and implementation

Our Group has deciphered the biological role of FGFR1 and FGFR4 in non-small cell lung cancer (NSCLC) and has developed new biomarkers with predictive roles for anti-FGFR therapy in NSCLC. The data show that the determination of FGFR1/4 gene amplification or expression alone is not sufficient to predict the efficacy of anti-FGFR therapy, but that a complementary determination of a biomarker expression may further optimise patient selection for this therapeutic strategy (manuscripts and patent applications have been submitted). Currently, the Group is: validating the results on a series of well-characterised PDX models; generating an antibody against the new biomarker in order to develop a diagnostic kit; carrying out the technical validation of the biomarker; and submitting a phase II trial proposal with an FGFR inhibitor in NSCLC patients with high expression of this biomarker.

The Group has also validated an NGS-based algorithm for the determination of genomic aberrations – in tumour tissue as well as in cfDNA – that could be used to guide treatment in clinical practice.

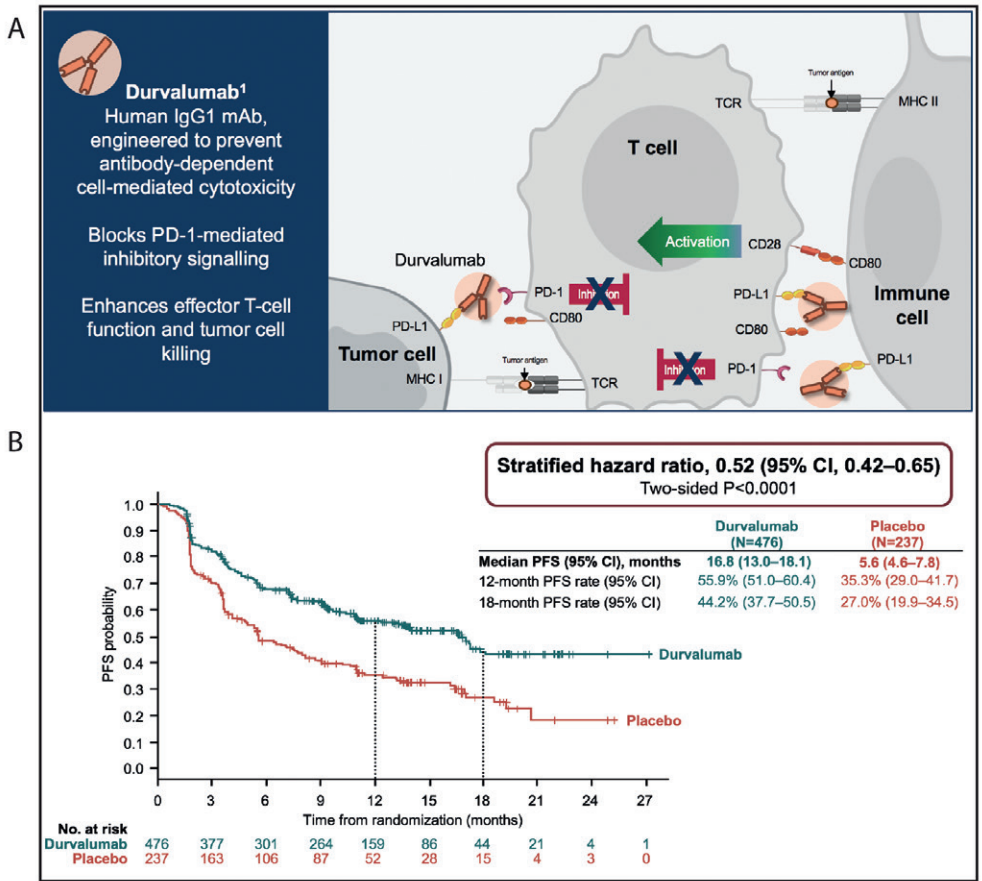
Early clinical trials

Our Group has significantly expanded its activity of testing new molecules and combinations in solid tumours, particularly in the area of immune-based approaches, and has participated in more than 25 projects in this area of research in 2017. Recently, we provided key delivery and feasibility data supporting the use of IV infusion of enadenotucirev (or therapeutic transgene-bearing derivatives of it) in clinical trials across a range of epithelial tumours, including the ongoing combination study of enadenotucirev with the checkpoint inhibitor nivolumab. This phase I study also provided insights into the potential immune-stimulating properties of enadenotucirev (R Garcia-Carbonero *et al.*, *J Immunother Cancer* 2017). Encouraging data on the novel combination of pembrolizumab plus ramucirumab in the cohort of non-small-cell lung cancer were updated at ASCO 2017, showing a response rate of 35 % and 9.7-months of progression-free survival in pretreated patients. Finally, data of a first-in-human trial with a novel T-cell bispecific antibody targeting carcinoembryonic antigen expressed on tumour cells and CD3 on T-cells, with or without atezolizumab, was communicated on at the ASCO annual meeting.

Changing standard-of-care treatments in clinical practice

The Lung Cancer Clinical Research Unit has led and has actively contributed to phase III trials whose results have had a significant impact on the clinical practice in the context of EGFR mutated (L Paz-Ares *et al.*, *Ann Oncol* 2017), ALK translocated (Soria JC *et al.*, *NEJM* 2017) or unselected (Carbone D *et al.*, *NEJM* 2017) stage IV lung cancer. More recently, Luis Paz-Ares communicated, at the ESMO 2017 Congress, the results of a Phase III trial showing a profound reduction in disease progression for stage III NSCLC patients treated with the anti PD-L1 agent *Durvalumab* following chemoradiation. ■

Figure (A) The anti PD-L1 mAb *Durvalumab* blocks PD-L1 binding to PD-1 and CD80. (B) Results of the randomised clinical trial PACIFIC, showing an improvement in progression-free survival (PFS) (HR 0.52; p<0.0001) in stage III Non-Small-Cell Lung Cancer (NSCLC) patients treated with *Durvalumab* (vs placebo) after chemoradiotherapy.



► PUBLICATIONS

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► AWARDS AND RECOGNITION

The Lilly Foundation Award for Clinical Biomedical Research 2017, Spain.

Ramiro Carregal- La Rosaleda Foundation International Prize for Oncology Research 2017, Spain.

BIOBANK

Manuel M. Morente
Director

Technicians
Nuria Ajenjo (TS)*, Inmaculada
Almenara (until October), M. Jesús
Artiga (TS), Francisco De Luna (TS)

*Titulado Superior (Advanced Degree)



OVERVIEW

The CNIO Biobank is a cross-service platform for CNIO researchers, as well as the general scientific community, and is geared towards the promotion of biomedical research in cancer and related diseases. The CNIO Biobank facilitates access to human samples for researchers, ensuring that both the acquisition and use of human samples complies with all the legal and ethical principles that protect donors’ rights.

The CNIO Biobank is a ‘biobank for biomedical research purposes’, as defined by the Spanish Law 14/2007 on Biomedical Research and the Royal Decree RD 1716/2011. It is therefore defined as a public, non-profit organisation that hosts several collections of human biological samples for biomedical research.

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

The Biobank has been authorised by the Health Authorities of the *Comunidad Autónoma de Madrid* – in accordance with the regulation established by RD1716/2011 – and is registered in the National Registry of Biobanks with reference B.000848.

RESEARCH HIGHLIGHTS

Biobanking

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

Management of other collections

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

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

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

Other services

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

During 2017, the CNIO-Biobank has supported 10 tissue requests from scientific research projects, independently of other services, corresponding to 650 cases distributed in 16 transferred tissue microarrays. Additionally, as the Spanish National Biobank Network Coordination Office, we have managed 30 tissue requests for scientific research projects of high complexity, plus 11 other requests for sample availability and/or documental support. We have also collaborated with the Familial Cancer Unit of the CNIO’s Human Cancer Genetics Programme in the acquisition of 49 clinical cases, as well as in the analysis and diagnosis of 64 new cases.



Figure Formalin-Fixed Paraffin-Embedded (FFPE) archives.

The mean impact factor of the 6 published articles in 2017, for which our Unit provided support, was 9.618.

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

Finally, the Unit has spearheaded many activities in the national and international biobanking scene through its participation and leadership in numerous forums, working groups and national and international scientific societies. These include the European, Middle Eastern and African Society for Biopreservation and Biobanking (ESBB), the International Society for Biological and Environmental Repositories (ISBER), international think tanks such as the Marble Arch International Working Group on Clinical Biobanking, BC-Net IARC-WHO/NCI initiative, and others. ■

PUBLICATION	AWARDS AND RECOGNITION
<ul style="list-style-type: none">• Doucet M, Becker KF, Björkman J, Bonnet J, Clément B, Daidone MG, Duyckaerts C, Erb G, Haslachner H, Hofman P, Huppertz B, Junot C, Lundeberg J, Metspalu A, Lavitrano M, Litton JE, Moore HM, Morante M, Naimi BY, Oelmueller U, Ollier B, Parodi B, Ruan L, Stanta G, Turano P, Vaught J, Watson P, Wichmann HE, Yuille M, Zaomi M, Zatloukal K, Dagher G (2017). Quality matters: 2016 annual conference of the National Infrastructures for Biobanking. <i>Biopreserv Biobank</i> 15, 270-276.	<ul style="list-style-type: none">• Member, Evaluation Panel ‘Cohorts Programme’, <i>Agence Nationale de la Recherche</i> (ANR), France.• Member, Nominating Committee, the European, Middle Eastern & African Society for Biopreservation and Biobanking (ESBB).• Member, Scientific and Organising Committee, <i>VIII Congreso Nacional de Biobancos</i>, Cartagena.• Course Director, ‘<i>Forum de Biobancos pediátricos</i>’, <i>Hospital Universitari Sant Joan de Déu (Barcelona)</i> & <i>Universitat de Barcelona</i>.

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ÓSCAR FERNÁNDEZ-CAPETILLO
Director of Innovation

“Innovation has become an intrinsic part of the CNIO culture. Besides from academic publications, our scientists are also actively involved in translating their discoveries back into society.”

The so-called ‘Translational Research’ only grows on the shoulders of excellent discoveries. At CNIO, our scientists cover a wide spectrum of topics related to cancer research; these include computational and structural biology, drug development, molecular and cellular biology, animal models, human genetics and clinical groups that are in close contact with cancer patients. Each of these activities can lead to know-how and/or goods that could be of interest beyond the boundaries of academic research, and it is the job of the Direction of Innovation to help our scientists in this transition. These exercises are not only necessary to translate our discoveries, but they also return back to the CNIO in the form of royalties that help the CNIO, its groups and the inventors themselves. Examples of these activities in 2017 include an Agreement with *PharmaMar* to provide CNIO scientists access

to a library of their natural compounds for usage in our Drug Screening projects; the licensing of Nicotinamide Riboside to *Stemtek Therapeutics* for its development as a cancer preventive and/or therapeutic agent, based on discoveries from the Group of Nabil Djouder; the creation of *Senolytic Therapeutics*, a company oriented towards the elimination of senescent cells, based on work from Manuel Serrano; and the first clinical trial in which cancer patients have been treated with a therapy developed at CNIO by Marisol Soengas’ Group. Besides from these achievements, we also kept a healthy level of activity in other areas related to Innovation, such as in establishing contracts with the Industry for the co-development of cancer-related projects, or in the licensing of antibodies that continues to be an important source of funding for CNIO.

BIOTECHNOLOGY PROGRAMME

FERNANDO PELÁEZ Programme Director



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

Faithful to its mission, a number of different technological innovations have been explored or implemented by the Programme's Core Units during the past year, often in collaboration with CNIO Groups. This year, the CNIO made a significant investment in upgrading the mass spectrometry (MS) technology available at the Proteomics Unit; the Centre acquired two modern MS instruments that will facilitate the performance of proteomics studies by improving sensitivity and throughput.

A significant proportion of the activities run by our Core Units are related to animal models. In line with our commitment to maintaining the highest possible standards related to animal research issues, the CNIO has this year joined the 'Agreement on Openness on Animal Research' promoted by the Federation of Scientific Societies in Spain (COSCE) that was launched in September 2016. An institutional statement on the use of research animals is available on the CNIO website.

This year, the Programme and its Core Units were actively involved in several networking activities. Two of us were elected as members of the Executive Committee of the Core Technologies for Life Sciences (CTLs), a new scientific association aiming to create a network of scientists working at core facilities in Europe with the goal of addressing issues that are common to these facilities. In addition, the Unit Heads were very active in participating in other networks and scientific societies from their corresponding fields.

Also, as an indication of our high commitment to training, education and outreach, the Programme has been deeply involved with the organisation of courses, workshops, visits from students, and specialised meetings. We collaborated with the 'CNIO and the city' project, organised by the Communications Department

“The access to cutting-edge technologies and associated expertise is nowadays essential for accomplishing the ambitious goals dictated by our mission of performing biomedical research of excellence.”

with funding from the FECYT (*Fundación Española para la Ciencia y la Tecnología*), organising a course for secondary school teachers that was highly successful and will be run again next year. Moreover, members of our staff participated in an increasing number of Masters and other training activities hosted at the CNIO and elsewhere.

This year, the Core Units were particularly active in attracting funding from external sources through activities related to innovation, including several contracts and agreements with private companies and public institutions, based on the technologies mastered by several of our Core Units. Also, the royalties derived from the sales of the antibodies produced by the Monoclonal Antibodies Unit have grown by about 5% over the previous year, reaching a new historical maximum.

Last but not least, 2017 has again been a very productive year, scientifically, for the Programme. The contribution of the Units to the overall scientific performance of the CNIO is reflected in about 20 publications, many of them appearing in top journals.

GENOMICS CORE UNIT

Orlando Domínguez
Core Unit Head

Technicians
Purificación Arribas, Antonio Íñigo
(since May), Guadalupe Luengo,



OVERVIEW

The Genomics Unit was established in 2000 for the purpose of providing the cutting-edge technology and services required by the CNIO research community. With the capacity to even interrogate whole genomes in a single assay, technologies such as high-density DNA microarrays and next-generation sequencing (NGS) reveal the genetic diversity of cancer and help to dissect molecular processes. Structural features, such as mutation repertoires, DNA-binding of protein factors, variations in chromatin structure/folding, as well as functional states such as transcriptomic profiles and changes (mRNA, miRNA) are being elucidated with these technologies in order to uncover basic mechanisms, therapeutic targets and prognostic biomarkers. We offer a broad range of products and services, including microarray whole genome gene expression, array comparative genomic hybridisation (aCGH), NGS library preparation for

“The Genomics Unit, with its genetic and genomic services, contributes to the understanding of the molecular processes of cancer by helping CNIO scientists in a large number of basic and applied research projects.”

exome sequencing, ChIPseq and RNAseq analysis, transgenic mouse genotyping, human cell line authentication and capillary DNA sequencing.

Jorge Monsech, Ana Belén Moreno
(until November) (PEJ)*, M.
Concepción Rodríguez (since May),
Ángeles Rubio, Delfina Sanguino

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RESEARCH HIGHLIGHTS

The Genomics Unit contributes to the advancement of research projects conducted by multiple CNIO Research Groups. We provide services at 2 levels of complexity. The genomic wide level is addressed by both deep-sequencing (NGS) and microarray technologies. NGS permits a variety of applications, such as whole genome or whole exome tumour sequencing, transcriptome analyses by RNAseq, or location of interacting protein factors on chromosomal DNA by ChIPseq. On the other hand, DNA microarray technology is a powerful platform for transcriptome determinations or for the detection of chromosomal copy number aberrations. Its use, however, has diminished over the last few years in favour of NGS. This year, the demand for NGS services has been stable and the number of samples processed has been similar to those processed in 2016. At the single locus level other services are provided. A traditional DNA capillary sequencing service, based on a 3730xl DNA Analyzer from Applied Biosystems, is being used to find and confirm mutations in candidate genes, or for the verification of cloned genes or inserts. The Unit also provides a transgenic mouse genotyping service, based on allele-specific quantitative PCR for a quick and efficient turnaround time. The catalogue of available tests for genetic modifications has expanded, upon demand, from 30 to close to 90 in 2017. The

genotyping service has seen an increase in demand in 2017 that nearly doubled that of former years.

An extended contribution, in the framework of a collaborator’s project, has led to a report being published with the co-authorship of some of the Unit’s members. A novel NGS analysis pipeline that facilitates forward genetic screenings on haploid mammalian cell lines has been described. The new RNA sequencing-based method, named TrapSeq, maps insertions that lead to productive trapping by recognising chimeric mRNAs containing gene-trap sequences spliced to an exon. The method provides a fast and cost-effective way that, not only identifies the insertion site but also, reveals its impact on the expression of the trapped gene (Mayor-Ruiz *et al.*). ■

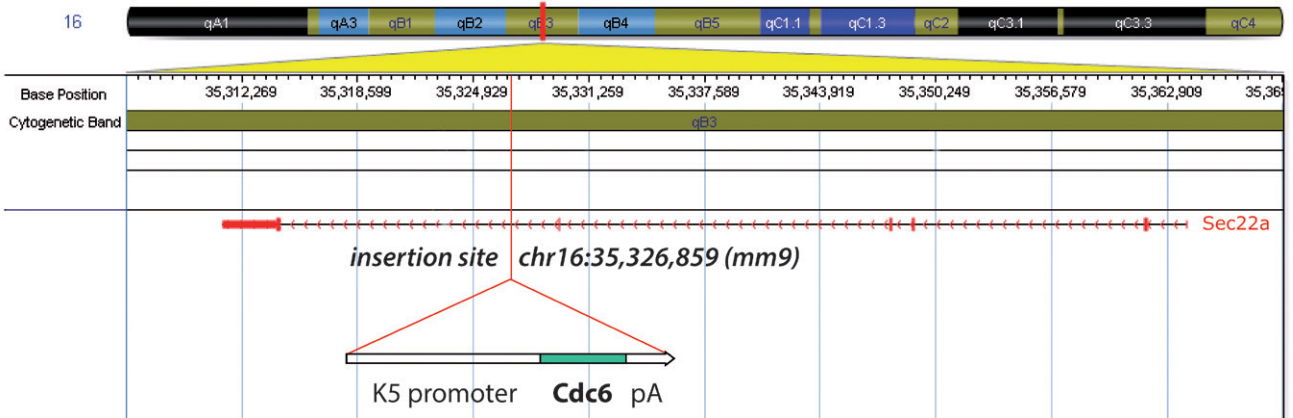


Figure This Unit is often requested to determine the insertion site of transgenes. Here, the gene coding for Cdc6, a key protein for DNA replication previously thought to have oncogenic potential, was inserted into the mouse genome to study the

effect of its deregulated function in mammalian tissues (Búa S *et al.*, *Cell Cycle*, 2015). The transgene was found to be inserted into an endogenous gene, as it often happens; in this case, the Sec22a locus.

► PUBLICATION

► Mayor-Ruiz C, Domínguez O, Fernández-Capetillo O (2017). TrapSeq: an RNA sequencing-based pipeline for the identification of gene-trap insertions in mammalian cells. *J Mol Biol* 429, 2780-2789.

TRANSGENIC MICE CORE UNIT

Sagrario Ortega
Core Unit Head

Graduate Student
Aleida Pujol

Technicians
Estefania Ayala, M. Carmen Gómez,
Jaime A. Muñoz (TS)*, Lucía M.



OVERVIEW

The laboratory mouse is the experimental model of choice for genetic studies and preclinical drug development in cancer. The Transgenic Mice Unit is dedicated to the genetic edition of the mouse germ line and to the generation of genetically modified mouse strains. Hundreds of mouse genes have been specifically modified at our Unit. In many cases, these modifications reproduce the genetic alterations found in human cancers and are introduced in the mouse to generate preclinical models of the disease, thereby contributing to the development of more efficient targeted therapies. In other instances, genetically modified mice are created for testing hypothesis *in vivo*, in a physiological context, with the aim of gaining new insight into the molecular mechanisms responsible for converting a normal cell into a malignant cell or for making a tumour grow and expand and subsequently invade other organs and become deadly. Cancer is an extremely complex

“A reporter mouse model, designed and created by the Transgenic Mice Unit to visualise tumour lymphangiogenesis by whole body imaging, has been crucial for elucidating mechanisms of melanoma dissemination and metastasis: *Nature* 546, 676 (2017).”

disease that cannot be sufficiently studied in a tissue culture plate. The generation of genetically modified mice is one of the basic pillars on which cancer research at the CNIO is based.

Pérez De Ayala (until November)
(PEJ-L) **, Patricia Prieto (TS) *,
Pierfrancesco Vargiu (TS) *

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

RESEARCH HIGHLIGHTS

The Transgenic Mice Unit has designed and created several mouse models to study the lymphatic system and its implication in tumour dissemination and metastasis. One of these models is a reporter strain in which the process of lymphatic vessel growth, or lymphangiogenesis, can be monitored, *in vivo*, by whole body imaging. In this knockin mouse model the fluorescent/ luminescent EGFP-Luciferase fusion reporter is expressed under the endogenous transcriptional control of the *Vegfr3* gene, a classical marker expressed predominantly, and almost exclusively, in lymphatic endothelium. This genetically modified strain enables tracing and quantification of lymphangiogenesis associated to tumour growth and dissemination. The response of the lymph nodes and lymphatic vessels to metastatic tumours that spread mostly through the lymphatic network leads to an activation of *Vegfr3* that can be monitored *in vivo* in this strain (Martínez-Corral, Olmeda *et al.*, *Proc Natl Acad Sci USA* 17, 2012). We have developed this reporter strain both in an immune-competent C57Bl6 background and also in an immune-suppressed nude background. The nude background allows the generation of xenograft models in combination with our lymphoreporter knockin allele.

In collaboration with the group of Marisol Soengas at the CNIO, our lymphoreporter mouse model has been applied to study mechanisms of melanoma metastasis leading to the discovery of Midkine as a novel potential therapeutic target for melanoma (Olmeda *et al.*, *Nature* 546, 2017).

Our reporter mouse model is a powerful tool for studying lymphatic vessels in physiological and pathological contexts and, combined with different mouse models of cancer such as genetically modified strains, xenograft models, etc., constitutes

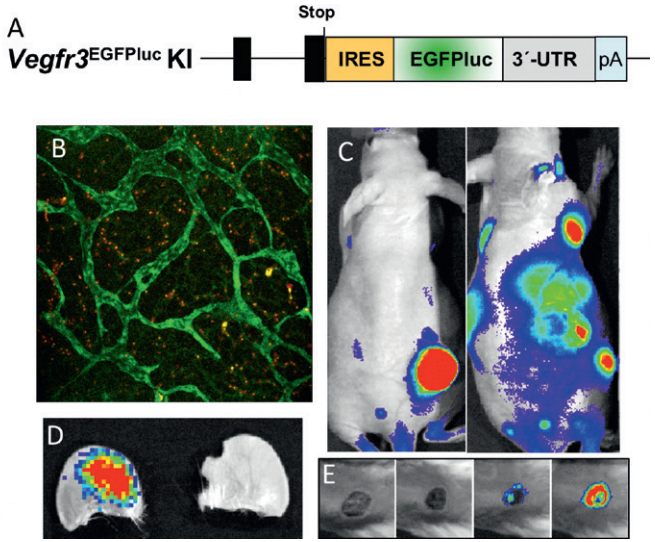


Figure Imaging of lymphatic vessels and lymphangiogenesis in the *Vegfr3*_GFP/Luc reporter mouse. **(A)** Schematic representation of the *Vegfr3*_EGFP/Luc reporter allele. **(B)** Skin lymphatic vessels in an E13.5 embryo (GFP direct fluorescence emission). **(C, D and E)** Bioluminescence imaging of tumour-, inflammation- and wound healing- induced lymphangiogenesis, respectively.

a unique platform for drug discovery and preclinical assays, not only in cancer and metastasis but also in many other areas such as inflammation or cardiovascular diseases (FIGURE). ■

• PUBLICATIONS

• Olmeda D, Cerezo-Wallis D, Riveiro-Falkenbach E, Pennacchi PC, Contreras-Alcalde M, Ibarz N, Cifdaloz M, Catena X, Calvo TG, Cañón E, Alonso-Curbelo D, Suarez J, Osterloh L, Graña O, Mulero F, Megías D, Cañamero M, Martínez-Torrecedradora JL, Mondal C, Di Martino J, Lora D, Martínez-Corral I, Bravo-Cordero JJ, Muñoz J, Puig S, Ortiz-Romero P, Rodríguez-Peralto JL, Ortega S, Soengas MS (2017). Whole-body imaging of lymphovascular niches identifies pre-metastatic roles of midkine. *Nature* 546, 676-680.

• Olbrich T, Mayor-Ruiz C, Vega-Sendino M, Gomez C, Ortega S, Ruiz S, Fernandez-Capetillo O (2017). A p53-dependent

response limits the viability of mammalian haploid cells. *Proc Natl Acad Sci USA* 114, 9367-9372.

• Muñoz S, Búa S, Rodríguez-Acebes S, Megías D, Ortega S, de Martino A, Méndez J (2017). In vivo DNA re-replication elicits lethal tissue dysplasias. *Cell Rep* 19, 928-938.

• Chirivella L, Kirstein M, Ferrón SR, Domingo-Muelas A, Durupt FC, Acosta-Umanzor C, Cano-Jaimez M, Pérez-Sánchez F, Barbacid M, Ortega S, Burks DJ, Fariñas I (2017). Cyclin-dependent kinase 4 regulates adult neural stem cell proliferation and differentiation in response to insulin. *Stem Cells* 35, 2403-2416.

MONOCLONAL ANTIBODIES CORE UNIT

Giovanna Roncador
Core Unit Head

Technicians
Álvaro García (until November)
(PEJ) *, Sherezade Jiménez

(until September), Lorena Maestre
(TS) **, Ana I. Reyes (until October)

*Plan de Empleo Joven (Youth Employment Plan)
**Titulado Superior (Advanced Degree)



OVERVIEW

Due to their high specificity and selectivity, monoclonal antibodies (mAbs) are exquisite tools that enable researchers to address basic questions in biology; they are currently one of the most important classes of reagents used in biomedicine.

The Monoclonal Antibodies Unit provides CNIO Research Groups with an “à la carte” generation of mAbs. We are highly specialised in the production of mouse and rat monoclonal antibodies. The Unit also offers mAb characterisation and validation, medium-scale mAb production and a service of *Mycoplasma* testing for the cell culture facility.

“The Unit is highly specialised in mAbs production and characterisation, providing CNIO researchers with reliable and well-validated reagents that give added value to their research projects.”

RESEARCH HIGHLIGHTS

During the last 17 years, the Monoclonal Antibodies Unit has generated a large number of mAbs against more than 135 different antigens, mostly targeting molecules for which mAbs are not commercially available. Many of those mAbs have been licensed to external companies, generating royalties that represent an important source of revenue for the CNIO.

Each year we prepare and update a detailed CNIO mAbs catalogue, which contains the datasheets of more than 80 thoroughly validated high-quality mAbs (accessible at <http://www.cnio.es/ing/servicios/anticuerpos/default.aspx>).

Research activities

In collaboration with Dr Miguel Ángel Piris, Associate Medical Chief of the Pathology Service at the *Fundación Jiménez Díaz*, we have produced and characterised a new monoclonal antibody against the TOX protein. TOX (Thymocyte Selection-Associated High-Mobility Group box) is a member of the family of HMG box proteins and is involved in the regulation of gene expression. This protein is present in many subsets of immune cells, suggesting that it plays significant roles in the immune system, including the development of CD4 T cells and NK cells, as well as lymph node organogenesis.

We have investigated the expression of TOX in normal and neoplastic lymphoid tissue using a novel murine monoclonal antibody. Using immunohistochemistry techniques, we found that the TOX mAb may help in the identification of neoplastic B and T cells and may be used to achieve a better understanding of the pathogenic role of TOX in inflammatory and malignant diseases.

EuroMAbNet and its commitment with Ab validation

In 2008, in collaboration with Oxford University, we founded EuroMAbNet (www.euromabnet.com), a non-profit organisation that includes internationally distinguished multidisciplinary academic laboratories focused on the generation and validation of mAbs.

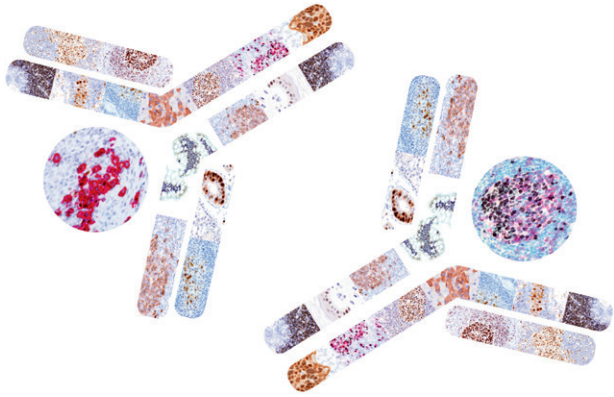


Figure Immunohistochemistry of several mAbs produced in the Monoclonal antibodies Unit.

The use of poorly characterised reagents is of major concern to the scientific community because of the perpetuation of serious scientific misconceptions that inevitably compromise the advancement of science. To help address mAbs unreliability, EuroMAbNet is strongly committed to improving the education and training of junior scientists. For this reason, we organise annual Antibody Validation Workshops (<https://www.euromabnet.com/meeting/>) to provide practical guidelines about the main principles underlying antibody validation (<https://www.euromabnet.com/guidelines/>). These workshops outline the problems generated by the use of poorly validated reagents and are designed to educate researchers, helping them to minimise the purchase of ineffective Abs, understand when additional validation is necessary, and to have an understanding of the information needed when publishing antibody-based data. ■

PUBLICATIONS

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MOLECULAR IMAGING CORE UNIT

Francisca Mulero
Core Unit Head

Technicians
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(PEJ-L) **, Elka Jesarela San Martín,
Gloria Visdominé



OVERVIEW

Molecular Imaging (MI) enables the visualisation of cellular function and the follow-up of molecular processes in living organisms without perturbing them. Molecular imaging offers significant advantages to the scientist over traditional research paradigms. While traditional studies of tumour response to a therapeutic agent involve a large cohort of animals analysed at multiple time points, molecular imaging allows characterisation of tumour development and response to a therapy within the same small set of animals imaged longitudinally at multiple time points, thereby reducing the number of mice and also increasing the statistical power of the study because each animal serves as its own control. Other advantages of molecular imaging procedures include the ability to interrogate the whole body, and to visualise the molecular target of interest in 3-dimensional space. Finally, Molecular Imaging is becoming a key bridging technology for

“Molecular imaging is rapidly growing, has tremendous potential to elucidate biological processes and pathways, and is able to translate scientific discoveries into the clinical setting.”

the translation of experimental preclinical findings into the clinical environment.

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

RESEARCH HIGHLIGHTS

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

We continue to test and incorporate new applications to the ImmunoPET strategy, combining the high specificity of antibodies with the high sensitivity of PET imaging; this year, we focused on the detection of pancreatic ductal adenocarcinoma (PDAC). In addition to conducting all the conventional tumour models follow-up – via different techniques such as Positron Emission Tomography-Computed Tomography (PET-CT), CT, and ultrasound imaging – we, this year, also contributed to the characterisation of metabolic models, in collaboration with the Growth Factors, Nutrients and Cancer Group. Using PET-CT, we determined the distribution of brown adipose tissue (BAT) and white adipose tissue (WAT) on body composition in mice, taking advantage of the ¹⁸F-FDG avidity of high metabolic tissues and using the CT to discriminate different densities between WAT and BAT (FIGURE).

We are actively involved with the mVision Foundation – created by the international consortium devoted to imaging research ‘m+visión’, led by MIT (The Massachusetts Institute of Technology) and the *Comunidad de Madrid* – in order to further develop the techniques that were already put in place

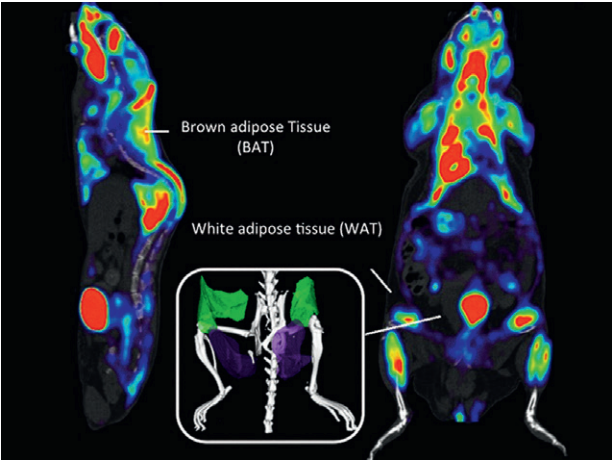


Figure Coronal and sagittal projections of PET-CT scans representing ¹⁸F-FDG uptake due to higher metabolism in BAT compared to WAT in perigonadal and inguinal fat pads. The square in the middle panel shows the 3D rendering of abdominal WAT (green, inguinal fat; purple, perigonadal fat).

by the Programme. The Unit also participates in projects with national companies and research groups in order to test and develop new probes for ImmunoPET. ■

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Book chapter

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► AWARDS AND RECOGNITION

- Advisory Board Chair, *Fundación mVision*, Spain.
- Member of Spanish PET Group, Spanish Society of Nuclear Medicine and Molecular Imaging (*SEMNM*).
- Project evaluator of the *Junta de Andalucía, Investigación, Desarrollo e Innovación Biomédica y en Ciencias de la Salud*, Spain.

FLOW CYTOMETRY CORE UNIT

Lola Martínez
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(PEJ-L)**, Miguel Ángel Sánchez
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**Titulado Superior (Advanced Degree)*
***Plan de Empleo Joven-Licenciado*
(Youth Employment Plan-Graduate)
(until December)



OVERVIEW

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

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

We count with 4 analysers and 3 high-speed cell sorters, containing different configurations of lasers and detectors, to cater for all our users’ needs. We also have an automated magnetic bead separation system (AutoMACS) and 2 automated cell counters.

“In order to understand cancer, it is becoming increasingly relevant to have information at the single cell level. At the Unit we are able to sort one single cell into 96 or 384 PCR plates for further genomic studies.”

Analysers are available to users upon appropriate training and cell sorters are operated by the Unit staff. Our sorters can separate up to 4- or 6- defined populations at a time, including single cell cloning. We can accept human samples to be sorted according to Biosafety regulations.

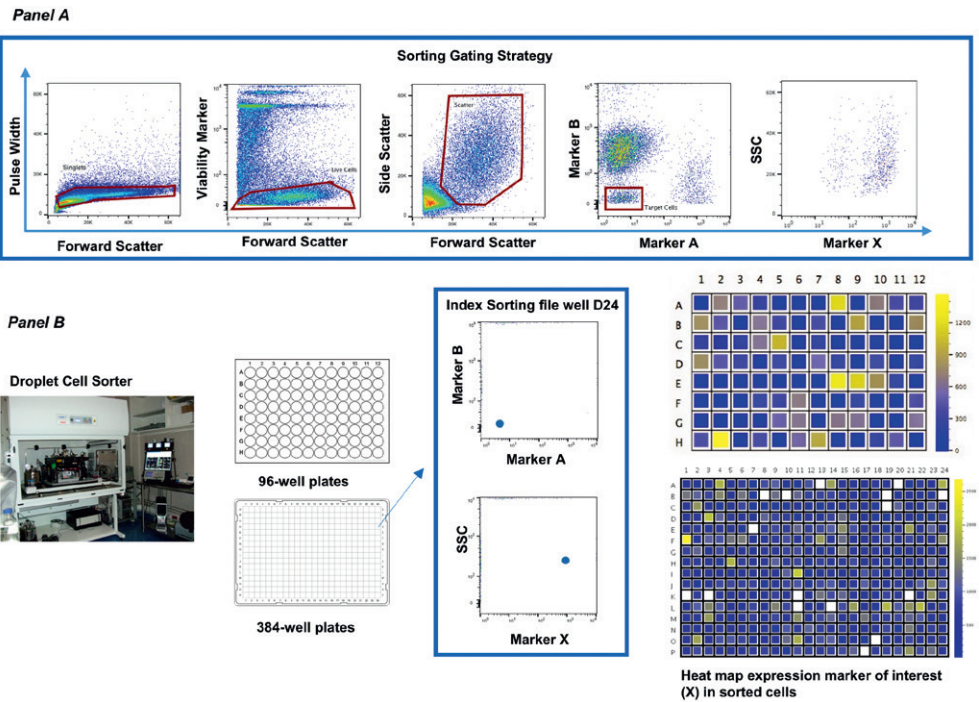
RESEARCH HIGHLIGHTS

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

- Cell proliferation studies (CFSE, Cell Trace Violet, BrdU or EdU, DNA content, etc.).
- Apoptosis studies (Annexin V, Mitochondrial Membrane Potential, Caspase 3, etc.).
- Multicolour Immunophenotyping panels (B and T cell development, Tregs, Inflammation, etc.).
- Functional Assays (side population detection, Ca²⁺ flux, intracellular pH, etc.).
- Cytometric Bead Arrays to measure several cytokines from cell extracts and plasma.
- Platelet studies.
- Extracellular vesicle detection (microvesicles and exosomes).
- Single cell sorting for RNA sequencing.

We have further developed our multicolour panels for the characterisation of the immune response by incorporating the new generation of Brilliant UV dyes from samples of haematopoietic tissues, pancreas, skin, liver, lung, as well as different tumour types. Modifications in our analytical and cell sorters have also been implemented to allow for this. These panels can still be combined with the detection of proliferation and cell death. In terms of our cell sorting capabilities, we included, at the end of the year, a MoFlo ASTRIOS in our portfolio of cell sorters. This cell sorter is equipped with 4 laser lines and 15 fluorescent detectors that enable the isolation of up to 6 different populations simultaneously. The optical configuration in the ASTRIOS will enable the usage of the new generation of Brilliant UV dyes. We also introduced, as part of our routine work, a successful calibration method for single cell deposition into 384 PCR plates. Throughout the year, we have also undertook lots of instrument testing in order to better characterise exosomes and microvesicles. ■

Figure Schematics Index sorting Figure. Panel (A) typical gating strategy of a primary sample stained with a combination of surface markers. Panel (B) index cell sorting schematics, a single file is generated for each sorted well so that information is retained regarding the cell sorted into the well; heat maps based on the expression levels of the marker of interest can be generated.



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cholesterol dysregulation. *J Exp Med* 214, 1387-1409.

- Sánchez-Luengo MA, Rovira M, Serrano M, Fernández-Marcos PJ, Martínez L (2017). Analysis of the advantages of cis reporters in optimized FACS-Gal. *Cytometry A* 91, 721-729.

AWARDS AND RECOGNITION

- 2017 International Visitors Leadership Programme “Hidden No More: Empowering Women Leaders in STEM”, recognising women global leaders. US Department of State.
- Tania López Briones received the “Best Student Poster Award”, XV Congress of the Iberian Cytometry Society, Lisbon (May 2017): *How to reliably choose settings for optimal resolution in a multicolour flow experiment.*

CONFOCAL MICROSCOPY CORE UNIT

Diego Megías
Core Unit Head

Technicians
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Pérez (TS) **

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(until December)
**Titulado Superior (Advanced Degree)



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

“The Confocal Microscopy Unit is fully committed to the dissemination of advanced microscopy methodologies that are useful for cancer research and society at large; we have organised courses, talks and visits, always with the aim of increasing our understanding of cell biology and the disorders of cells that lead to cancer.”

RESEARCH HIGHLIGHTS

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

In addition, the Unit has implemented the use of high throughput technologies applied to confocal microscopy using two different systems:

- An Opera (Perkin Elmer) High Content Screening (HCS) system, which allows running HCS experiments on fixed and live cells in multiwell plates, and enables the monitoring of cell dynamics (translocation, cell division, etc.) through the use of fluorescence.
- A Matrix Screening Application integrated into the SP5 confocal systems, enabling high throughput feeding of the instrument, not only in multiwell plates but also in tissue sections.

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

The Confocal Microscopy Unit continues to dedicate significant efforts towards the development and implantation of High Content Screening technology at CNIO; during this year, we carried out several medium size screenings with up to 5,000 compounds. In order to boost the output of our results, we reinforced our image and data analysis capabilities by hiring a dedicated person, thereby giving us the possibility of establishing more advanced routines.

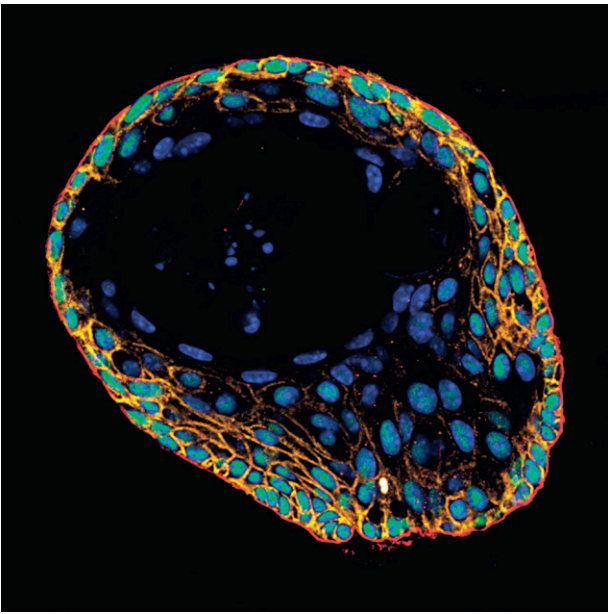


Figure The ‘hidden bladder skeleton’ image has been awarded in the Scientific International Immunofluorescence Contest from Abcam Company (Catarina Santos, CNIO Epithelial Carcinogenesis Group).

The Unit is promoting and helping with the latest sample preparation protocol development, bringing knowledge in tissue clearing as well as in expansion microscopy. Moreover, Microfluidics, used for live cell assays in perfusion chambers, has also experienced a great increase in performance and demand. The field of intra-vital microscopy is already available and we are now running several projects for studies of metastasis and skin alterations. ■

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PROTEOMICS CORE UNIT

Javier Muñoz
Core Unit Head

Graduate Student
Ana Martínez



OVERVIEW

Proteins are the molecular effectors of cells and catalyse almost all biological processes. The levels of protein abundance, together with their modification states and interactions, adapt dynamically to external or internal (genetic) stimuli and thus define the cell’s functional state and determine its phenotype. Mass spectrometry-based proteomics is the most powerful tool to study the proteome, providing fundamental information of basic biology. In addition, recent improvements in sensitivity and throughput now allow the analysis of larger cohorts of samples including biopsies, thus fully integrating proteomics into the clinical research toolbox. All these efforts have led to the provision of new insights into the molecular mechanisms underlying cancer development as well as the identification of novel biomarkers.

“The possibility to identify and profile secreted proteins, even in the exosomes, with mass spectrometry, enables the identification of novel biomarkers.”

Technicians
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de Embún (TS) *, Eduardo Zarzuela (PEJ-L) **

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

RESEARCH HIGHLIGHTS

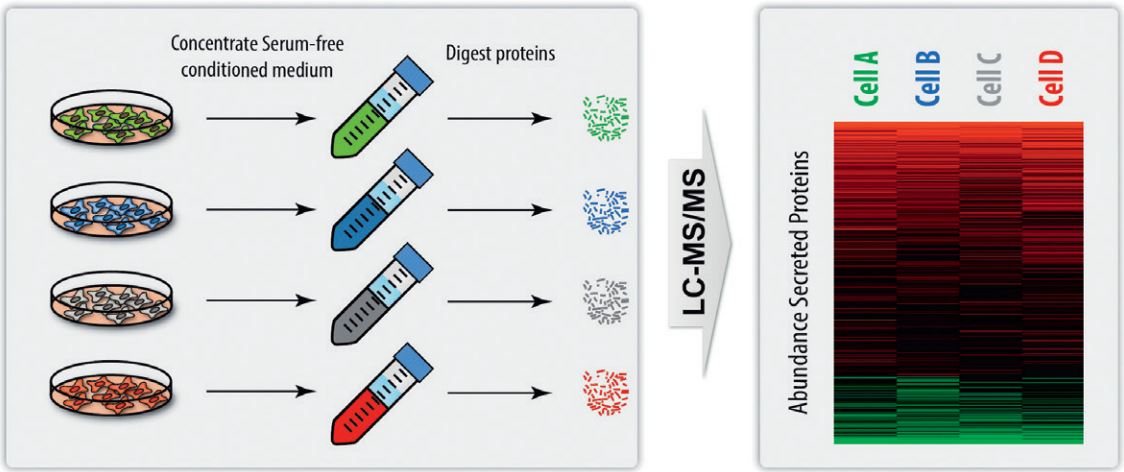


Figure Secretome analysis by mass spectrometry. Serum-free conditioned media from different biological conditions (cell lines, treatments, tissues...) can be collected and concentrated in micro-filters. Secreted proteins are then trypsin-digested and analysed by high-resolution liquid

chromatography-tandem mass spectrometry (LC-MS/MS). Proteins are identified and quantified by means of a label-free strategy in order to obtain the profiles of secretomes across the biological conditions under study.

In 2017, the Unit introduced a new immuno-purification approach to profile lysine acetylation in cells and tissues (Martinez-Val *et al.*). This method is being used to study the effects of several inhibitors against deacetylases and their effect in cancer. Furthermore, in collaboration with the Brain Metastasis Group, we are exploring ultra-sensitive proteomic strategies to analyse metastatic brain lesions obtained from micro-dissected tissues. In addition, we have conducted a large phosphoproteomic analysis to identify possible genes involved in certain platelet activation defects (in collaboration with the Cell Division and Cancer Group). Together with the Tumour Suppression Group, we are dissecting at the molecular level the transition of primed embryonic stem cells (ESCs) into the ground-state of pluripotency. The results of this project show that targeting the transcriptional regulator Mediator, shifts ESCs towards a state that significantly resembles that of naïve ESCs. On a different note, a lot of the evidence shows that the proteins secreted into the extracellular space, or even encapsulated in micro-vesicles such as exosomes, have important organotropic effects in cancer and most importantly in metastasis. Bearing this in mind, we are mining the secretomes of different types of samples in order to identify potential new biomarkers and molecular targets. Indeed, in collaboration with the Melanoma Group, we have identified the protein Midkine as a systemic

inducer of neo-lymphangiogenesis, which defines the prognosis of melanoma patients (Olmeda *et al.*). Finally, in collaboration with the Technical University of Dresden, we are performing several proteomic analyses on stomach organoids carrying different sets of cancer-inducing mutations in order to study their applicability as a novel platform for drug screening. ■

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HISTOPATHOLOGY CORE UNIT

Vacant
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Pizarro (since February), Paula
Tejedor (until February), Irene Roda,
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Plan) (until December)



OVERVIEW

Pathology is devoted to the study of the structural, biochemical and functional changes in cells, tissues and organs that underlie disease. By using molecular, immunological and morphological techniques, pathology aims to serve as the bridge between basic science and clinical medicine.

The Histopathology Unit offers assistance and expertise through a full range of services covering from paraffin embedding and tissue sections to histochemical stains, research and diagnostic immunohistochemistry (IHC) testing, antibody validation, and *in situ* hybridisation (ISH), as well as the construction of tissue microarrays. Furthermore, the Unit offers other added-value services – for which assistance is provided by a team of highly specialised technicians – such as: laser-capture microdissection; slide digitalisation for bright-field, polarisation light and fluorescence; image analysis; and quantification. The

“The availability of diverse automated platforms for the performance of immunohistochemistry techniques is a key factor facilitating the reproducibility and high throughput of those techniques demanded by our users, and one of the indicators of excellence of the Unit.”

Unit collaborates with researchers at any stage of their scientific career in the histological characterisation of phenotypically relevant animal models of disease, thus providing them with the pathology expertise required for the success of their projects.

RESEARCH HIGHLIGHTS

In 2017, the Unit maintained the usual outstanding levels of throughput in terms of number of requests of services, which included nearly 34,000 paraffin-embedded blocks, more than 26,000 histochemical techniques, around 9,000 routine IHC techniques, and more than 6,000 requests for histological slide scanning and image analysis, in addition to a small number of other services such as tissue microarray preparation and ISH.

This year, two of the technological areas mastered by the Unit were subjected to a significant upgrade and improvement. First, the laser microdissector was substantially upgraded in terms of software as well as hardware elements. The new version of the instrument now enables the selection of the areas to be dissected by colour detection, based on the selection system implemented in the AxioVision imaging software. Also, we incorporated a new platform for automated immunostaining, the Roche Discovery Ultra. This new system is able to perform IHC and ISH in an independent manner, which substantially increases its flexibility and capacity to adapt to the fluctuating needs of our customers. It also allows revealing the stains with chromogenic and fluorescent markers with up to five colours, and we have actually included several new chromogenic systems based on the peroxidase system. This has also made it possible to increase the number of immunohistochemical markers in both human and mouse tissues, particularly on samples from patient-derived xenograft (PDX) models. Other technological

developments that took place in the Unit this year include the set-up of histological techniques on cells grown in tissue culture plates for the study of glucogen deposits using P.A.S. technique.

The Unit is increasingly involved in clinical trials that require assistance regarding complex IHC techniques on human tissues; these are conducted in collaboration with several pharmaceutical companies. Also, our commitment to training and educational activities is reflected in our participation in a number of training programmes at different levels, ranging from modules of ‘*Formación Profesional*’ for Pathology technicians to several Masters, as well as the course focusing on secondary school teachers that is organised through the ‘CNIO & the City’ project.

Due to the great importance that our users place on quality and reproducibility, the Unit participates in several External Quality Assessment Schemes, such as NordiQC and UK NEQAS, which evaluate the quality of the staining techniques performed at the Unit and in which more than 800 laboratories participate worldwide. In 2017, our Unit incorporated a new companion diagnostic module from NordiQC focusing on PD-L1; this was done due to the high demand and increasing clinical importance of this marker. Overall, the Unit scored very high in all the evaluated techniques, and two of the protocols developed by the Unit were incorporated into the ‘Best Methods section’ of the UKNEQAS Cellular Pathology Technique. ■

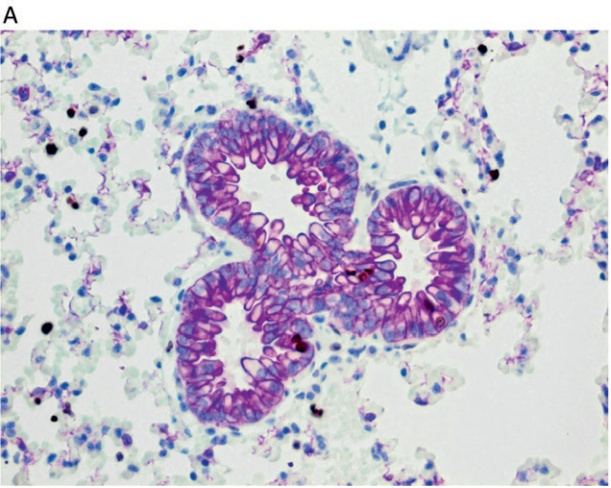


Figure Techniques developed by the Histopathology Core Unit. (A) Section of mouse lung tissue stained with double immunohistochemistry using two different chromogenic systems against two markers, brown for Ki-67 and purple for CK-19. (B) Image of the same tissue for the automatic detection of Ki-67 positive cells for laser-microdissection.

PUBLICATIONS

Olmeda D, Cerezo-Wallis D, Riveiro-Falkenbach E, Pennacchi PC, Contreras-Alcalde M, Ibarz N, Cifdaloz M, Catena X, Calvo TG, Cañón E, Alonso-Curbelo D, Suarez J, Osterloh L, Graña O, Mulero F, Megías D, Cañamero M, Martínez-Torrecuadrada JL, Mondal C, Di Martino J, Lora D, Martínez-Corral I, Bravo-Cordero JJ, Muñoz J, Puig S, Ortiz-Romero P, Rodríguez-Peralto JL, Ortega S, Soengas MS (2017). Whole-body imaging of lymphovascular niches identifies pre-metastatic roles of midkine. *Nature* 546, 676-680.

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ANIMAL FACILITY

Isabel Blanco
Core Unit Head

Management
Vivotecnia Management & Services



“The Animal Facility provides CNIO researchers with all the support required to work with mouse models, which are critical to understand the processes involved in tumour generation and development, as well as for the validation of new targets and therapeutic approaches.”

The CNIO has a state-of-the-art Animal Facility, managed by Vivotecnia Management & Services. The Animal Facility’s primary responsibility is the supply, husbandry and quality control of laboratory animals used by the Research Programmes in their experimental protocols. The strict compliance to national, EU and international recommendations regarding the use and care of animals in research is of paramount importance to the CNIO.

The high standards achieved by the CNIO with regards to the use and care of animals for experimentation have been recognised by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC), which granted us full accreditation in October 2016. AAALAC International is a private non-profit organisation that promotes the humane treatment of animals in science through voluntary accreditation and assessment programmes. Nearly 1,000 companies, universities, hospitals, government agencies and other research institutions in 44 countries have earned AAALAC accreditation, which is considered one of the top international recognitions in this field.

The CNIO Animal Facility was established to assist researchers in the development and analysis of *in vivo* models. We are currently collaborating with as many as 28 Research Groups, Sections and Units from different Research Programmes.

Our Animal Facility has the capacity to house 19,000 type IIL cages. Our mouse lines are maintained and bred in the Facility’s barrier area, which assures Specific Pathogen Free (SPF) health status through a comprehensive health surveillance programme. Microbiological and environmental parameters in the animal areas are constantly monitored. All mouse strains housed in the barrier are either generated within the barrier or introduced by rederivation. We also have an additional area with a capacity for 1,800 type II cages dedicated for the use of non-replicative strains of adenovirus, lentivirus and retrovirus, as well as for xenograft models. In this area, mice are housed in ventilated racks with integration of Individually Ventilated Caging (IVC) units in the building ventilation systems. Mice are always manipulated in Type II biosafety cabins.

Daily operations and husbandry procedures are highly automated in order to safe-guard our personnel from any associated risks; robotic devices perform the potentially hazardous tasks such as the processing of dirty bedding, the washing/filling of cages and bottles, etc. These automated systems maximise the productivity and ensure the quality standards in our washing and sterilising areas. All records concerning breeding protocols and animal inventory are computerised and stored in a web-based application accessible via the CNIO intranet.

The Animal Facility currently harbours more than 40,000 mice representing more than 3,000 genetically modified mouse lines, either as live animals or as cryopreserved embryos or sperm, carrying close to 400 gene targeted alleles and more than 200 transgenic integrations. The Facility also provides access to

more than 50 tool strains, including constitutive and inducible Cre strains, Flp strains, reporter strains, Tet transactivator strains and others.

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

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

In addition to mice, the Animal Facility hosts over 100 specimens of the frog *Xenopus laevis*, which are used to obtain eggs for chromosome dynamics studies. Also, in 2017, we introduced a small rat colony for a project involving the generation of monoclonal antibodies directed against mouse antigens, as new tools for cancer research.

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

The *Orden ECC/566/2015* stipulates that all animal procedures are to be carried out by qualified people in the possession of the corresponding accreditation as issued by the competent authority. The Animal Facility offers CNIO’s new staff a short course focused on the training of personnel performing work with laboratory animals; this is complementary to the online courses that are a requisite to gain access to the facility.

In line with our commitment to maintaining the highest possible standards related to animal research issues, the CNIO has joined the Agreement on Openness on Animal Research, promoted by the Federation of Scientific Societies in Spain (COSCE) in collaboration with the European Animal Research Association (EARA), which was launched on September 2016. An institutional statement on the use of research animals can be consulted on the CNIO website. ■

EXPERIMENTAL THERAPEUTICS PROGRAMME

JOAQUÍN PASTOR Programme Director



“The number, diversity and results of collaborations established by basic researchers with the Experimental Therapeutics Programme are positive indicators of its integration and added value to CNIO’s Chemical-Biology and Drug Discovery activities.”

The Experimental Therapeutics Programme (ETP) serves as a bridge between basic research results in cancer biology (i.e. novel therapeutic targets and hypothesis) and the development of potential antitumour drugs. This is achieved through the application of early drug discovery phases in order to obtain advanced compounds ready for *in vivo* Proof of Concept (PoC) studies. These molecules are subject to standard *in vivo* characterisation by our Group as well as more detailed studies at the basic research labs where the therapeutic target emerged. The best candidates obtained by applying this operating model are then ready for clinical development in partnership with pharmaceutical companies. ETP also helps in the validation of innovative targets by providing high quality chemical probes to the basic research laboratories. Moreover, we participate in the identification of novel targets (for projects based on phenotypic screenings) by using our expertise in target deconvolution phases, including the development of affinity probes.

Currently, the most advanced targeted project is dedicated to CDK8 inhibitors. In this project, we have selected our first leads, ETP-27 and ETP-93, which have yielded positive results in efficacy studies in haematological cancer xenograft models after oral administration. Interestingly, our chemical series displays dual CDK8/Haspin or specific CDK8 activities depending on the substitution pattern. ETP-18 represents a highly selective CDK8 inhibitor, which is undergoing pharmacokinetic (PK) studies.

Other targeted projects dedicated to the kinases Haspin and Mastl (in collaboration with Marcos Malumbres, CNIO Cell Division and Cancer Group) are focused on the discovery and generation, for the first time, of specific chemical probes to interrogate their therapeutic potential. During 2017, we generated a chemical series of Haspin inhibitors by application of structure-based design strategies. Compound ETP-949 proved to be a low nanomolar and highly specific inhibitor after profiling against more than 450 kinase targets. These compounds will serve as important tools to study the therapeutic potential of the pharmacological inhibition of this mitotic and epigenetic kinase. We have continued with the exploration of several previously identified families of Mastl inhibitors; we have now generated a preliminary SAR (Structure Activity Relationship) and have improved their potency up to a low nanomolar range. Currently, we are working to control their selectivity.

On the other hand, we are collaborating with the CNIO Telomeres and Telomerase Group (Maria Blasco) in the development of TRF1 target for cancer therapy; in this context, we have contributed to the discovery of novel TRF1 inhibitors. During 2017, ETP has

helped to decipher the connection of the PI3K/AKT axis as a modulator of TRF1 localisation at telomeres by using a chemical biology approach. Moreover, we have been able to identify other potential cell signalling pathways as TRF1 regulators, currently under investigation, after the screening of our ETP-library of antitumour compounds. Furthermore, we are still working on the deconvolution of the molecular mechanism of a previously identified chemical series. Interestingly, several affinity probes have been prepared and will be used for cell localisation and pull down experiments in order to shed light on the mechanism of action of these compounds.

ETP has collaborated in a project dedicated to the discovery of novel targets and modulators against Cancer Stem Cells (Manuel Serrano). Our team previously designed and synthesised affinity probes to help in target deconvolution studies. The use of these probes has enabled the identification of a potential mechanism of action, which is currently under *in vivo* validation studies.

Finally, we have also collaborated with other CNIO basic researchers to carry out several screening campaigns, both targeted and phenotypic. For instance, to discover novel senolytic compounds (Manuel Serrano), brain metastasis blockers (Manuel Valiente), mTOR pathway modulators with unexplored mechanisms of action (Alejo Efeyan), alternative Ras pathway modulators (Óscar Fernández-Capetillo) and treatments for malignant peripheral nerve sheath tumours (MPNST) (Héctor Peinado), bladder cancer (Francisco Real), and EGFR deficient mutant KRas mouse tumours (Mariano Barbacid/Carmen Guerra). ETP-MedChem has also contributed with the synthesis of valuable tool compounds for research in the field of lung fibrosis (Erwin Wagner) and cell haploidy stabilisation (Óscar Fernández-Capetillo).

MEDICINAL CHEMISTRY SECTION

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OVERVIEW

The Medicinal Chemistry Section is part of the Experimental Therapeutics Interdisciplinary Programme that is dedicated to early Drug Discovery activities in the oncology field. Medicinal Chemistry activities start with the identification of hits through high throughput screening campaigns from targeted or phenotypic assays, and lead on to further activities related to the design, synthesis and optimisation of the compounds in order to obtain novel lead compounds with *in vivo* activity in appropriate animal models. For hits obtained from phenotypic screenings we introduce an additional target identification step in order to decipher the mechanism of action responsible for the observed phenotype. Our Group has experience in the design and synthesis of affinity probes for target deconvolution studies. These molecules enable the detection of the cellular localisation of the target of interest through imaging techniques and enable its isolation through pull-down experiments. Additionally, as a complementary alternative, we are developing proteolysis targeting chimeras (PROTACs) as promoters of cellular protein degradation in order to establish their applicability across diverse drug discovery projects.

“We developed first generation HASPIN-selective inhibitors as chemical probes through the application of structure-based design strategies in order to determine the therapeutic potential of the pharmacological inhibition of this mitotic and epigenetic kinase.”

RESEARCH HIGHLIGHTS

Project advances in 2017:

Cyclin-dependent protein kinase 8 inhibitors (CDK8i) project

During 2017, we were involved in the optimisation of ETP-27; a lead compound identified and developed in our programme, with demonstrated *in vivo* proof-of-concept in the acute myeloid leukaemia model, MOLM-13 xenograft. Fine tuning optimisation has been done and we have identified ETP-93, an orally bioavailable compound with longer half-life than ETP-27. ETP-93 showed oral levels in both plasma and tumour, biomarker modulation (pSTAT1) up to 8 hours after oral administration in PK/PD studies, and a significant tumour growth inhibition of 50% in a 10 day short-term study at 50 mpk P.O. in MOLM-13. The compound is rather selective (S(35): 0.08) in a 468 kinase panel (KINOMEScan™ platform) and only 1 main off-target has been identified. Currently, we are evaluating the positive contribution of this activity to the antiproliferative profile of the compound. Additionally, in this series we have identified very selective CDK8 inhibitors with good *in vivo* PK, for example ETP-18, which has been selected for further *in vivo* efficacy studies.

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

Two different hits were identified from an HTS campaign with active full length human MASTL protein. About 100 analogues have been synthesised around them to establish Structure-Activity-Relationships (SAR), identifying compounds in the single digit nanomolar range from both chemical series. Selected compounds have been profiled in a small set of kinases to determine their selectivity and we have identified some main off-targets in the compounds. Currently, we are focussing our efforts on trying to obtain high quality selective chemical probes that will be used in pharmacological inhibition studies to decipher the whole therapeutic potential of MASTL.

HASPIN inhibitors

During the exploration of 2 chemical series we were able to obtain very potent biochemical and cellular low nanomolar haspin inhibitors, while removing the off-target activities present in the original hits. After the synthesis of about 90 compounds, we profiled the selectivity of 2 representative molecules from each chemical series, ETP-949 and ETP-885, in a 468 kinase panel (KINOMEScan™) obtaining a high level of selectivity for both of them (S(35) of 0.025 and 0.007). Currently, one of these

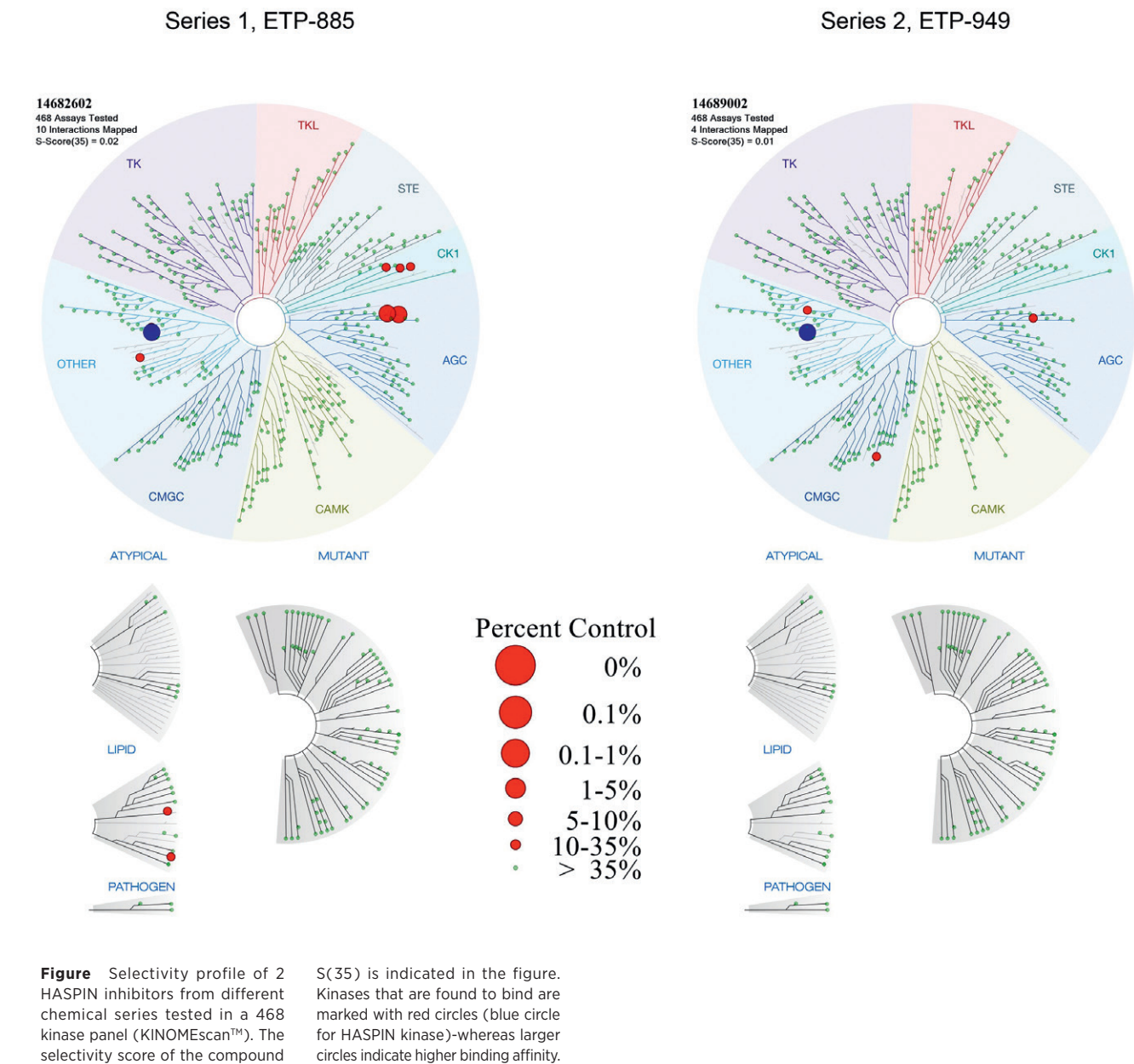
chemical series is under *in vivo* characterisation with the aim of identifying a high quality Haspin inhibitor for pharmacological target validation studies. Additionally, a third chemical series was generated in 2017, including intellectual property in the design.

Inhibition of Cancer Stem Cell (CSC) proliferation

This project is undertaken in collaboration with the CNIO Tumour Suppression Group. After the identification of compounds able to modulate CSC proliferation, stemness and, at sublethal doses, inhibit the tumour initiating capacity of pancreatic CSCs, we generated affinity probes by introducing a minimalist linker, retaining the activity; these probes were used in deconvolution studies in 2017. The presence of the minimalist linker increased the efficiency of these studies by enabling the formation of a covalent linkage with the binding proteins after photo-irradiation treatment. Additionally, we have scaled up the hit compound to evaluate its *in vivo* pharmacokinetic properties.

Telomeric repeat binding factor 1 (TRF1) inhibitors

This is a collaborative project undertaken with the CNIO Telomeres and Telomerase Group. During 2017, we focused on the synthesis of different affinity probes of hit compound ETP-946 in order to identify the putative molecular target responsible for the observed TRF1 modulation. Among them, the ETP-455 affinity probe showed similar TRF1 modulation to the hit compound and was selected for deconvolution studies. ETP-455 contains a reversible linker with a terminal alkyne reactive group that helps in cell localisation and pull down experiments by using a click chemistry reaction with functionalised (azide reactive group) fluorophores and/or biotinylated derivatives. Preliminary cell localisation experiments using imaging techniques have been performed, demonstrating a rather specific localisation of the chemical probe that can be removed by competition with ETP-946. ■



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- Bejarano L, Schuhmacher AJ, Méndez M, Megías D, Blanco-Aparicio C, Martínez S, Pastor J, Squatrito M, Blasco MA (2017). Inhibition of TRF1 telomere protein impairs tumor initiation and progression in glioblastoma multiforme mouse models and in patient-derived xenografts. *Cancer Cell* 32, 590-607.
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PATENT

- Pastor Fernández J, Martínez González S, Blanco Aparicio C, Hernández Higuera AI, Gómez de la Oliva CA, Rivero Buceta V, Riesco Fagundo RC (2017). Preparation of tricyclic pyrolopyranopyridines as protein kinase inhibitors. *WO2017033019A1* 20170302.

BIOLOGY SECTION

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(TS) *, Javier Klett (TS) *, M. Carmen Rodríguez De Miguel (TS) *
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***Plan de Empleo Joven (Youth Employment Plan) (until December)*

Visiting Scientist
Ann Louw (January-June, University of Stellenbosch, South Africa)

OVERVIEW

In the Experimental Therapeutics Programme, we are working on both targeted and phenotypic-based drug discovery projects as well as on exploratory screening projects carried out in collaboration with other CNIO Groups. Furthermore, we conduct screenings with an ETP-antitumour library in order to identify potential new treatments in tumour types or metastatic settings for which there is an unmet medical need regarding new therapies. On the other hand, this library is used to identify novel signalling pathways that modulate a target responsible for an interesting phenotype. The newly identified signalling pathways are validated by using a chemical-biology approach, through which a set of inhibitors for the target, with quite dissimilar structures, are interrogated against the expected phenotype. In order to reach a conclusion, we establish correlations between cellular modulation of the target/pathway and the desired phenotype. Finally, the CNIO Group that develops the screening also performs all the biological validation required to confirm the new hypothesis.

“In collaboration with the CNIO Telomeres and Telomerase Group, we have identified new signalling pathways that modulate the levels of TRF1 at telomeres. Using a chemical-biology approach, we have contributed to the elucidation that the PI3K/AKT pathway regulates TRF1.”

RESEARCH HIGHLIGHTS

During 2017, our Section was involved in several projects:

Cyclin-dependent kinase 8 (CDK8)

We characterised ETP-93, a second improved dual lead compound, and ETP-18, a nanomolar selective CDK8/CDK19 inhibitor, as it was described in the Medicinal Chemistry Section.

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

These projects are undertaken in collaboration with the CNIO Cell Division and Cancer Group. For MASTL, we tested 98 new

compounds in our biochemical assay with active human full length MASTL protein. We identified low nanomolar inhibitors that were profiled in a small internal kinase panel, revealing off-targets that were removed. The more potent and selective inhibitors are under cellular characterisation. For HASPIN, we tested in a biochemical assay 90 compounds belonging to 3 different chemical series. Active compounds were also profiled in the small internal kinase panel. Cellular inhibition of phosphorylation of H3T3 was evaluated for those inhibitors with a biochemical IC_{50} below 100nM. We identified nanomolar biochemical and cellular HASPIN inhibitors and representative molecules for two chemical series that have been profiled in a panel of 468 kinases (KinomeScan), thereby obtaining a high level of selectivity for both of them ($S(35)$ of 0.025 and 0.007). These compounds are under further cellular evaluation and *in vivo* PK.

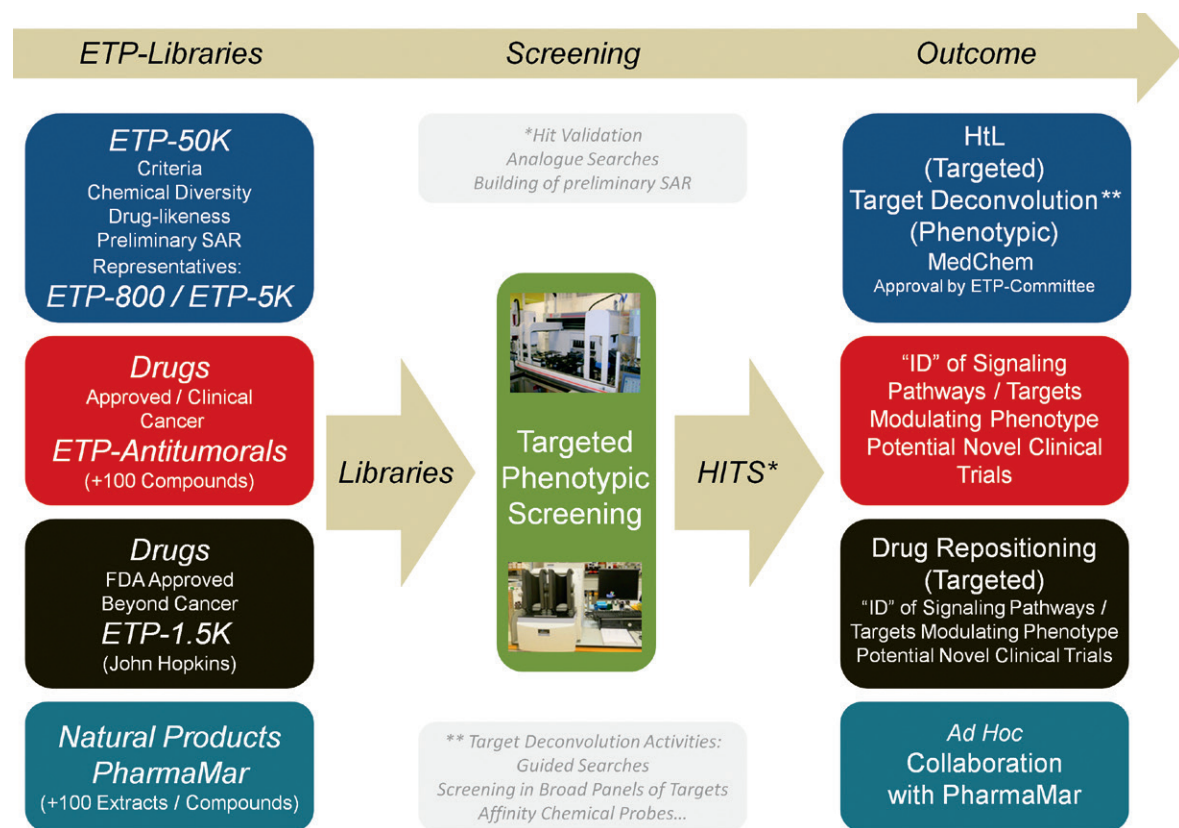


Figure ETP- libraries and their potential outcome after screening. ETP has 4 different collections of diverse origin covering chemically diverse small molecules, drugs that are approved or in clinical trials for cancer treatment or for other illnesses, as well as natural products. The outcome of a targeted or phenotypic screening using one of these

libraries could enable the discovery of new drugs for a specific target, the identification of new targets associated with a phenotype, or a more translational result such as the postulation of new clinical trials for unmet medical needs. Moreover the screening of the PharmaMar library could trigger a collaboration to further develop results.

Telomeric repeat binding factor 1 (TRF1)

This project is carried out in collaboration with the CNIO Telomeres and Telomerase Group. A phenotypic assay to measure the association of TRF1 to telomeres was used to test 44 compounds, which include ETP-946 analogues and its corresponding chemical probes. Moreover, we used one of these chemical probes, ETP-455, to perform cellular localisation assays of the putative target of ETP-946. This information has been used to design pull-down experiments that are currently ongoing. Furthermore, we have tested the ETP-antitumour library to identify new signalling pathways that modulate TRF1. We are validating these new pathways by using a chemical-biology approach that we previously applied to uncover that the PI3K/AKT pathway regulates TRF1.

Cancer stem cells (CSC) and screening to identify new senolytic drugs

These projects are carried out in collaboration with the CNIO Tumour Suppression Group. For the CSC project, we collaborated on the pharmacokinetic characterisation of the most interesting identified hit, as well as in the *in vivo* validation studies. For the new senolytics screening project, ETP-Biology provided support for testing and analysing the ETP-antitumour and ETP-5K libraries in an assay that compares the viability of tumour cells and senescent cells. After validation of the hits identified by single point and the testing of analogues, four hits were selected for further characterisation.

Brain metastasis screening

The CNIO Brain Metastasis Group developed an *ex vivo* assay to search for drugs that kill human brain metastasis in mice. One class of drugs identified in the screening was further characterised; we contributed to the experimental design and the analysis of tumour levels in PK/PD experiments in order to help design the administration schedule for the efficacy study performed by our collaborators.

Screening to identify new Ras modulators

The CNIO Genomic Instability Group has developed an assay to search for drugs that modulate the Ras pathway. ETP-Biology has provided support in running the experiments with both the ETP-800 and ETP-5K libraries and by testing the analogues. Two interesting hits were identified and we performed their ADME profiles; they are currently under pharmacokinetic characterisation and will eventually progress to *in vivo* PoC studies.

Screening to identify New mTOR modulators

The CNIO Metabolism and Cell Signalling Group developed an innovative assay to search for drugs that modulate the mTOR pathway. ETP-Biology provided support in running the experiments with both the ETP-800 and ETP-5K libraries. The identified hits are under further characterisation.

Collaborations with other CNIO Groups

ETP-Biology has provided support by testing and analysing the ETP-antitumour library, either alone or in combination, in order to identify: i) novel treatments or combinations in MPNST cell lines in collaboration with the Microenvironment and Metastasis Group; ii) novel treatments or combinations in bladder cancer cell lines in collaboration with the Epithelial Carcinogenesis Group; iii) novel treatments or combinations in brain cancer cell lines in collaboration with the Brain Tumour Group; and iv) novel treatments of KRas mutant NSCLC mouse cell lines with and without EGFR in collaboration with the Experimental Oncology Group. ■

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CNIO - LILLY CELL SIGNALLING THERAPIES SECTION

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**Titulado Superior (Advanced Degree)*



SCOPE OF THE ELI LILLY - CNIO PARTNERSHIP

Eli Lilly and CNIO are collaborating on the identification and validation of novel targets in cancer immuno-metabolism. Our Section is funded through a research contract with Eli Lilly and focuses on the identification of small molecular weight molecules that regulate the metabolism of malignant cells, with the objective of killing them, either directly, acting synergistically with other anti-tumour agents, or activating the anti-tumour immune response. Exploring how to better target these mechanisms would lead to better and more efficient therapeutic options.

A combination of *in vitro* and *in vivo* approaches is being utilized to obtain a complete understanding of the metabolic reprogramming regulated by oncogenes like RAS, as well as the characterisation of the metabolic status of tumours. For this purpose, we have developed a series of biochemical and cell-based assays exploiting

advanced techniques such as extracellular flux analysis (Seahorse technology), NMR and metabolomics. Finally, each target goes through an *in vivo* validation process using xenografts, allografts and mouse models developed at the CNIO that includes the use of non-invasive *in vivo* imaging technologies, as well as the immune histochemical characterisation of tumours for different metabolic, immune and tumour-specific markers. The final step is the validation in human samples from healthy donors or patients using PBMCs or tumour tissue arrays.

SCIENTIFIC CONTEXT

The observation of an altered metabolic state in cancer cells dates back to the early 20th century when Otto Warburg observed that cancer cells preferentially utilise glycolysis over oxidative phosphorylation for growth, even in the presence of normal oxygen levels (Warburg 1956), a phenomenon known as the ‘Warburg effect’. Warburg argued that, ‘this altered metabolic state was the underlying cause for cancer’. The molecular mechanisms driving an altered tumour metabolism have only recently begun to be understood as a result of large-scale genomic sequencing as well as advances in metabolomic profiling technologies. Recent studies have shown that many oncogenes, including MYC and RAS, impart an altered metabolic phenotype in cancer cells through the regulation of genes involved in central metabolic pathways such as glycolysis, fatty acid metabolism, oxidative phosphorylation, nucleotide synthesis and the one carbon pool (reviewed by Gilmour & Velasco, 2017).

Cellular metabolism is a fine tuned process; tumours rely heavily on specific metabolic pathways to obtain their energy while using other pathways to grow in order to give tumour cells a growth

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

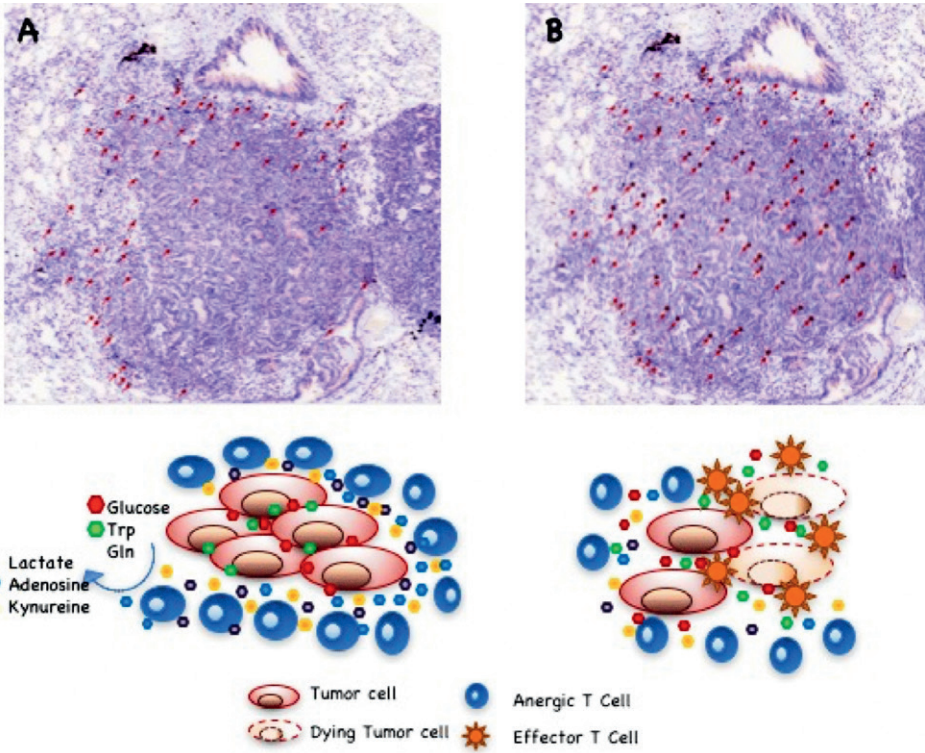


Figure Targeting cancer metabolic immune suppression. (A) T cells remain in the periphery of tumours staying away from internal areas. An immune histochemical analysis of CD3 positive T cells in a K-Ras^{G12}/p53^{-/-} lung adenocarcinoma is shown. Tumour cells produce a battery of immunosuppressive metabolites such as lactic acid, kynurenine or adenosine that result in an anergic T cell phenotype, while consuming key metabolites such as glucose or tryptophan necessary for a proper T effector (Teff) cell activity. As a result, T cells are incapable of mounting an effective anti-tumour immune response. (B) Metabolic regulation of the tumour and/or tumour microenvironment would diminish the production of immune suppressive metabolites and increase the levels of metabolites such as glucose, or tryptophan necessary for a proper immune T cell response. Teff cells would be able to invade and attack the tumour, destroying it. The image depicts a virtual anti-tumour T cell response in the same lung adenocarcinoma.

PUBLICATION

- Gilmour R, Velasco S. (2017). Back to the Future: Therapeutic targeting of cancer cell metabolism. *SLAS Discov* 22, 333-337.

CNIO - LILLY EPIGENETICS SECTION

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(since October), Jacinto Sarmentero
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*Titulado Superior (Advanced Degree)



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

SCOPE OF THE CNIO - ELI LILLY PARTNERSHIP

Eli Lilly and CNIO are collaborating on the identification and validation of novel targets in cancer epigenetics. Our Section is funded through a research contract with Eli Lilly and focuses on the identification of small molecular weight molecules able to modulate the epigenome of malignant cells and ultimately block

the growth and spread of tumours. Potential targets are being validated *in vitro* and *in vivo* using animal models developed at the CNIO. Furthermore, we are currently setting up biochemical and cell-based assays with the aim of understanding the mechanism of action of such targets at the molecular level (FIGURE).

SCIENTIFIC CONTEXT

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

and other human diseases. The epigenome is regulated in a highly dynamic fashion by the coordinated action of regulators able to write, erase and read histone and DNA modifications. Thus, contrary to genetic mutations, epigenetic aberrations can be reversed through the targeting of the appropriate epigenetic regulators. Indeed, drugs targeting DNA methyltransferases and histone deacetylases have successfully demonstrated anticancer properties and are currently used in the clinic. Therefore, identifying the molecular function of critical epigenetic regulators and their complex relationship with the cancer epigenome (FIGURE), as well as the development of small molecular inhibitors of their activities holds great promise for the therapeutics of cancer. ■

During the past few years several studies, including our own, have suggested that the deregulation of the chromatin-modifying machineries can lead to aberrant gene expression causing cancer

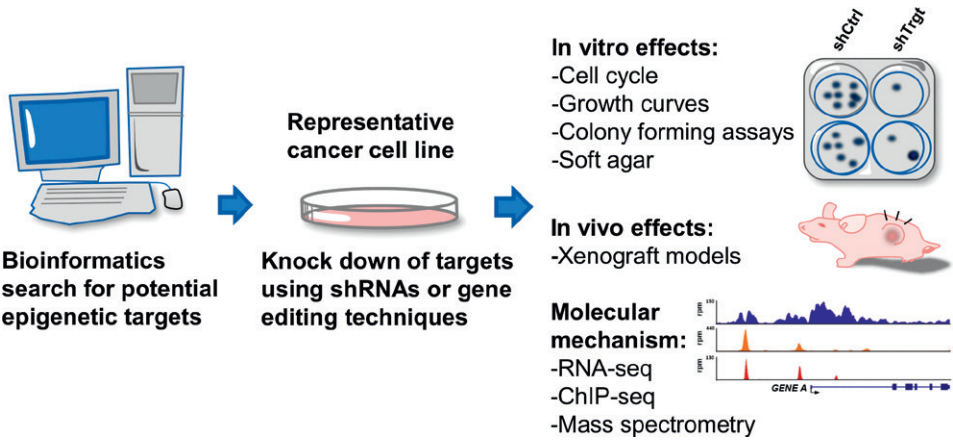


Figure In vivo and in vitro strategies for target validation.

PUBLICATION

Barrero MJ (2017). Epigenetic Strategies to Boost Cancer Immunotherapies. *Int J Mol Sci* 18, 1108.

TECHNOLOGY TRANSFER AND VALORISATION OFFICE

Anabel Sanz
Director

Technology Transfer Manager
M. Cruz Marín



Technician
Rocío Manzano (PEJ-L)*
**Plan de Empleo Joven- Licenciado (Youth
Employment Plan-Graduate) (until
December)*

At the CNIO, the best science is coupled with the desire to make a true impact through our research efforts, all for the benefit of cancer patients and the health system. The Technology Transfer and Valorisation Office (TTVO) contributes to these endeavours by ensuring appropriate intellectual protection and by fuelling the technologies that arise from our research into companies and entrepreneurs that can develop them further and thereby impact society.

The TTVO carries out the all-round management and follow-up of all aspects – including relationships with stakeholders – in order to ensure the appropriate intellectual property protection and commercial viability of the research results generated by CNIO’s scientist. Additionally, the Office proactively follows up on the progress of scientific activity at the CNIO in order to identify projects with high transfer potential. The TTVO plays a managerial and advisory role throughout the entire process, whereby it safeguards the efficient use of the patent system, identifies appropriate commercial partners for a timely development of technologies, negotiates licenses, monitors the activities of licensees regarding the achievement of milestones, and oversees the payment of royalty fees.

Other activities include: 1) working with researchers to enable them to exchange ideas, research results and materials with other researches from both academic and/or commercial entities; and 2) negotiating complex intellectual property terms related to sponsored research or strategic engagements with companies. The TTVO Office managed 305 agreements related to research results and CNIO’s intellectual property in 2017. About two thirds of these were of an international nature. We provide advice and guidance to researchers wishing to valorise their research results in order to make them more attractive to companies willing to develop technological, diagnostic and therapeutic solutions. In 2017, 16 valorisation projects were monitored. Valorisation of CNIO’s research results through alliances with industry remains a priority. The Technology Transfer Office offers support to CNIO researches to leverage on public-private partnerships for valorisation purposes. In 2017, CNIO scientists, in collaboration with local industrial leaders, presented 5 innovative projects under the framework of private-public partnership funding schemes, such as the national *Retos Colaboración* programme.

The inventions of CNIO scientists that have the potential to be transferred to the market are protected through patents. The CNIO’s patent portfolio is composed of 30 families, 5 of which are novel inventions brought about in 2017; these cover a wide range of applications such as novel targets, tissue regeneration, nano-

“We work closely with the CNIO faculty and researchers in order to understand how their ideas might be useful to patients as well as to ensure that all matters related to intellectual property protection and commercialisation are taken care of.”

systems to deliver cancer drugs to the tumour site, and methods to treat metastasis in the brain. Licensed patents are managed by our licensees and the rest is managed by the TTVO. Patents and unpatented research tools are licensed to increase their availability to the scientific community, as well as to create opportunities for our business partners and to provide a financial return on public investment. In 2017, CNIO entered into 7 new licence agreements with commercial partners for the exploitation of research results and tools. The royalty fees collected from licenses in 2017 exceeded 550,000 euros. This income reverts back to CNIO research activities as well as to the inventors themselves. A total of 52 inventors, well above 10% of CNIO’s researchers, have contributed towards and benefited from this achievement.

In 2017, a remarkable step was taken under the leadership of Manuel Serrano; namely, the start-up of a new company based on his research in the field of senescence, which has applications in cancer treatment and other diseases such as pulmonary fibrosis.

Fostering an innovation culture among our scientists is one of our priorities. With the support of *Fundación Banco Santander*, we uphold our collaboration with the prestigious IE Business School, through which many of our investigators – 3 new ones in 2017 – have already obtained training in market-oriented innovation strategies.

All the above mentioned achievements stand testament to the excellence and hard work of the CNIO scientists and to CNIO’s unwavering encouragement of innovation and technology transfer activities.

Communication

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COMMUNICATIONS

NURIA NORIEGA Head of Communications

Communications Officer
Cristina de Martos (TS)*

Science Outreach Officer
Pablo Camacho (since May) (TS)*

**Titulado Superior (Advanced Degree)*



The Communications Department takes the Centre’s activities beyond its doors in a truthful, accurate, and at the same time, attractive manner. Through different channels, we aim to draw the attention of the media and society in general towards the endeavours and achievements of the Centre’s researchers. Over the course of this past year, their advancements and discoveries have been well received and have attracted broad media coverage, with over 2,300 appearances in the press (online and on paper), and appearances on radio and TV, which represent an increase of 63% compared to the previous year.

In an increasingly competitive news environment, in which the specific weight of scientific news stories has declined in recent years, several of our articles have been in the forefront of Spain’s most prominent media channels and international media outlets such as the BBC and Der Standard, among others. The paper about the visualisation of melanoma metastasis written by Marisol Soengas and David Olmeda and published in *Nature* journal in June, and more recently, the article about a new strategy to halt glioblastoma growth, published in *Cancer Cell* by Maria A. Blasco and Leire Bejarano, have generated extensive attention. Both articles appeared on the front pages of several printed and digital publications, as well as on radio and television. But these were not the only ones to be publicly commended. Other stories also captured the public’s attention, such as the research led by Nabil Djouder and Marta Brandt that was published at the end of the year in *Cell Metabolism*, or the award granted by the American Prostate Cancer Foundation to Elena Castro, which broke records in terms of social media traffic and reach. These spotlighted accomplishments represent valuable tools when it comes to achieving our goal of reaching out to a broader audience. With a community of over 13,000 followers on Twitter and over 34,000 on Facebook, we have a great platform of communication that we seek to make full use of in order to ascertain the concerns and interests of society. This will help us to establish an enriching dialogue that will ultimately highlight and spread the idea that research in general and cancer research in particular have an intrinsic value that is essential for the betterment of society.

To this end, the Communications Department also drives and supports other initiatives developed by the Centre that pursue the dissemination of scientific knowledge, education, and the fostering of scientific values. In this regard, the Communications Department established an agreement with the TV & Media Group *Atresmedia*, one of the leading media groups in Spain, and the AXA Foundation to promote medical and scientific research

“Communication is a key factor for ensuring the success of Research & Development activities that, in turn, play an essential role in the advancement of society.”

on the occasion of World Cancer Research Day. Through this agreement, the event ‘Present and Future of Cancer Research’ took place at *El Matadero* – one of the most vibrant and cultural venues in Madrid – with Nobel Prize-winner Harald zur Hausen as guest of honour, and with the participation of CNIO’s Director Maria A. Blasco, Ángela Nieto from the Institute of Neurosciences (CSIC-UMH), and Pilar Garrido, Chief Oncologist at the Hospital Ramón y Cajal. The event, hosted by the well-known journalist of laSexta TV Mamen Mendizabal, served to communicate the message: research is key in the prevention and fight against cancer.

Following this spirit, the CNIO participated in the “la Caixa” Banking Foundation publicity campaign that revolved around the unique concept of ‘*Crucial people*’, the objective of which was to give a voice to outstanding people. This homage to remarkable individuals was covered by the written press, TV, radio, cinemas and digital media in order to promote the role of research and the importance of supporting research for society.

Last but not least, during 2017, the CNIO joined the Google Ad Grants for registered non-profit organisations that share Google’s philosophy of community service to help the world in areas such as science and technology, education, health, etc. Google Ad Grants is an advertising programme that offers free online positioning through the tool Google AdWords for non-profit organisations.

The CNIO Friends initiative to raise funds for cancer research was again greatly successful in 2017. Beyond other activities, the CNIO brought out the book *Excelentes*, which presents artistic photographs and personal stories by some of the most influential people who have visited the Centre in recent years like Nobel laureates Elizabeth Blackburn and Paul Nurse, the physicist Ignacio Cirac, or the palaeontologist Juan Luis Arsuaga. The funds collected through this book, on sale at stores such as *El Corte Inglés*, *VIPS* and the CNIO store, go directly towards the CNIO Friends initiative; these results motivate us even more to keep working on the important cause of cancer research.

PRESS CLIPPINGS



1 ABC, January 30, 2017
2 Telemadrid informativos, February 4, 2017

3 La Sexta Noche, La Sexta, February 18, 2017
4 El Global, March 27, 2017

5 QUO, April, 2017
6 Gaceta Médica, April 3, 2017
7 Diario Médico, April 10, 2017

8 El Mundo, May 6, 2017
9 El Correo, May 16, 2017



10 Heraldo, May 23, 2017
11 Especial informativo Antena 3, May 24, 2017
12 El Mundo, June 5, 2017

13 La 1 noticias, TVE, June 29, 2017
14 La Razón (front page), June 29, 2017

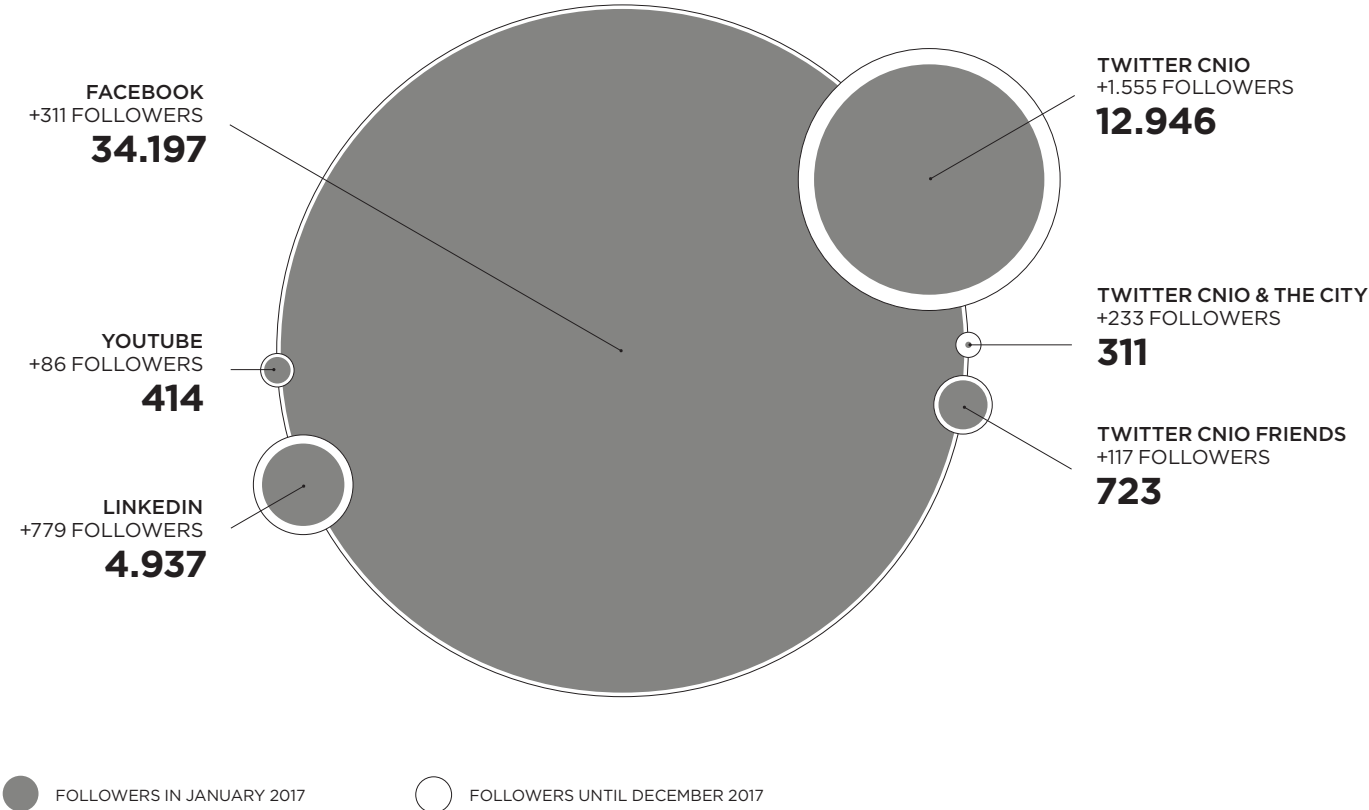
15 El Mundo, July 11, 2017
16 Diario Médico, July 21, 2017
17 El Progreso, July 31, 2017

18 BBC, September, 2017
19 La Razón, September 23, 2017



20 Más vale tarde, La Sexta, September 25, 2017
21 La Razón, November 14, 2017
22 SINC, November 24, 2017
23 El País Retina, El País, November 25, 2017
24 La 1 noticias, TVE, November 27, 2017
25 La Opinión, December 5, 2017
26 Buenos días Madrid, Telemadrid, December 18, 2017
27 Diario Médico, December 22, 2017

2017 SOCIAL NETWORK DATA



FACEBOOK

DATA PER POST

LIKES	187,63
SHARED	41,62
COMMENTS	4,43
REACH	5.716,18
LINK CLICKS	93,15

LINKEDIN

DATA PER POST

REACH	4.245,8
INTERACTIONS	31,95
CLICKS	49,54

TWITTER CNIO

DATA PER TWEET

LIKES	6,74
RETWEETS	5,43
ANSWERS	0,32
REACH	2.154,53
LINK CLICKS	8,17

YOUTUBE

GENERAL DATA

UPLOADED VIDEOS	88
UPLOADED IN 2017	8
VIEWS IN 2017	21.028
LIKES	172
SHARED	367

INVITED GUEST SPEAKERS (Distinguished Seminar Series)



Elaine Fuchs, January 13, 2017



Kari Alitalo, April 28, 2017



Vera Gorbunova, May 5, 2017



Paola Scaffidi, October 6, 2017



Peter Carmeliet, October 20, 2017

SOCIAL EVENTS



On occasion of International Women's Day, a performance of 'A Room of One's Own', a stage adaptation of Virginia Woolf's essay was given. The performance was directed by María Ruiz and starred Clara Sanchís. It was promoted by the CNIO's Women in Science Office (WISE). March 7, 2017.



Carmen Vela, Secretary of State for Research, Development and Innovation, as well as President of the Board of the CNIO; María A. Blasco, CNIO's Director; and José María Fernández Sousa-Faro, Chairman of *PharmaMar*, signed an agreement for the implementation of new screening tests to characterise potential anti-tumour compounds of marine origin. March 17, 2017.



On the occasion of World Cancer Research Day, the CNIO organised the event 'Present and Future of Cancer Research' together with *Atresmedia* Corporation and *AXA* Foundation. Attendees enjoyed an inspiring talk by Harald zur Hausen, winner of the Nobel Prize in Medicine in 2008, plus a panel discussion with Prof. zur Hausen, CNIO Director María A. Blasco, Ángela Nieto (Institute of Neurosciences, CSIC-UMH) and Pilar Garrido (*Hospital Ramón y Cajal*). September 25, 2017.



In 2017, the CNIO once again participated in the European Researchers' Night, which is funded by the EU Framework Programme for Research & Innovation, and Horizon 2020 - Marie Skłodowska-Curie actions. More than 60 CNIO volunteers and 240 visitors attended the event aimed at raising awareness about science and promoting scientific culture in society. September 29, 2017.



The CNIO celebrated its traditional Lab Day by recognising the work of its researchers, especially the youngest ones. Organised by the Dean's Office for Academic Affairs, it was a day to enjoy the spirit of team building, and especially, of being proud to belong to the CNIO community. December 18, 2017.

CNIO & THE CITY

CAROLINA POLA Director of International Affairs



Communications Officer
Cristina de Martos (TS)*

Science Outreach Officer
Pablo Camacho (since May) (TS)*

*Titulado Superior (Advanced Degree)

In 2017, the Spanish National Cancer Research Centre launched a new *mission to inform*. A project that aims to centralise all of CNIO’s efforts in terms of bringing science and research to the general public. The CNIO & The City initiative was launched halfway through the year, forming a bridge between our laboratories, our scientists and society. Specifically, this project, jointly funded by the Spanish Foundation for Science and Technology (FECYT) and “la Caixa” Social Projects, is aimed at students, secondary schools and the further education of teachers. The objective, in addition to explaining the work that we do and conveying the importance of research for development and evolution, is to awaken a passion for science in young minds. The accessibility of research centres through outreach events, citizen science and co-creation is an aspect of growing interest and importance for European institutions, funding bodies and evaluators. It is essential that both research projects and institutions themselves consider these aspects as important in order to achieve new standards of scientific excellence in the future.

The CNIO has a rich history when it comes to *science outreach* activities; this ranges from its participation in European Researchers’ Night to guided visits for secondary schools. These activities all require the commitment of the staff that take part in them and make them possible. CNIO & The City is the product of this undeniable vocation to inform and the desire to push

“Bringing science closer to society, stimulating scientific vocations and providing young people with role models are some of the Centre’s objectives and the spirit for which CNIO & The City stands.”

this facet to a new level. The project, the content of which is available on the <http://cnioandthecity.cnio.es> website, is divided into two main parts: FORMACNIO, devoted to teachers, and EDUCACNIO, devoted to students. Both the teachers’ course (held in October) and the ‘Laboratory immersion’ and ‘My first scientific project’ (in progress) activities are proving hugely popular with the participants who have expressed their gratitude and enthusiasm on numerous occasions. The commitment of the researchers who participated on a voluntary basis is, without doubt, the key to the project’s success and we are hoping that this will help us to repeat the initiative in 2018.

The integration of science in societal culture is a long-standing demand in Spain since the Enlightenment. This change must be led by research centres that possess the knowledge. That is our commitment, through CNIO & The City and other present and future outreach initiatives.



International Affairs

INTERNATIONAL AFFAIRS

CAROLINA POLA Director of International Affairs



A focus on innovation and new research alliances has been the main goal of the Department of International Affairs (IAs) in 2017. The start of the year strongly set the tone with the hosting of the first Innovative Medicines Initiative (IMI) Workshop in Oncology. This event to support the participation of CNIO and other Spanish cancer institutions in IMI, the largest European public-private initiative for drug discovery was organised in collaboration with the Spanish Centre for the Development of Industrial Technology (CDTI). Different actors from the Spanish oncology community gathered together at our Centre to discuss the strategy in cancer with high-rank influencers of the European programme. Putting ourselves even more firmly on the map of cancer research and innovation, the CNIO achieved an important milestone in Spain by establishing a research collaboration agreement with the largest Spanish and most international biopharmaceutical company, *PharmaMar*. This represents a remarkably successful case of knowledge transfer in relation to the support of innovation.

In the context of furthering our international impact on public-private collaborations, we have invested efforts in expanding and consolidating our international network for innovation. The joint effort of the IAs and the Department of Innovation to actively engage collaborators in forums such as BIO-Europe 2017 in Berlin, conveys the importance of the interdepartmental work to achieve common goals. Other international networks dedicated to drug discovery, such as the Milner Therapeutics Alliance, now include the CNIO as affiliate academic institution.

At the CNIO, basic discoveries and scientific creativity are at the forefront of research progress, and as such, the Centre strives to form alliances with centres of excellence that can serve as springboards for other research collaborations. In 2017, and with the invaluable support of the *Ramon Areces* Foundation, the 1st CNIO-Weizmann Institute of Science Joint Symposium on ‘New Insights in Cancer Discovery’ was launched, creating the appropriate setting for knowledge exchange. The CNIO will continue nurturing this tripartite alliance with the Weizmann Institute of Science in order to set the basis for a fruitful collaboration among scientists from both institutions.

“Our international scope embraces the interests of all our different stakeholders, crucial for our development as a centre of excellence. These partnerships with both public and private entities will help us build our roadmap for the future.”

Establishing communication channels with international delegations is part of the strategy of IAs, as we can leverage their power to facilitate the creation of partnerships of value for the CNIO and represented countries. This year we established a fruitful relationship with the US Embassy in Spain, resulting in the participation of a CNIO female Head of Unit in the international programme for women leaders in STEM called “Hidden No More” to represent Spain in this initiative. We are tapping other embassies to forge new partnerships and to develop new projects that can add value to the scientific community in an international context.

Last but not least, we believe that having a strong impact abroad is just as important as being influential in networks that are composed of Spanish Research Centres of Excellence; in this light, the *Severo Ochoa* and *Maria de Maetzu* alliance (*SOMMa*) was recently launched to promote their members internationally and to further boost the high-profile reputation for the institutions. The CNIO is actively participating, through the IAs, as leader of the ‘work package of Outreach’. The goals shared by the alliance and our Centre are framed within the European ‘Responsible Research & Innovation’ (RRI) concept, which continues to guide the institutional strategy and science policy of the CNIO.

CNIO Offices

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DEAN’S OFFICE

MARÍA S. SOENGAS
Dean for Academic Affairs

Participants
Mónica Álvarez, Daniela Cerezo,
Marta Contreras, Guillermo de
Cárcer, Ana Losada, Jorge Martínez,

David Olmeda, Francisco Real,
Mercedes Robledo, Manuel Valiente



The CNIO is recognised for the relevance and international projection of our Scientific Programmes. Key to this success is a solid core of undergraduate students, predoctoral and postdoctoral fellows, medical residents and a broad spectrum of visiting scientists. In fact, personnel in training constitute over 60% of the workforce in our institute. As such, the CNIO dedicates particular emphasis to career development, supported in part by highly competitive PhD and Postdoctoral Programmes. Agreements are also in place with multiple universities and medical centres, in order to ultimately bridge the gap between academic and clinical environments. Also very successful are our undergraduate summer internships, as well as diverse exchange and visitor programmes.

Part of the CNIO mission is to nurture and foster the development of our scientists-in-training in order to maximise their chances of success. Likewise, we acknowledge that career options extend beyond the bench and therefore we pay special attention to the

areas of public communication, management of intellectual property, and the creation of *start-ups* or *spin offs*. These activities are performed in concert with CNIO’s Training Programmes, as well as with the Innovation and Communication Offices, which are deeply committed to providing the best environment for our personnel. We are most grateful to the *Fundación Jesús Serra* for their continuous support to strengthen career development programmes at the CNIO.

We believe that an informed society is better prepared to understand (and if needed, face) the diseases that constitute human cancer. Therefore, we are actively involved in knowledge dissemination. For example, over 60 CNIO PhD students and postdoctoral fellows volunteered this year took part in the fifth edition of our ‘*Meet a Scientist, Become a Scientist*’ event. This is an open doors activity that we hold as part of the *Marie Skłodowska Curie European Researchers’ Night* and was attended by over 250 participants of all ages, who learned about the daily life at

our centre and have the opportunity to run an experiment side by side with our researchers.

A particularly inspirational event this year was our Annual CNIO Lab Day. We were fortunate to host *Eduardo Oliver*, a founding member of the Society of Spanish Researchers in the UK (SRUK/CERU), and *Patricia Salama*, a highly accomplished European Patent Attorney of the Elzaburu Law firm. Eduardo Oliver summarised how the efforts of a small group of postdoctoral fellows coalesced in a successful organisation that today spans multiple cities in the UK and has branches in multiple countries, including Spain. It was a pleasure to hear him explain the networking strategies that SRUK/CERU has in place for the career development of young researchers. He thus emphasised the value of seeking and cultivating the right mentors. In turn, Patricia Salama spoke about her personal voyage from working at the bench to helping scientists translate their findings into valuable intellectual property. Her tips and advice on the aspects of planning and timing public disclosure of marketable results were also very appreciated by the audience. We also had 7 outstanding talks given by CNIO trainees that covered exciting discoveries in the fields of epidemiology, epigenetics, proteomics, metastasis and drug development. Progress made in other basic and translational aspects of cancer were covered in over 60 posters, which together emphasised the breadth of research covered by our different Scientific Programmes.

Another main highlight of the Lab Day was the announcement of the recipients of our ‘*Director’s List Awards*’. These are recognitions of outstanding contributions made by our personnel in 3 categories: (1) predoctoral fellows with publications of the highest scientific impact; (2) excellence in research by postdoctoral and staff investigators; and (3) altruistic volunteering to further the mission of the Centre related to training, scientific divulgation and outreach.

1. Awards for Excellence in Research by Predoctoral Fellows

We are grateful to the Agüera-Nieto family for a generous donation in the name of their mother Antonia Nieto to support an award to acknowledge the PhD student authoring the article with the highest impact in a scientific journal. This year, the “*Antonia Nieto Award*” went to Leire Bejarano, for an impressive work in *Cancer Cell* on new discoveries in the field of glioblastoma, a highly lethal brain cancer. We are again moved by the commitment

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

of the Agüera-Nieto family, their energy and suggestions for the *Amigos del CNIO* initiative.

Additional awards in the PhD category went to Marta Brandt (*Cell Metabolism*), Metehan Cifdaloz (*Nature Communications*), Teresa Olbrich (*PNAS*) and Laura Remacha (*Clin Cancer Res*).

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

The awardee was David Olmeda, for the development of animal models to visualise melanoma metastasis *in vivo*, and the unravelling of how these systems lead to the identification of putative markers of poor patient prognosis in this disease (*Nature*).

3. Outstanding Contribution to Outreach and Awareness

The recipient was Jorge Martínez-Torrecuadrada, for his tireless efforts in the organisation of the 2017 European Researcher’s Night as well as for his numerous contributions to several open doors activities held over the years. The award was presented by Valle Salnés, the General Director of *Juegaterapia*.

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

CNIO WOMEN IN SCIENCE OFFICE

Lola Martínez
Coordinator

Members
Work-Life Balance Coordinator:
Marinela Méndez
Seminars and Events Coordinator:
Nicole Dölker (until July), Isabel
López de Silanes (since September),
Maria A. Blasco, Cristina de Martos,



The CNIO Women in Science Office (WISE) was established in 2012. Our main objectives are to give visibility to women, to raise awareness regarding the importance of gender equality, to help correct imbalances in the career ladder at the CNIO community, to try to promote and support women in their professional careers, as well as to come up with ideas and policies to improve the life/work balance at the CNIO. The WISE Office is composed of CNIO volunteers from across all the areas present in the Centre including the Director. All of us share the belief that women are still underrepresented in leadership positions in science and we are committed to ensuring gender equality within the research domain.

Several studies and statistics from different organisations within Spain and the European Union still display the ‘scissors’ graphic when it comes to gender distribution and career ladder positions. There is a clear lack of female talent in leadership positions, which, together with an average 20% salary gap between men and

women, are clear indicators that there remains a lot of work to be done in terms of promoting female talent and facilitating access to areas that are still not traditionally open to women. Still today, women are underrepresented in academia. Although data from Universities here in Spain and other European countries show that the percentage of women undertaking university studies is over 50%, and the representation of women at the pre-doctoral and post-doctoral stages is similar, those percentages diminish significantly as we move up the scientific career ladder: a poor 25% of women are represented at the Principal Investigator level, and it is even lower at the levels of Department Directors and beyond. This year, once again, no women were awarded Nobel Prizes in any scientific discipline, despite the fact that there were several women nominated for their key discoveries. Sadly, the acknowledgement of women when it comes to prizes continues to be very low and, in particular, when we consider the most prestigious ones; is there simply not enough female talent present in the scientific fields, or is it still just harder for

Raquel García-Medina, Diego Megías,
Francisca Mulero, Fernando Peláez,
Carolina Pola, Alejandra Tavera

them to be recognised for their achievements? We believe it is mainly due to the second cause and so we continue with our endeavours that take the form of a successful seminar series where we give all CNIO members and others (including, since last year, students from different high schools in Spain) the opportunity to listen to and meet women who embody roles and positions that are traditionally not held by women. With this initiative, it is our mission to promote scientific vocations among girls – it is worrying to see the lack of female students in the so-called STEM careers – as well as to positively change the cultural and gender stereotypes that exist among teenagers. A real change is needed, and we are convinced that it needs to come through the education of our younger generations.

The Office counts with two main working groups:

- Life/Work Balance – aimed to promote and support initiatives to help improve the delicate balance between professional and personal life at CNIO.
- Seminars and Events – aimed to raise and stimulate institutional awareness of gender issues, and to provide networking opportunities to all CNIO researchers.

In 2017, the WISE office seminar series continued to host several top female leaders from different areas. Some of the talks given during 2017 include:

- How the current design of parental leaves is hampering the professional development of women. The ‘PLENTY of rights’ proposal. Teresa Jurado and Mariano Nieto. PPIINA (Spanish Platform for Equal and Non-Transferable Birth and Adoption Leaves), Madrid, Spain. February 28.
- A room of one’s own. A play based on Virginia Woolf’s speech directed by María Ruiz and interpreted by Clara Sanchís, to celebrate International Women’s Day. March 7.
- Situation of women’s sport in Spain. Personal experience. Natalia Flores Sanz. Former Spanish National indoor football player. May 23.
- Leave your comfort zone and be disruptive. Katharina Miller. European lawyer and founder of 3CCompliance, Madrid, Spain. September 19.
- Micromachisms, the daily machismo that conditions the life of women. Ana Requena. Journalist and columnist. October 17.
- The future of women lies in our hands. Margarita de Cos. Head Major Donors Relations WWF Spain and CEO of RAINSOFT SL, Madrid, Spain. December 12.

“Here at the WISE Office, we share the views expressed by the African activist and writer Malebo Sephodi: “we do criticise the narrative that excludes women and continually puts men in the forefront.” Let’s continue working together towards Scientific Excellence, recognising and promoting talent, leaving all gender barriers behind us.”

We also participated in several initiatives such as ‘CNIO and The City’, a science outreach project, co-financed by the *FECYT* (Spanish Foundation for Science and Technology) and *Obra Social “la Caixa”*, with the aim of creating closer links between society and the education system. We are also working on establishing a fruitful collaboration with ESMO (European Society for Medical Oncology) in order to expand their survey work on behalf of Women in the Oncology field. The Office has also participated, in a consultancy capacity, in the elaboration of a non-law proposal regarding gender; the proposal aims to integrate the gender perspective in scientific contents as well as to increase knowledge on illnesses that mainly affect women, focusing on their causes and preventive measures. The proposal was presented to Congress during the 1st quarter of the year. This year, we are also very pleased to announce that one of our members has been selected to participate, along with 47 other women from all over the world, in the International Visitor Leadership Programme run by the U.S. State Department “Hidden No More: Empowering Women Leaders in STEM”.

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SCIENTIFIC MANAGEMENT

ISABEL BARTHELEMY Scientific Management Director



Raquel Ares, Sonia Cerdá, Almudena del Codo, M. Dolores Liébanes, Ana Merino, Juan Ramón Molina (until June), Mercedes Moro, Victoria López, Leyre Vergés

The Scientific Management Department at the CNIO is committed to assisting with the facilitation of all those key areas that help our scientists to better focus their efforts on their research. The Department encompasses various Offices: Projects and Consortia, Education and Training Programmes, Scientific Events, Scientific Publishing, and Library and Archives.

The mission of the Projects' Office is to guide the CNIO scientists through all stages related to the application and management processes of externally-funded projects, whether they be financed through either public and/or private institutions, or stem from either national or international funding bodies. The Office coordinates the internal call alerts, manages the ethical certification for projects involving animal experimentation or human samples, supports scientists with the preparation of the project proposals, manages the ongoing projects, and contacts the funding agencies to resolve any issues or deal with questions.

The Training Office is the central point for training at the CNIO; it aids the recruitment process, serves as an advocate for all fellows, provides administrative support, and creates educational and learning opportunities. It is responsible for helping PhD students, Postdoctoral scientists and post-resident MDs by announcing call alerts and providing the relevant key information; helping foreign students with their paperwork at the foreign office; organising the summer training call; and, in general, in collaboration with the Personnel Department, managing student's grants.

The Events Office organises CNIO meetings, such as the CNIO-*"la Caixa"* Foundation Frontiers Meetings, the Distinguished Seminars series, the external Scientific Advisory Board (SAB) meeting, CNIO Progress Reports, as well as Faculty retreats, among others. The Office also helps scientists by providing advice for the organisation of specific events, including science outreach events, and supervises the CNIO guided visits.

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

The Library administers the electronic subscriptions of over 300 scientific journals at the CNIO and manages journal article requests for journals that the CNIO is not subscribed to. The Library also provides information regarding reference management software.

“All our efforts are dedicated towards providing our scientists with the best possible framework and to taking care of all formalities so that they, in turn, can focus on making a difference through top-notch science.”

COMPETITIVE FUNDING


The CNIO attracts a substantial proportion of its funding from external sources. Most of this funding comes from national and international funding bodies. In 2017, researchers at the CNIO were involved in 135 projects that received extramural funding.






In 2017, the CNIO actively participated in a total of 42 collaborative projects: 20 were international collaborative projects (3 of which are coordinated by the CNIO) and 22 were collaborative projects at the national level (7 of them are coordinated by the CNIO). The international collaborative projects were funded by institutions such as the European Commission through the 7 Framework Programme and Horizon 2020, the Interreg SUDOE Programme, the US National Institutes of Health (NIH), the US Department of Defense (DoD), the Melanoma Research

Alliance, the Paradifference Foundation and the Worldwide Cancer Research.

In addition to these collaborative projects, researchers at the CNIO attracted funding for projects that are carried out by individual groups. In 2017, 27 of these projects received international funding, while 66 of them received national funding (mainly from the State Research Agency, Spanish Ministry of Economy, Industry and Competitiveness, as well as the Institute of Health Carlos III). The international individual projects are funded by the European Commission (4 ERC grants and 8 Marie Curie Actions), the Worldwide Cancer Research, the Howard Hughes Medical Institute (HHMI) and the European Foundation for the Study of Diabetes (EFSD).

INTERNATIONAL GRANTS COLLABORATIVE PROJECTS

EUROPEAN COMMISSION	
	
7 TH FRAMEWORK PROGRAMME (2007-2013)	
COST ACTION	
PRINCIPAL INVESTIGATOR	PROJECT TITLE
Malats, Núria (coordinator)	COST Action EU Pancreas: An integrated European platform for pancreas cancer research: from basic science to clinical and public health interventions for a rare disease (Ref.: COST BM1204)
EURATOM	
PRINCIPAL INVESTIGATOR	PROJECT TITLE
Serrano, Manuel (until April 30th)	RISK-IR: Risk, Stem Cells and Tissue Kinetics-Ionising Radiation (Ref.: 323267)
INTEGRATED PROJECT (IP)	
PRINCIPAL INVESTIGATOR	PROJECT TITLE
Valencia, Alfonso (until February 28th)	RD-CONNECT: An integrated platform connecting registries, biobanks and clinical bioinformatics for rare disease research (Ref.: 305444)
ERA-NET ON TRANSLATIONAL CANCER RESEARCH (TRANSCAN)	
PRINCIPAL INVESTIGATOR	PROJECT TITLE
Malats, Núria	Bio-PaC: Biomarkers of tumor recurrence in pancreatic cancer (financed by ISCIII, Ref.: AC14/00025)
ERA NET NEURON II: NETWORK OF EUROPEAN FUNDING FOR NEUROSCIENCE RESEARCH	
PRINCIPAL INVESTIGATOR	PROJECT TITLE
Malumbres, Marcos	MicroKin: Deciphering the multifaceted pathways underlying MCPH pathogenesis in the mouse and human (financed by MEIC, Ref.: PCIN-2015-007)
HORIZON 2020 (2014-2020)	
RESEARCH INFRASTRUCTURES, INCLUDING E-INFRASTRUCTURES	
PRINCIPAL INVESTIGATOR	PROJECT TITLE
Valencia, Alfonso (until February 28th)	ELIXIR-EXCELERATE: Fast-track ELIXIR implementation and drive early user exploitation across the life-sciences (Ref.: 676559)
Valencia, Alfonso (until February 28th)	OpenMinTeD: Mining INfrastructure for TExt and Data (Ref.: 654021)
MARIE SKŁODOWSKA-CURIE ACTIONS (MSCA)	
PRINCIPAL INVESTIGATOR	PROJECT TITLE
Soengas, María S.	ITN IMMUTRAIN: Training network for the immunotherapy of cancer (Ref.: 641549)

<div>INTERREG SUDOE PROGRAMME¹</div> <div></div>	SOCIETAL CHALLENGE 1: HEALTH, DEMOGRAPHIC CHANGE AND WELLBEING	
	PRINCIPAL INVESTIGATOR	PROJECT TITLE
	Benítez, Javier	BRIDGES: Breast cancer risk after diagnostic gene sequencing (Ref.: 634935)
<div>MELANOMA RESEARCH ALLIANCE (MRA)</div>	PRINCIPAL INVESTIGATOR	PROJECT TITLE
	Valencia, Alfonso (until February 28th) Al-Shahrour, Fátima (since March 1 st)	ONCONET: European Network for Translational Research and Innovation in Oncology /Réseau Européen de Recherche translationnelle et d'Innovation en oncologie (Ref.: SOE1/P1/F0082)
<div>THE PARADIFFERENCE FOUNDATION</div>	PRINCIPAL INVESTIGATOR	PROJECT TITLE
	Soengas, María S. (coordinator)	Imaging and targeting dormant and pro-metastatic melanoma lesions <i>in vivo</i> (Ref.: 401181)
<div>US CONGRESSIONALLY DIRECTED MEDICAL RESEARCH PROGRAMS (CDMRP)/US DEPARTMENT OF DEFENSE</div> <div></div>	PRINCIPAL INVESTIGATOR	PROJECT TITLE
	Peinado, Héctor	Organ-tropic metastatic secretomes and exosomes in breast cancer (Ref.: W81XWH-13-1-0427)
<div>US NATIONAL INSTITUTES OF HEALTH (NIH)</div> <div></div>	PRINCIPAL INVESTIGATOR	PROJECT TITLE
	Muñoz Fernández, Inés	Targeting Mdm2-MdmX E3 ligase for treatment of drug-resistant lymphoma (Ref.: R01CA208352)
	Peinado, Héctor	Characterization and functional analysis of breast cancer secreted exosomes in malignant progression (Ref.: U01CA169538)
	Peinado, Héctor	Exosome-mediated transfer of c-MET to bone marrow progenitors promotes metastasis (Ref.: R01CA169416)
	Valencia, Alfonso (until February 28th) Tress, Michael (from March 1 st on)	GENCODE 2: Integrated human genome annotation: generation of a reference gene set (Ref.: U41HG007234)
<div>WORLDWIDE CANCER RESEARCH (WCR, FORMERLY AICR)</div> <div></div>	PRINCIPAL INVESTIGATOR	PROJECT TITLE
	Malats, Núria (coordinator)	Oral microbiotic profiles and its association with risk of pancreatic ductal adenocarcinoma (Ref.: 15-0391)
<div>ELIXIR (EUROPEAN LIFE-SCIENCE INFRASTRUCTURE FOR BIOLOGICAL INFORMATION)</div> <div></div>	PRINCIPAL INVESTIGATOR	PROJECT TITLE
	Valencia, Alfonso (until February 28th)	The ELIXIR Beacon project (Ref.: ES-2015-BEACON)
	Valencia, Alfonso (until February 28th)	Genomic data management for TraiT using EGA (Ref.: ES-2015-TRAIT)

1. This Programme is cofunded by the European Regional Development Fund (ERDF)



INTERNATIONAL GRANTS INDIVIDUAL PROJECTS

EUROPEAN COMMISSION



7TH FRAMEWORK PROGRAMME (2007-2013)

EUROPEAN RESEARCH COUNCIL (ERC)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Fernández-Capetillo, Óscar	ERC Consolidator Grant RSHEALTH: Investigating the causes and consequences of replication stress in mammalian health (Ref.: 617840)

MARIE CURIE ACTIONS (MCA)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Al-Shahrour, Fátima	PERSMEDOMICS: Bioinformatics and integrative genomics for a novel personalized cancer therapy (Ref.: 334361)
Peinado, Héctor	WHRI COFUND ADIPOMET: Analyzing the crosstalk of tumor and adipose tissue during metastasis (Ref.: 608765 WHRI-309)
Plaza, Iván	WHRI COFUND: Structure-Function studies of oncogenic RET kinase Fusions in non-small cell Lung Cancer (NSCLC): from structure to targeted therapy (Ref.: 608765 WHRI-612)
Ramón, Santiago Moreno, María	WHRI COFUND CAD_FL: Revealing the functional mechanism of CAD and its potential as a therapeutic target (Ref.: 608765 WHRI-222)
Real Arribas, Francisco Paliwal, Sumit	WHRI COFUND: NFIC as a novel regulator of pancreatic acinar differentiation and homeostasis (Ref.: 608765 WHRI-609)
Squatrito, Massimo	GLIDD: DNA Damage Response (DDR) signaling in tumor formation and therapeutic resistance of gliomas (Ref.: 618751)
Wagner, Erwin F., Gago, Nuria	WHRI COFUND STEM-PSO: Unraveling the contribution of Epidermal and Non-Epidermal Progenitor (Ref.: 608765 WHRI-319)

HORIZON 2020 (2014-2020)

EUROPEAN RESEARCH COUNCIL (ERC)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Barbacid, Mariano	ERC Advanced Grant THERACAN: Novel therapeutic strategies to treat pancreatic and lung cancer (Ref.: 695566)
Efeyan, Alejo	ERC Starting Grant NutrientSensingVivo: The Physiology of Nutrient Sensing by mTOR (Ref.: 638891)
Serrano, Manuel (until April 30th)	ERC Advanced Grant CELLPASTICITY: New Frontiers in Cellular Reprogramming: Exploiting Cellular Plasticity (Ref.: 669622)

MARIE SKŁODOWSKA-CURIE ACTIONS (MSCA)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Soengas, María S.	METMEL: Long range-acting drivers of premetastatic niches in melanoma (Ref.: 753442)

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

HOWARD HUGHES MEDICAL INSTITUTE (HHMI)



MELANOMA RESEARCH ALLIANCE (MRA)

Melanoma Research Alliance

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Soengas, María S.	Prognostic and therapeutic impact of lymphovascular niches in melanoma (Ref.: 348673)
Valiente Cortés, Manuel	Blocking melanoma brain metastasis by targeting the environment (REF.: 498103)

PROSTATE CANCER FOUNDATION

PROSTATE CANCER FOUNDATION

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Olmos, David	Integration of clinical, molecular and biological characteristics to define an aggressive subtype of prostate cancer based on deficient homologous recombination
Castro, Elena	Prospective study of lethal prostate cancer clinical and genomic evolution in DNA repair deficient tumours

WORLDWIDE CANCER RESEARCH (WCR, FORMERLY AICR)



PRINCIPAL INVESTIGATOR	PROJECT TITLE
Blasco, Maria	Targeting telomeres in cancer (Ref.: 16-1177)
Lietha, Daniel	Targeting regulatory mechanisms for allosteric cancer drug discovery (Ref.: 15-1177)
Malumbres, Marcos	New therapeutic strategies by inhibiting Mastl in breast tumors (Ref.: 15-0278)
Peinado, Héctor	Evaluation of obesity as a novel risk factor in metastasis (Ref.: 16-1244)
Pérez Moreno, Mirna A.	Defining the role of macrophage-derived Wnts in squamous cell carcinoma (Ref.: 15-1219)
Soengas, María S.	Harnessing endo/exocytosis for a coordinated targeting of melanoma cells, their vasculature and the immune system (Ref.: 15-1374)

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



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

INTERNATIONAL HUMAN FRONTIER SCIENCE PROGRAM ORGANIZATION



PRINCIPAL INVESTIGATOR	PROJECT TITLE
Llorca, Óscar	Photochemical trap and high-resolution imaging of transient chromatin complexes from living cells (Ref.: RGP0031/2017)

THE COMPANY OF BIOLOGISTS (COB)



PRINCIPAL INVESTIGATOR	PROJECT TITLE
Djouder, Nabil	Event CNIO CFM: Molecular Chaperones in Cancer (Ref.: EA1287)

BEUG FOUNDATION FOR METASTASIS RESEARCH



PRINCIPAL INVESTIGATOR	PROJECT TITLE
Valiente Cortés, Manuel	Altered brain vessels as a novel target in brain metastasis

NATIONAL GRANTS COLLABORATIVE PROJECTS

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



SUB-PROGRAMME OF COOPERATIVE HEALTH RESEARCH THEMATIC NETWORKS/ SUBPROGRAMA DE REDES TEMÁTICAS DE INVESTIGACIÓN COOPERATIVA (RETICS)²

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Rodríguez, Sandra	Red Temática de Investigación Cooperativa en Cáncer (RTICC) (Group, Ref: RD12/0036/0037)
Malats, Núria	Red Temática de Investigación Cooperativa en Cáncer (RTICC) (Group, Ref: RD12/0036/0050)

SUB-PROGRAMME OF GRANTS FOR RESEARCH SUPPORT PLATFORMS IN HEALTH SCIENCES AND TECHNOLOGY/ SUBPROGRAMA DE AYUDAS PARA PLATAFORMAS DE APOYO A LA INVESTIGACIÓN EN CIENCIAS Y TECNOLOGÍAS DE LA SALUD³

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Benítez, Javier	Plataforma de recursos biomoleculares y bioinformáticos, PRB ² (Group, Ref: PT13/0001/0005)
Morente, Manuel M. (coordinator)	Plataforma de Biobancos (Coordination node and group, Ref: PT13/0010/0001)
Muñoz, Javier	Plataforma de recursos biomoleculares y bioinformáticos, PRB ² (Group, Ref: PT13/001/0010)
Valencia, Alfonso (until February 28th)	Plataforma de recursos biomoleculares y bioinformáticos, PRB ² (Group, Ref: PT13/0001/0030)

RESEARCH PROJECTS IN HEALTH ⁴

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Blasco, María A.	Cellular aging in first episode early-onset psychosis (Collaboration with <i>Gregorio Marañón</i> Hospital, Ref.: PI14/00397)
Blasco, Maria A.	Safety and efficacy of gene therapy with telomerase in acute myocardial infarction. Impact on ventricular remodeling in an experimental porcine model. (Collaboration with <i>Gregorio Marañón</i> Hospital, Ref. DTS15/00095)
Morente, Manuel (coordinator)	Optimization of tissue samples for the development and validation of disease biomarkers: the OPTIMARK project. (Ref.: PI16/00946)
Olmos, David (coordinator)	THERATLAS Project: integration of early and adaptative genetic events to establish therapeutic subgroups in Castration-Resistant Prostate Cancer (Ref.: PI16/01565)

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



STATE RESEARCH AGENCY/
AGENCIA ESTATAL DE
INVESTIGACIÓN (AEI)
MINISTRY OF ECONOMY,
INDUSTRY AND
COMPETITIVENESS
/ MINISTERIO DE
ECONOMÍA, INDUSTRIA Y
COMPETITIVIDAD (MEIC)



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

EXCELLENCE NETWORKS/ REDES DE EXCELENCIA

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Efeyan, Alejo (Coordinator)	METABOCANCER: crosstalk between systemic and cellular metabolism in cancer (Ref.: SAF2016-81975-REDT)

CHALLENGES-COLLABORATION/RETOS-COLABORACIÓN⁵

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Djouder, Nabil	NRCANCER: <i>desarrollo de nueva terapia antitumoral basada en nicotinamida-ribosido</i> (Ref.: RTC-2016-5431-1)
Soengas, María S.	<i>Ensayo Clínico Fase I de BO-110: un nuevo tratamiento para melanoma avanzado y otros tumores</i> (Ref.: RTC-2014-2442-1)

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



PRINCIPAL INVESTIGATOR	PROJECT TITLE
Barbacid, Mariano (coordinator)	A multifaceted approach to target pancreatic cancer (Ref.: GC16173694BARB)
Benítez, Javier	Cancer and immunodeficiency in children (Ref.: CEI14142070LOPE)
Malats, Núria Real, Francisco X. (coordinator)	Invasive bladder cancer: towards precision medicine (Ref.: GCB14142293REAL)
Gómez, Gonzalo Peinado, Héctor Soengas, María S. (coordinator)	Distinct routes of metastatic dissemination in different melanoma subtypes. Implications in the validation of new tumor biomarkers and therapeutic targets (Ref.: GCB15152978SOEN)
Valiente, Manuel	Study of the molecular mechanisms involved in primary (glioblastoma) and secondary (metastasis) brain tumors to identify novel therapeutic targets and anti-cancer agents, biomarkers to select treatments and novel non-invasive methods for molecular diagnosis (Ref.: GCTRA16015SEOA)

LA MARATÓ TV3
FOUNDATION / FUNDACIÓN
LA MARATÓ TV3



PRINCIPAL INVESTIGATOR	PROJECT TITLE
Barbacid, Mariano	Molecular analysis of Capicua, a novel tumor suppressor involved in RTK signalling and transcriptional repression (Ref.: 20131730/31)
Fernández-Capetillo, Óscar	Exploring synthetic lethal interactions between PARP and the DNA damage response in cancer treatment (Ref.: 20134130/31)
Soengas, María S.	Role of RNA binding proteins in melanoma progression: searching for new diagnostic markers and therapeutic targets (Ref.: 20131430/31)

MADRI+D FOUNDATION
/ FUNDACIÓN PARA EL
CONOCIMIENTO MADRI+D



PRINCIPAL INVESTIGATOR	PROJECT TITLE
Dean's Office for Academic Affairs Soengas, María S.	European Researchers' Night 2016-2017, organized by Madri+d Foundation and founded by European Commission, H2020 Programme (Ref: MadridERN2016-2017, GA 721631)

5. This Programme is cofunded by the European Regional Development Fund (ERDF)



NATIONAL GRANTS INDIVIDUAL PROJECTS

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



RESEARCH PROJECTS IN HEALTH⁶

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Benítez, Javier	Biologic and genetic bases of telomere shortening in hereditary breast cancer. Searching for new high susceptibility genes in <i>BRCAX</i> families with short telomeres (Ref.: PI12/00070)
Benítez, Javier	Massive sequencing contributes to decipher the genetic bases of families with rare tumors (Ref.: PI16/00440)
Cascón, Alberto	Exome sequencing of trios, mother-father-proband, in pediatric patients with multiple pheochromocytomas/paragangliomas (Ref.: PI12/00236)
Cascón, Alberto	Next generaton sequencing of genes directly and indirectly involved in the Krebs cycle, applied to pheochromocytomas/paragangliomas with hypermethylated phenotype (Ref.: PI15/00783)
Cigudosa, Juan C. (until 31 st January)	Genetic diagnostics by next-generation-sequencing in myeloid neoplasias: step towards its clinical use and characterization studies on the mutation genomic and functional pathological effects (Ref.: PI12/00425)
Malats, Núria	Aetiology of pancreas cancer: Application of “omics” technologies in the assessment of risk factors (Ref.: PI12/00815)
Malats, Núria	Building and validation of risk prediction models for pancreas cancer. The application of a multi-omics approach (Ref.: PI15/01573)
Molina, María Esther	Dietary patterns, antioxidants and biomarkers of oxidant-antioxidant status in the EPIC-Granada and EPIC-Gipuzkoa (European Prospective Investigation into Cancer and Nutrition) cohort (Ref.: PI12/00002)
Quintela, Miguel Ángel	Tumor-tolerant immune reprogramming secondary to hypoxia-inducing antiangiogenics in breast cancer: physiopathogenic mechanisms and therapeutic utility (Ref.: PI16/00354)
Robledo, Mercedes	Prognostic profiles in endocrine tumours identified by next generation sequencing, and definition of markers with clinical utility (Ref.: PI14/00240)
Rodríguez, Sandra	Ewing Sarcoma Model: induction of the t(11;22) translocation in human mesenchymal stem and iPS cells by the CRISPR-Cas9 system and study of the cellular context and other secondary events role (Ref.: PI14/01884)
Urioste, Miguel	PTEN-hamartoma tumour syndrome research: Phenotypic spectrum, associated cancers, molecular basis and search of new gene (Ref.: PI14/00459)

STATE RESEARCH AGENCY/
AGENCIA ESTATAL DE
INVESTIGACIÓN (AEI)
MINISTRY OF ECONOMY,
INDUSTRY AND
COMPETITIVENESS
/ MINISTERIO DE
ECONOMÍA, INDUSTRIA Y
COMPETITIVIDAD (MEIC)



NATIONAL R&D&I PLAN 2008-2011

SUB-PROGRAMME FOR NON-TARGETED FUNDAMENTAL RESEARCH PROJECTS / SUBPROGRAMA DE PROYECTOS DE INVESTIGACIÓN FUNDAMENTAL NO ORIENTADA

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Uluçkan, Özge	PsorTACEmiR21: Investigating the role of microRNA21/ TIMP-3/TACE in psoriasis - evaluating the potential therapeutic implications (Ref.: SAF2012-39670)

6. This Programme is cofunded by the European Regional Development Fund (ERDF)



NATIONAL PLAN FOR SCIENTIFIC AND TECHNICAL RESEARCH AND INNOVATION (2013-2016)	
CENTRES AND UNITS OF EXCELLENCE “SEVERO OCHOA” SUB-PROGRAMME/ <i>SUBPROGRAMA DE APOYO A CENTROS Y UNIDADES DE EXCELENCIA ‘SEVERO OCHOA’</i>	
PRINCIPAL INVESTIGATOR	PROJECT TITLE
Blasco, Maria A.	Centre of Excellence “Severo Ochoa” (Ref.: SEV-2015-0510)
R&D EXCELLENCE PROJECTS/ <i>PROYECTOS DE I+D EXCELENCIA’</i>	
PRINCIPAL INVESTIGATOR	PROJECT TITLE
Méndez, Juan	REPLICON: Molecular mechanisms that control eukaryotic DNA replication (Ref.: BFU2013-49153-P)
Ramón, Santiago (until March 23 rd)	CADstructure: Structural determination of the architecture of CAD, an antitumoral target that controls the biosynthesis of pyrimidines (Ref.: BFU2013-48365-P)
Ruiz, Sergio	RSHIPS: Replicative stress during somatic cell reprogramming (Ref.: SAF2013-49147-P)
Ruiz, Sergio	Role of RAS signaling in pluripotency and totipotency (Ref.: SAF2016-80874-P)
CHALLENGES-RESEARCH/ <i>RETOS-INVESTIGACIÓN</i> ^a	
PRINCIPAL INVESTIGATOR	PROJECT TITLE
Barbacid, Mariano	PANTHER: A three prong strategy to fight pancreatic ductal adenocarcinoma (Ref.: SAF2014-59864-R)
Blasco, Maria A.	TeloHealth: Telomeres, telomerase and disease (Ref.: SAF2013-45111-R)
Djouder, Nabil	MILC: Metabolic inflammation in liver cancer (Ref.: SAF2013-46089-R)
Djouder, Nabil	URI loss in intestinal pathologies (Ref.: SAF2016-76598-R)
Efeyan, Alejo	NUTRIENTOR: Physiology of nutrient sensing and signaling by the mTOR complex 1 (Ref.: SAF2015-67538-R)
Fernández-Capetillo, Óscar	BREAKINGRAD: Exploring the limits of radioresistance in mammals (Ref.: SAF2014-59498-R)
Lietha, Daniel	Structural studies elucidating the activation mechanism of Focal Adhesion Kinase (Ref.: BFU2016-77665-R)
Llorca, Óscar (since July 1st)	RuvBL1-RuvBL2 ATPases in DNA/RNA surveillance and human diseases: molecular and structural mechanisms (Ref.: SAF2014-52301-R)
Losada, Ana	COHESIN: Cohesin function and regulation: a multidisciplinary approach (Ref.: BFU2013-48481-R)
Losada, Ana	Molecular mechanisms of variant cohesin function (Ref.: BFU2016-79841-R)
Malumbres, Marcos	Cyclexit: Physiological and therapeutic relevance of mitotic kinases and phosphatases (Ref.: SAF2015-69920-R)
Méndez, Juan Ramón	Control of eukaryotic DNA replication (Ref.: BFU2016-80402-R)
Muñoz, Javier	steMS: Understanding ground state pluripotency of embryonic stem cells through mass spectrometry-based proteomics (Ref.: SAF2013-45504-R)

7, 8. This Programme is cofunded by the European Regional Development Fund (ERDF)



Muñoz, Javier	Epigenetic modifiers in pluripotency: a proteomic analysis of non-histone protein methylation (Ref.: SAF2016-74962-R)
Ortega, Sagrario	HaploEScancer: Haploid ES cells for cancer research (Ref.: SAF2013-44866-R)
Osorio, Ana	IPAGEN: Exploring the mechanism of action of PARP inhibitors in breast and ovarian cancer patients. Identification of new genetic predictors of response (Ref.: SAF2014-57680-R)
Peinado, Héctor	METASTAXOMES: Role of tumor-secreted exosomes in lymph node microenvironment reprogramming during metastasis (Ref.: SAF2014-54541-R)
Pérez Moreno, Mirna A.	ESSENCE: Extrinsic control of the skin stem cell niche in homeostasis and cancer (Ref.: BFU2015-71376-R)
Ramón, Santiago (until March 23 rd)	Structure and functional characterization of CAD, an anti-tumoral target controlling the synthesis of pyrimidines (Ref.: BFU2016-80570-R)
Real, Francisco X.	TRANS-PDAC: Transcriptional control of pancreatic cancer development (Ref.: SAF2015-70553-R)
Rodríguez, Cristina	PREDICT: Identification of genetic markers and physiopathologic factors predictive of the peripheral neuropathy of paclitaxel and of other oncologic drugs: massive sequencing of candidate genes (Ref.: SAF2015-64850-R)
Serrano, Manuel (until April 30th)	CANCERAGE: Cancer and ageing-associated diseases: new frontiers and new strategies (Ref.: SAF2013-48256-R)
Soengas, María S.	MEL-STOP: Vesicular trafficking in melanoma progression and treatment response (Ref.: SAF2014-56868-R)
Valencia, Alfonso (until February 28th)	EPIC: Expression Patterns of Inverse Comorbidity (Ref.: BFU2015-71241-R)
Valiente, Manuel	ReACTIVE BrainMET: Dissecting the role of reactive astrocytes in brain metastasis (Ref.: SAF2014-57243-R)
Wagner, Erwin F.	CANPSOR: Investigating Cancer Risk in Psoriasis (Ref.: SAF2015-70857-R)
EXPLORA PROJECTS	
PRINCIPAL INVESTIGATOR	PROJECT TITLE
Blasco, Maria A.	Non canonical treatment for neurodegenerative diseases: telomerase gene therapy (Ref.: SAF2015-72455-EXP)
NETWORKS AND SCIENTIFIC MANAGERS-EUROPE / <i>EUROPA REDES Y GESTORES</i>	
PRINCIPAL INVESTIGATOR	PROJECT TITLE
Blasco, Maria A.	CNIO in Horizon 2020: support for proposal preparation and project management (Ref.: EUC2014-51617)
YOUNG RESEARCHERS PROGRAM/ <i>PROGRAMA JÓVENES INVESTIGADORES</i> ^a	
PRINCIPAL INVESTIGATOR	PROJECT TITLE
Álvarez, Mónica	GPGenCan: Functional relevance of Greatwall/PP2A pathway in the maintenance of genomic stability: therapeutic implications in cancer (Ref.: SAF2014-60442-JIN)
Lecona, Emilio	UBQREP: Modulation of DNA Replication by ubiquitination of chromatin proteins (Ref.: BFU2014-55168-JIN)

9. This Programme is cofunded by the European Regional Development Fund (ERDF)



SPANISH FOUNDATION FOR SCIENCE AND TECHNOLOGY / FUNDACIÓN ESPAÑOLA PARA LA CIENCIA Y TECNOLOGÍA (FECYT)



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



ASTRAZENECA FOUNDATION / FUNDACIÓN ASTRAZENECA



LEUKEMIA AND LYMPHOMA FOUNDATION / FUNDACIÓN LEUCEMIA Y LINFOMA



SPANISH ASSOCIATION OF GASTROENTEROLOGY / ASOCIACIÓN ESPAÑOLA DE GASTROENTEROLOGÍA



SCIENTIFIC INFRASTRUCTURES/INFRAESTRUCTURAS CIENTÍFICO-TECNOLÓGICAS ¹⁰	
PRINCIPAL INVESTIGATOR	PROJECT TITLE
Muñoz, Javier	<i>Sistema cromatográfico UHPLC acoplado a Espectrómetro de Masas de alta resolución para estudios de proteómica avanzada</i> (Ref.: CNIO15-EE-2855)
Pisano, David G.	<i>Clúster SMP de Análisis HPC</i> (Ref.: CNIO15-EE-3845)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Pola, Carolina	CNIO and the City – <i>Construyendo un Puente a la Sociedad</i> (Ref.: FCT-16-11115)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Efeyan, Alejo	Grant for emerging Groups: Nutrient signaling in the pathogenesis and treatment of B cell Lymphoma (Ref.: LABAE16001EFEY)
Squatrito, Massimo	Grant for emerging Groups: Novel therapeutic approaches for therapy-resistant malignant brain tumors (Ref.: LABAE16015SSQUA)
de Cárcer, Guillermo	Grant for emerging Groups: Identification of new biomarkers for breast cancer: mechanisms of sensitivity and resistance to cell cycle drugs (Ref.: LABAE16017DECÁ)
Squatrito, Massimo	“Idea Semilla” Grant: Identification of biomarkers of tumor treating fields (TTFields) in glioblastoma (Ref.: IDEAS185SQUA)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Quintela, Miguel Ángel	<i>Reprogramación inmune en cáncer de mama preexpuesto a antiangiogénicos inductores de apoxia</i>

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Ortega, Ana	“Marcos Fernández” Grant: Functional characterization of RagC mutations in Follicular Lymphoma pathogenesis

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Molina, M ^a Esther	<i>Evaluación del valor pronóstico de diabetes mellitus tipo II (DM2) en pacientes con cáncer de páncreas</i>

FERO FOUNDATION / FUNDACIÓN FERO




ATRESMEDIA COORPORATION / ATRESMEDIA COORPORACIÓN



OLGA TORRES FOUNDATION / FUNDACIÓN OLGA TORRES



FUNDACIÓN PROYECTO NEUROFIBROMATOSIS



BBVA FOUNDATION / FUNDACIÓN BBVA



FUNDACIÓN PFIZER



PRINCIPAL INVESTIGATOR	PROJECT TITLE
Peinado, Héctor	Liquid biopsy by nanoplasmonic detection of exosomes: predicting response to (immuno- and radio)-therapy
Valiente, Manuel	Predictive biomarkers for brain metastasis in small cell lung cancer

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Fernández-Capetillo, Óscar	<i>Premio Constantes y Vitales en la categoría “Joven talento en investigación biomédica” 2015</i> (A3M 2015)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Djouder, Nabil	Understanding the role of growth factors and nutrients in inflammatory bowel disease and colon cancer

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Peinado, Héctor	<i>Uso de exosomas circulantes como marcadores de progresión en neurofibromatosis y para la determinación de nuevas estrategias terapéuticas</i>

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Squatrito, Massimo	Leonardo Grant: Precision glioma mouse models by somatic genome editing with the RCAS-CRISPR-Cas9 system
Valencia, Alfonso (until February 28th)	BIG DATA Grant PerMed: Precision Medicine from Big Data to Cognitive Computing CNIO (Ref.: 76/2016)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Peinado, Hector	Tumour exosome integrins determine organotropic metastasis

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



EDUCATION AND TRAINING PROGRAMMES

One of the principal goals of the CNIO is to increase its training capacity in order to give students and professionals the opportunity to advance their careers in the healthcare sector. During 2017, the CNIO signed several new agreements with

TRAINING PROGRAMMES	PARTICIPANTS IN EDUCATION AND TRAINING PROGRAMMES				
	2013	2014	2015	2016	2017
Training of PhD students	116	108	105	110	112
Post-doctoral training	67	55	48	51	44
Training for MDs	21	14	25	17	21
Laboratory training for MSc/BSc students	36	73	80	95	99
Laboratory training for technicians	19	21	27	26	20

TRAINING OF BSC/MSC STUDENTS

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

An annual Summer Training Programme for undergraduate students, from any country, who are in their last years of study in the biomedical field. The Programme encompasses 8 weeks of full-time laboratory training (292.5 hours). During this time, the students actively participate in research projects in one of the CNIO groups. During 2017, 7 students from 4 different countries participated in this programme.

Additionally, students can apply for laboratory training throughout the academic year by directly contacting the Heads of CNIO individual Research Groups or Units. This year, 99 students participated in these programmes, of which 3 ended up joining the CNIO as pre-doctoral students.

Spanish Universities and other institutions, namely with the *Universidad de Extremadura*, *Universidad Complutense de Madrid*, *Universidad Internacional de la Rioja*, *Fundación Banco Santander*, *Fundación La Caixa*, *FCT Puerta de Hierro* and *CESUR*.

TRAINING OF PHD STUDENTS

The training of PhD students in cutting-edge cancer research is of key importance to the CNIO. The Centre offers many opportunities for bright and dynamic university graduates, of all nationalities, to pursue an ambitious PhD project. To attest this, 21 students obtained their PhD degrees in 2017 and 21 others joined the CNIO in the same year. Over 21% of the 112 students working at the CNIO in 2017 were graduates from foreign universities, thus contributing to the internationalisation of the Centre.

Since 2008, the *Fundación “la Caixa”* offers international fellowships to PhD students to enable them to carry out their thesis projects in biomedical research in Spanish centres of excellence. The CNIO was chosen, as one of such centres, to launch a programme for outstanding young pre-doctoral students from all over the world who have an interest in pursuing an ambitious PhD project. Since 2013, the Ministry of Economy, Industry and Competitiveness has undertaken efforts to link the “*la Caixa*”/CNIO International PhD Programme to distinguished research centres accredited as “Severo Ochoa” Centres of Excellence. In 2017, a new doctoral fellowship programme of “*la Caixa*” Foundation, named INPhINIT, was launched to recruit outstanding international students: 2 pre-doctoral students received one of these 3-year contracts. The *Fundación “la Caixa”* also launched a call to carry out a doctorate at Spanish universities and research centres; CNIO was chosen as a host institution. During 2017, 1 pre-doctoral student received this fellowship.

The distribution of students across the CNIO's Research Programmes in 2017 was as follows: 53% of students worked in the

Molecular Oncology Programme, 13% in the Structural Biology Programme, 13% in the Cancer Cell Biology Programme, 10% in the Human Cancer Genetics Programme, 1% in the Experimental Therapeutics Programme, 2% in the Biotechnology Programme, and 8% in the Clinical Research Programme.



FUNDING OF PHD TRAINING	NO.
SPANISH ORGANISATIONS	88
State Research Agency, Ministry of Economy, Industry and Competitiveness / <i>Agencia Estatal de Investigación (AEI), Ministerio de Economía, Industria y Competitividad (MEIC)</i> (Predoctoral fellowships)	39
State Research Agency, Ministry of Economy, Industry and Competitiveness / <i>Agencia Estatal de Investigación (AEI), Ministerio de Economía, Industria y Competitividad (MEIC)</i> (I+D Projects)	9
Ministry of Education, Culture and Sport / <i>Ministerio de Educación, Cultura y Deporte (MECD)</i> (Predoctoral fellowships)	6
Institute of Health Carlos III / <i>Instituto de Salud Carlos III (ISCIII)</i> (I+D Projects)	4
"la Caixa" Banking Foundation / <i>Fundación Bancaria "la Caixa"</i> (Predoctoral fellowships)	17
Spanish Association Against Cancer (AECC) / <i>Fundación Científica de la AECC</i> (I+D Projects)	2
Community of Madrid / <i>Comunidad de Madrid</i>	2
<i>Cris</i> Foundation / <i>Fundación Cris</i>	3
<i>FERO</i> Foundation / <i>Fundación FERO</i>	1
CNIO	5
INTERNATIONAL ORGANISATIONS	24
European Commission Framework Programme / H2020	1
Marie Skłodowska-Curie actions of the European Commission	2
European Research Council	6
Fundação do Ministério de Ciência e Tecnologia de Portugal (FCT)	1
China Scholarship Council	2
European Foundation for the Study of Diabetes	1
Melanoma Research Alliance	1
<i>Boehringer Ingelheim</i>	2
<i>Consejo Nacional de Ciencia y Tecnología</i> (Mexico)	2
Howard Hughes Medical Institute	1
Pfizer	5
TOTAL	112

POST-DOCTORAL TRAINING

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

In 2017, 44 postdoctoral fellows worked at the CNIO. Notably, more than one third of these fellows were from outside of Spain, many coming from very prestigious international institutions.

In 2017, the *Fundación Banco Santander* signed a new annual agreement with the CNIO to continue the highly competitive fellowship programme aimed at supporting outstanding young scientists who have been trained in the UK or in the USA, and who wish to start or continue their postdoctoral training at the CNIO. One young scientist from the City University of New York (CUNY) was awarded the *Banco Santander* Foundation-CNIO Fellowship in early 2017.

Thanks to the donations received through the ‘CNIO Friends’ platform, launched in 2017, the second call of the Postdoctoral Contract ‘CNIO Friends’ Programme resulted in the recruitment of 2 scientists for a 2-year period each. Also, thanks to a ‘Juegaterapia-CNIO Friends’ Postdoctoral Contract in 2017, one scientist was able to continue with her project related to paediatric oncology.

FUNDING SOURCES OF POST-DOCTORAL RESEARCHERS	NO.
SPANISH ORGANISATIONS	28
State Research Agency, Ministry of Economy, Industry and Competitiveness / <i>Agencia Estatal de Investigación (AEI), Ministerio de Economía, Industria y Competitividad (MEIC)</i> (Postdoctoral Fellowships)	3
State Research Agency, Ministry of Economy, Industry and Competitiveness / <i>Agencia Estatal de Investigación (AEI), Ministerio de Economía, Industria y Competitividad (MEIC)</i> (I+D Projects)	4
Institute of Health Carlos III / <i>Instituto de Salud Carlos III (ISCIII)</i> (I+D Projects)	1
Institute of Health Carlos III / <i>Instituto de Salud Carlos III (ISCIII)</i> (Postdoctoral Fellowhips)	2
Spanish Association Against Cancer (<i>AECC</i>) / <i>Fundación Científica de la AECC</i> (Postdoctoral Fellowhips)	3
Spanish Association Against Cancer (<i>AECC</i>) / <i>Fundación Científica de la AECC</i> (I+D Projects)	1
Community of Madrid / <i>Comunidad de Madrid</i>	1
<i>Banco Santander</i> Foundation / <i>Fundación Banco Santander</i>	2
<i>La Marató TV3</i> Foundation/ <i>Fundació La Marató TV3</i>	1
<i>Celgene</i>	1
CNIO	9
INTERNATIONAL ORGANISATIONS	16
European Commission Framework Programme / H2020	1
European Research Council	3
Marie Skłodowska-Curie actions of the European Commission	2
Melanoma Research Alliance	1
Worldwide Cancer Research UK	5
US Department of Defense	1
<i>Daiichi Sankyo</i> Agreement	1
Pfizer	2
TOTAL	44

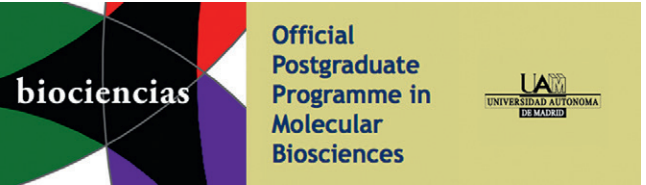
POSTGRADUATE PROGRAMMES

In addition, the CNIO – in collaboration with academic institutions across Spain – provides access to a variety of postgraduate programmes that cover the areas of Cellular &

Molecular Biology, Molecular Biomedicine, Biotechnology, Biocomputing & 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). These trainings fall under 4 Official Postgraduate Programmes, namely, the Doctorate in Biosciences, Master’s in Molecular and Cell Biology, Master’s in Molecular Biomedicine, and Master’s in Biotechnology. CNIO also collaborates with the UAM, as a Partner Institution of UAM’s Doctoral School (EDUAM), and is a member of the Management Committee.



Master’s Degree in Biocomputing and Computational Biology

The Master’s in *Bioinformática y Biología Computacional* 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

The CNIO and the CEU-San Pablo University in Madrid (USP-CEU) co-organise a Postgraduate Training Programme in Clinical and Applied Cancer Research: the *Máster Universitario en Investigación Clínica y Aplicada en Oncología*.



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

The CNIO collaborates with the Biochemistry and Molecular Biology Department at the University of *Alcalá 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 an 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, Clinical Diagnostic Laboratory, and Archiving/Recording; it is organised through agreements with 19 institutions that provide secondary education for laboratory technicians in Spain. It provides students with hands-on

knowledge in cellular and molecular biology techniques. The programme consists of 14 weeks (370-400 hours) of laboratory training for students. Of the 20 students who participated in this programme in 2017, 2 were hired by the CNIO.

TRAINING FOR MDS

In line with CNIO’s commitment to bridge the ‘bench to bedside’ gap, the Centre offers 3 training opportunity programmes to MDs and other health care professionals. Training usually consists of

a 3-month period during residency. In 2017, 24 medical residents from 18 different hospitals enjoyed the benefits of rotations within the different Groups and Units at the CNIO.

ADVANCED TRAINING OF SCIENTISTS THROUGH EXTRAMURAL PROGRAMMES

During 2017, the *Ramón y Cajal* Programme supported 8 scientists. This special initiative, established in 2001 by the former Spanish Ministry of Science and Technology (currently the State Research Agency of the Spanish Ministry of Economy, Industry and Competitiveness) aims to encourage Spanish or foreign scientists working abroad to return to or relocate to Spain. Successful candidates are selected on the basis of their potential capacity to lead independent projects and groups, or

to contribute successfully to the ongoing research in the existing groups. Ten other scientists were funded by similar programmes, including the *Juan de la Cierva* programme (Spanish Ministry of Economy, Industry and Competitiveness, 4 contracts); *Miguel Servet* (1 contract) and *SEOM-Río Hortega* (contract funded by the Spanish Society of Medical Oncology, 1 contract) programmes of the Institute of Health Carlos III; and the Spanish Association Against Cancer (*AECC*, 4 contracts).

VISITING RESEARCHER PROGRAMME

The *Jesús Serra* Foundation, part of the *Catalana Occidente* Group, aims to help eminent international specialists work together with CNIO researchers for a few months in order for them to expand their knowledge in areas of common interest. During 2017, Raul Rabadan, from Columbia University in New York (USA) was beneficiary of the *Jesús Serra* Foundation’s Visiting Researcher Programme. In addition, Wolfgang Weninger, from the Royal Prince Alfred Hospital of Sydney (Australia) was also a beneficiary.



‘SCIENCE BY WOMEN’ PROGRAMME

Thanks to this Programme, launched by the Spanish “Fundación Mujeres por África”, 2 African Scientists stayed at the CNIO as visiting scientists: Ann Louw, from the University of Stellenbosch in South Africa, worked in the Experimental Therapeutics Programme from January to June 2017; and Chiaka Anumudu, from the University of Ibadan in Nigeria, worked in the Genetic and Molecular Epidemiology Group from August 2017 to January 2018.



SCIENTIFIC EVENTS

CNIO-”LA CAIXA ”FOUNDATION FRONTIERS MEETINGS

CNIO-”la Caixa” Foundation Frontiers Meetings are the main international conferences organised by the CNIO and ”la Caixa” Foundation. They focus on specific, cutting-edge aspects of cancer research, thus providing a unique platform for an intensive and dynamic exchange and debate of scientific ideas. The invited speakers – 20 internationally renowned leaders in oncology –

present their latest findings during 2 and a half days. Up to 100 additional participants are selected, via a widely publicised call for applications, based on their potential to make relevant contributions to the conference by presenting hot topics as posters or short talks.

PRIMARY AND SECONDARY BRAIN TUMORS 19-22 FEBRUARY 2017

ORGANISERS

- **Massimo Squatrito**, Spanish National Cancer Research Centre (CNIO), Madrid, Spain
- **Manuel Valiente**, Spanish National Cancer Research Centre (CNIO), Madrid, Spain
- **Richard Gilbertson**, CRUK Cambridge Institute, UK
- **Michael Weller**, University Hospital Zurich, Switzerland

SESSIONS

- Primary Adult Brain Tumors (I)
- Primary Adult Brain Tumors (Ii)
- Secondary Adult Brain Tumors (I)
- Secondary Adult Brain Tumors (Ii)
- Primary And Metastatic Pediatric Tumors

SPEAKERS

- **Suzanne Baker**, Brain Tumor Research Division, St. Jude Children’s Research, Hospital, Memphis, US
- **Gabriele Bergers**, Vesalius Research Center, Leuven, Belgium
- **Priscilla Brastianos**, Massachusetts General Hospital, Harvard Medical School, Boston, US
- **Amy B. Heimberger**, The University of Texas MD Anderson Cancer Center, Houston, US
- **Eric Holland**, Nancy and Buster Alvord Brain Tumor Center, Fred Hutchinson Cancer Research Center, UW Medicine, US
- **Luis F. Parada**, Brain Tumor Center, Albert C. Foster, MSKCC, New York, US
- **Stefan Pfister**, DKFZ, Heidelberg, Germany
- **Matthias Preusser**, Comprehensive Cancer Center Vienna, Medical University of Vienna, Austria

- **Nicola Sibson**, CRUK/MRC Oxford Institute for Radiation Oncology, UK
- **Joan Seoane**, Vall d’Hebron Institute of Oncology, Barcelona, Spain
- **Riccardo Soffiatti**, University and City of Health and Science University Hospital of Turin, Italy
- **Michael Taylor**, The Arthur and Sonia Labatt Brain Tumour Research Centre, The Hospital for Sick Children, Ontario, Canada
- **Roeland Verhaak**, The University of Texas MD Anderson Cancer Center, The Jackson Laboratory for Genomic Medicine, US



- **William A. Weiss**, UCSF, Helen Diller Family Comprehensive Cancer Center, San Francisco, US
- **Wolfgang Wick**, DKFZ, Heidelberg, Germany
- **Frank Winkler**, DKFZ, Heidelberg, Germany

In addition, 11 short talks were selected among participants’ contributions and 49 posters were presented.

MOLECULAR CHAPERONES IN CANCER 2-4 MAY 2017

ORGANISERS

- **Nabil Djouder**, Spanish National Cancer Research Centre (CNIO), Madrid, Spain
- **Wilhelm Krek**, Institute for Molecular Health Sciences, Zurich, Switzerland
- **Paul Workman**, The Institute of Cancer Research, London, UK
- **Xiaohong Helena Yang**, *Cancer Cell*, Cambridge, US

SESSIONS

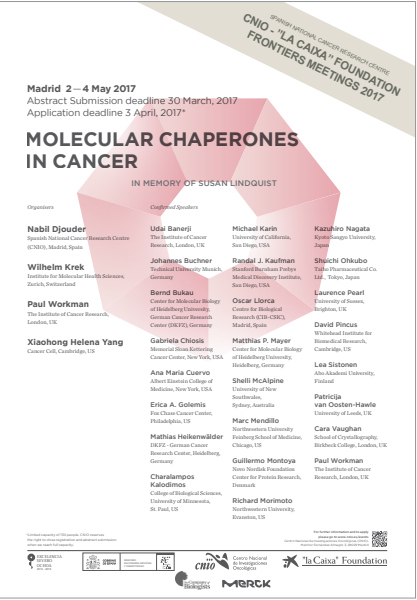
- Protein Quality Control
- Folding, Misfolding and Aggregation
- Stress Mechanisms in Cancer
- Chaperones in Cancer
- Targeting Chaperones: Chaperonotherapy

SPEAKERS

- **Udai Banerji**, The Institute of Cancer Research, London, UK
- **Johannes Buchner**, Technical University Munich, Germany
- **Bernd Bukau**, Center for Molecular Biology of Heidelberg University, German Cancer Research Center (DKFZ), Germany
- **Gabriela Chiosis**, Memorial Sloan Kettering Cancer Center, New York, US
- **Ana Maria Cuervo**, Albert Einstein College of Medicine, New York, US
- **Erica A. Golemis**, Fox Chase Cancer Center, Philadelphia, US
- **Mathias Heikenwälder**, DKFZ - German Cancer Research Center, Heidelberg, Germany
- **Charalampos Kalodimos**, College of Biological Sciences, University of Minnesota, St. Paul, US
- **Michael Karin**, University of California, San Diego, US
- **Randal J. Kaufman**, Stanford Burnham Prebys Medical Discovery Institute, San Diego, US
- **Oscar Llorca**, Centre for Biological Research (CIB-CSIC), Madrid, Spain
- **Matthias P. Mayer**, Center for Molecular Biology of Heidelberg University, Heidelberg, Germany
- **Shelli R. McAlpine**, University of New Southwales, Sydney, Australia

- **Marc Mendillo**, Northwestern University Feinberg School of Medicine, Chicago, US
- **Guillermo Montoya**, Novo Nordisk Foundation Center for Protein Research, Denmark
- **Richard Morimoto**, Northwestern University, Evanston, US
- **Kazuhiro Nagata**, Kyoto Sangyo University, Japan
- **Shuichi Ohkubo**, Taiho Pharmaceutical Co., Ltd., Tokio, Japan
- **Laurence Pearl**, University of Sussex, Brighton, UK
- **David Pincus**, Whitehead Institute for Biomedical Research, Cambridge, US
- **Lea Sistonen**, Abo Akademi University, Finland
- **Patricija van Oosten-Hawle** University of Leeds, UK
- **Cara Vaughan**, School of Crystallography, Birkbeck College, London, UK
- **Paul Workman**, The Institute of Cancer Research, London, UK

In addition, 10 short talks were selected among participants’ contributions and 18 posters were presented.



OTHER MEETINGS & CONFERENCES

The CNIO annually hosts various international meetings and conferences. Within this category, the 6 international events were held in 2017.

MEETING CNIO –CELL AND GENE THERAPY CATAPULT
30 MARCH 2017

CNIO PARTICIPANTS

- **Maria A. Blasco**
- **Oscar Fernández-Capetillo**
- **Anabel Sanz**
- **Marcos Malumbres**
- **Maria Salazar**

CELL AND GENE THERAPY CATAPULT PARTICIPANTS

- **Ricardo Baptista**
- **Almira Bartolome**
- **Sara Cebrián**

II SYMPOSIUM CNIO-IBIMA: PROSTATE CANCER AND OTHER GENITOURINARY TUMOURS
20-21 APRIL 2017

ORGANISERS

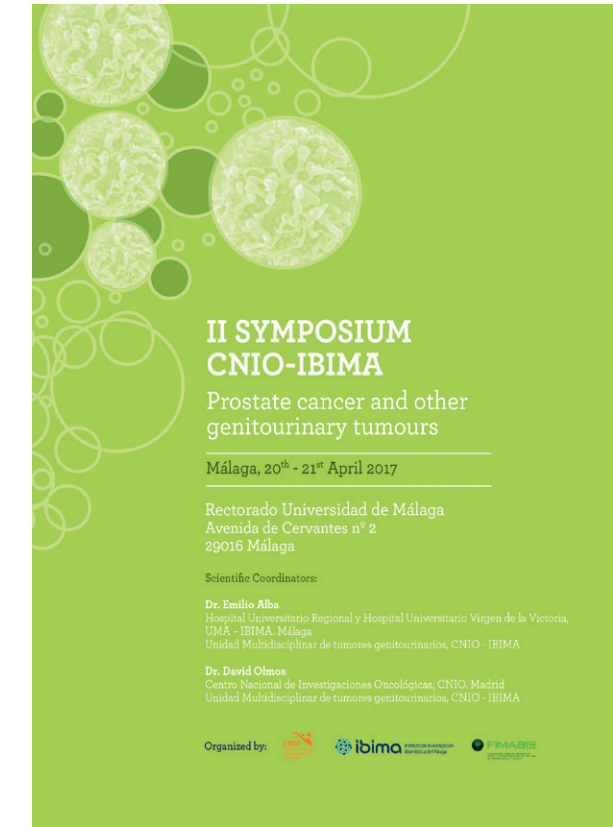
- **CNIO-IBIMA-FIMABIS**

SESSIONS

- Germ –Cell Tumours
- Renal Cancer
- Bladder Cancer
- What's New in the Biology of Prostate Cancer Mts

SPEAKERS

- **Emilio Alba**, *Hospital Regional Universitario* and *Virgen de la Victoria* University Hospital, UMA – IBIMA
- **Isabel Hierro**, *Hospital Regional Universitario* and *Virgen de la Victoria* University Hospital
- **Enrique González-Billalabeitia**, Morales Meseguer University Hospital
- **Ferran Algaba**, Puigvert Foundation
- **Emilio Julve**, *Hospital Regional Universitario* and *Virgen de la Victoria* University Hospital
- **Christophe Massard**, Gustav Roussy Institute
- **Francisco Javier Machuca**, *Hospital Regional Universitario* and *Virgen de la Victoria* University Hospital
- **Ana Medina**, Oncologic Center of Galicia
- **Álvaro Montesa**, *Hospital Regional Universitario* and *Virgen de la Victoria* University Hospital
- **Guillermo de Velasco**, *12 de Octubre* University Hospital
- **Emilio García-Galisteo**, *Hospital Regional Universitario* and *Virgen de la Victoria* University Hospital
- **Álvaro Montesa**, *Hospital Regional Universitario* and *Virgen de la Victoria* University Hospital



- **Francisco Real**, Spanish National Cancer Research Centre
- **Bernardo Herrera**, *Hospital Regional Universitario* and

- *Virgen de la Victoria* University Hospital
- **Maribel Sáez**, *Hospital Regional Universitario* and *Virgen de la Victoria* University Hospital
- **José Antonio Medina**, *Hospital Regional Universitario*
- **Mª José Requena**, Reina Sofía University Hospital
- **Raquel Correa**, *Hospital Regional Universitario* and *Virgen de la Victoria* University Hospital
- **Antonio Conde**, *Hospital Provincial* of Castellón
- **David Hernández**, *Hospital Regional Universitario* and *Virgen de la Victoria* University Hospital
- **José Rubio**, Valencia Oncology Institute Foundation
- **Gerhardt Attard**, The Royal Marsden NHS Foundation Trust
- **Elena Castro**, Spanish National Cancer Research Centre
- **Joaquín Mateo**, The Royal Marsden NHS Foundation

- Trust
- **Hishima Beltrán**, Cornell University
- **Jose Ángel Arranz**, Gregorio Marañon University Hospital
- **Joan Carles**, Vall d’Hebron Oncology Institute
- **Javier Puente**, *Hospital Clínico San Carlos*
- **José Menéndez Vidal**, Reina Sofía University Hospital
- **Ignacio Durán**, Virgen del Rocía University Hospital
- **Rosa Villatoro**, Costa del Sol Hospital
- **David Lorente**, Hospital Universitario y Politécnico de La Fe
- **José Luis Pérez Gracia**, University of Navarra Clinic
- **David Olmos**, Spanish National Cancer Research Centre
- **Emilio Alba**, *Hospital Regional Universitario* and *Virgen de la Victoria* University Hospital

NEW INSIGHTS IN CANCER DISCOVERY
26-27 SEPTEMBER 2017

ORGANISERS

- **Maria Blasco**, Spanish National Cancer Research Centre (CNIO), Spain
- **Oskar Fernández-Capetillo**, Spanish National Cancer Research Centre (CNIO), Spain
- **Moshe Oren**, Weizmann Institute of Science, Israel
- **Ravid Straussman**, Weizmann Institute of Science, Israel

CO-ORGANISERS

- **Ramón Areces Foundation**

SPEAKERS

SPANISH NATIONAL CANCER RESEARCH CENTRE, MADRID, SPAIN

- **Maria Blasco**
- **Oskar Fernández-Capetillo**
- **Manuel Valiente**
- **Rafael Fernández Leiro**
- **Marisol Soengas**
- **Francisco X. Real**
- **Marcos Malumbres**
- **Alejo Efeyan**



WEIZMANN INSTITUTE OF SCIENCE, REHOVOT, ISRAEL

- **Moshe Oren**
- **Ravid Straussman**
- **Itay Tirosh**
- **Liran Shlush**
- **Sima Lev**
- **Varda Rotter**
- **Yifat Merbl**
- **Yosef Yarden**

CIBERER
21 NOVEMBER 2017

Jornada de investigación cooperativa 2017. Reunión Anual Programa de Cáncer Hereditario, Enfermedades Hematológicas y Dermatológicas.



ONCO EMERGENCE FORUM
14-15 DECEMBER 2017

ORGANISERS

- University Toulouse III Paul Sabatier
- CNIO
- Biocat
- Navarrabiomed
- Universidad de Coimbra

SESSIONS

- Liquid biopsies as Prognostic biomarkers in solid tumours, new diagnostic approaches and implication in clinical studies

SPEAKERS

- Patrice Deneffe, Directeur de l’Institut ROCHE de Recherche et de Médecine Translationnelle
- María José Serrano, Virgen de las Nieves Hospital. Pfizer. University of Granada. Centre for Genomics and Oncological Research (GENYO)
- Atocha Romero, Puerta de Hierro University Hospital
- Artur Paiva, The Coimbra Hospital and University Centre
- Catherine Panabieres, LCCRH – CHU of Montpellier
- Gilles Favre, The University Cancer Institute of Toulouse
- Gabriel Capella, The Catalan Institute of Oncology. Bellvitge Biomedical Research Institute
- Hugh Ilyne, DestiNA Genomics
- Simon Perera del Rosario, Anaxomics Biotech
- Eduardo Pareja, Era7 Bioinformatics
- María Esther Molina, Spanish National Cancer Research Centre
- Jean-Philippe Nougayrède, Digestive Health Research Institute
- Sara Ramio Pujol, GoodGut
- Benjamin Lelouvier, Vaioomer
- Fay Betsou, IBBL (Integrated Biobank of Luxembourg)

- Raquel Bermudo Gascón, HCB-IDIBAPS Biobank, IDIBAPS
- Manuel Rodríguez Maresca, Andalusian Public Health System
- Manuel Morente, Spanish National Cancer Research Centre
- Taycir Skhiri, FHU OncoAge
- Joaquim Pereira, Hospital Santa Maria, IMM
- Maya Lauriol, Canceropôle Grand Sud-Ouest
- David Olmos, Spanish National Cancer Research Centre
- Henriqueta Breda, Faculty of Medicine of University of Coimbra
- Eva Martinez-Balibrea, Catalan Institute of Oncology Germans Trias I Pujol Research Institute
- María Tabernero, IMDEA Food Institute
- Alboukadel Kassambara, Institute of Human Genetics, CNRS-UM and University hospital of Montpellier
- Carlos Cano Gutiérrez, University of Granada; Tanglegen
- Francisco Caramelo, Faculty of Medicine of the University of Coimbra; Spiral Data Lda
- Fátima Al-Shahrour, Spanish National Cancer Research Centre
- Joaquín Dopazo, Progreso y Salud Foundation
- Sacha Beaumeunier, SeqOne
- Janet Piñero, Hospital del Mar Medical Research Institute (IMIM); Barcelona Biomedical Research Park
- Josep LL. Gelpi, Barcelona Supercomputing Center & University of Barcelona
- Lucas Jurado Fasoli, Oncodieta
- Anabel Sanz, Spanish National Cancer Research Centre
- Ana Catarina Mamede, ResearchTrial
- Marta Soler, Biocat
- Francisco Ambrósio, Faculty of Medicine of the University of Coimbra
- Emmanuel Le Boudier, Eurobiomed; Transferts

METABOCANCER KICK OFF MEETING
14 DECEMBER 2017

ORGANISER

- CNIO

METABOCANCER NETWORK MEMBERS

- Alejo Efeyan
- Anna Bigas
- Arkaitz Carracedo
- Guadalupe Sabio
- José Cuezva
- Marc Claret
- María Mittelbrunn

- Mariona Graupera
- Ruben Nogueiras
- Xosé Bustelo

GUEST PARTICIPANTS

- Guillermo Velasco
- Miguel López
- Nabil Djouder
- Pablo José Fernández Marcos
- Roger Gomis
- Toño Enríquez

TRAINING COURSES AND WORKSHOPS

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

them to stay abreast of recent developments in specialised techniques. This is achieved through training courses and hands-on workshops organised by CNIO scientists and technologists.

INNOVATIVE MEDICINES INITIATIVE WORKSHOP ON ONCOLOGY
12 JANUARY 2017

SPEAKERS

- Marta Gómez Quintanilla, IMI. CDTI
- Ion Arocena, ASEBIO
- Javier Urzay, FARMAINDUSTRIA
- Alfonso Beltrán, FIPSE. ISCIII
- Maria A. Blasco, CNIO
- Pierre Meulien, IMI
- Carmen Eibe, PHARMAMAR

- Andrés Cervantes, Hospital Clínico Universitario de Valencia
- Joaquín Arribas, CIBERONC
- Alfonso Valencia, INB
- Jan van de Loo, European Commission
- César Hernández, AEMPS
- Marta Gómez Quintanilla, Health & IMI – Horizon 2020

2ND EUROMABNET: ANTIBODY VALIDATION WORKSHOP
26 MAY 2017

ORGANISER

- European Monoclonal Antibody Network EuroMAbNet

SPEAKERS

- Giovanna Roncador, Spanish National Cancer Research Centre
- Pablo Engel, University of Barcelona



- Friedrich Koch-Nolte, Institute of Immunology Hamburg
- Alison Banham, University of Oxford
- Vladka Čurin Šerbec, Blood Trasfusion Centre of Slovenia
- Alejandra Solache, Abcam
- Matt Baker, Thermo Fisher Scientific

ADVANCE CELL SORTING COURSE
3-4 SEPTEMBER 2017

ORGANISERS/SPEAKERS

- Rui Gardner, Head of the Flow Cytometry Core Facility, Memorial Sloan Kettering Cancer Center, NY, USA

- Lola Martinez, Head of the Flow Cytometry Unit, CNIO, Madrid. Spain

HOW TO SUCCESSFULLY PERFORM & ANALYSE A MULTICOLOR FLOW EXPERIMENT WORKSHOP
7-8 SEPTEMBER 2017

ORGANISERS/SPEAKERS

- Lola Martinez, Head of the Flow Cytometry Unit. CNIO. Madrid. Spain

- Andrea Valle, Application Specialist FCS Express, DeNovo Software. Milano. Italy

JOIN OUR WORKSHOP FEATURING LEICA TCS SP8 DIVE AND LEICA DMi8 S
14-16 NOVEMBER 2017

ORGANISER

- LEICA Microsystems – CNIO

The purpose of the Distinguished Seminars Series is to invite outstanding and internationally renowned scientists to give a seminar and to meet with researchers at the CNIO. Distinguished Seminars are recurrent events that are open to the general public and are held throughout the year, usually on Fridays at noon in the CNIO Auditorium. Each Distinguished Seminar series includes world-leading scientists who address topics that are of general interest to the CNIO faculty.

serves to, annually, invite 3 to 4 opinion leaders from other areas of science, technology, and literature; the overarching goal is to enable the CNIO to present its know-how as well as its vision on contemporary and future technological, societal and cultural challenges. These 'out-of-the-box' seminars are sponsored by the '*Fundación Banco Sabadell*'. The breadth of expertise and topics covered creates a multidisciplinary and intellectually challenging environment that goes far beyond the frontiers of cancer research.

DATE	SPEAKER	ORGANISATION	TITLE	
JANUARY				
13/01/2017	Elaine Fuchs	Howard Hughes Medical Institute, The Rockefeller University, NY, US	Stem cells in silence, action and cancer	
20/01/2017	Raul Mostoslavsky	Kristine and Bob Higgins MGH Research Scholar Massachusetts General Hospital Cancer Center Harvard Medical School, Boston, US	Linking Epigenetics, Metabolism and Cancer: lessons from SIRT6	
27/01/2017	Benjamin L. Ebert	Brigham and Women's Hospital, Boston, US	The genetics of myeloid neoplasia: from clonal hematopoiesis to acute leukemia	
FEBRUARY				
17/02/2017	Nuria Oliver	DataPop Alliance, New York, US	The Future of the Mobile Phone	Fundación BancoSabadell BS
MARCH				
10/03/2017	Tom Kirkwood	Newcastle University Institute for Ageing, UK; University of Copenhagen Center for Healthy Aging, Copenhagen, Denmark	Why and how are we living longer?	Fundación BancoSabadell BS
17/03/2017	Reinhard Faessler	Max Planck Institute of Biochemistry, Munich, Germany	Identification of a molecular switch that controls cell migration speed	
24/03/2017	Ioannis Aifantis	NYU School of Medicine, US	Missing Lnkns: The impact of the non-coding genome in acute leukemia	
31/03/2017	José Luis Sanz	Autonomous University of Madrid (UAM), Spain	I left my heart in the Jurassic: Dinomania today	Fundación BancoSabadell BS
APRIL				
07/04/2017	Jacob (Yaqub) Hanna	Weizmann Institute of Science, Rehovot, Israel	Molecular Mechanisms for Inducing, Maintaining and Resolving Distinct States of Pluripotency	
28/04/2017	Kari Alitalo	Institute of Biomedicine Biomedicum Helsinki, University of Helsinki, Finland	Therapeutic Potential of Vascular Growth Factors	
MAY				
05/05/2017	Vera Gorbunova	University of Rochester, New York, US	Mechanisms of longevity and cancer resistance in long-lived mammals	Fundación BancoSabadell BS
19/05/2017	Oscar Marín	New Hunt's House King's College, London, UK	Getting excited about inhibition: molecular and functional diversity of brain interneurons	Fundación BancoSabadell BS
JUNE				
16/06/2017	Guillermo Oliver	Feinberg Cardiovascular Research Institute, Northwestern University, Chicago, US	The lymphatic vasculature in the 21st century: developmental mechanisms and functional roles	



SEPTEMBER			
08/09/2017	Timothy Rebbeck	Dana Farber Cancer Institute and Harvard T.H.Dana Farber Cancer Institute and Harvard T.H. Chan School of Public Health, Boston, US	Cancer Prevention and Early Detection in the Precision Medicine Era
15/09/2017	David J. Kwiatkowski	Brigham and Women's Hospital, Dana-Farber/ Harvard Cancer Center, Boston, US	The TSC/mTOR pathway in germline disorders and cancer
29/09/2017	Hongtao Yu	Howard Hughes Medical Institute, UT Southwestern Medical Center, Dallas, US	Mitotic checkpoint regulators in chromosome segregation and insulin signaling
OCTOBER			
06/10/2017	Paola Scaffidi	The Francis Crick Institute, London, UK	Epigenetics and functional intratumor heterogeneity
20/10/2017	Peter Carmeliet	Vesalius Research Center, Leuven, Belgium	Angiogenesis revisited: role and (therapeutic) implications of endothelial metabolism
DECEMBER			
01/12/2017	Victor G. Corces	O. Wayne Rollins Research Center, Emory University, Atlanta, US	Transcription and the three-dimensional organization of the genome

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 45 ad-hoc seminars were organised by CNIO researchers in 2017.

DATE	SPEAKER	ORGANISATION	TITLE
JANUARY			
17/01/2017	Karin Verspoor	The University of Melbourne, Australia	Application of Literature Mining for Protein Function Prediction
19/01/2017	Georg Winter	CeMM, Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria	“-taming the beast- small molecule induced target protein degradation”
FEBRUARY			
08/02/2017	Erika Pastrana	Nature Communications, New York, US	Publishing in the Nature journals: an insider's view
09/02/2017	Mauricio Rojas	McGowan Institute for Regenerative Medicine University of Pittsburgh Medical Center University of Pittsburgh, US	Idiopathic pulmonary fibrosis as a disease of Aging
13/02/2017	Anna Bigas	Hospital del Mar Medical Research Institute ,IMIM, Barcelona, Spain	Notch and Wnt in normal and leukemic hematopoiesis
16/02/2017	Åsmund Flobak	Norwegian University of Science and Technology, The Cancer Clinic, St Olav's University Hospital, Trondheim, Norway	Rationalizing drug combination screening for improved clinical trials and precision oncology

16/02/2017	Jean J. Zhao	Dana-Farber Cancer Institute, Harvard Medical School, Boston, US	Precision Oncology: From mouse models to human therapy
16/02/2017	David Dominguez-Sola	The Tisch Cancer Institute & The Graduate School of Biological Sciences, Icahn School of Medicine at Mount Sinai, NY, US	FOXO1 and the unforeseen path to lymphomagenesis
21/02/2017	Luis Martí Bonmatí and Ángel Alberich	The university hospital and polyclinic La Fe, QUIBIM, Valencia, Spain	Introduction to personalized quantitative Imaging Dr. Bonmatí / Radiomics Dr. Alberich
24/02/2017	Manel Cascallo	VCN Biosciences, Barcelona, Spain	Oncolytic adenoviruses expressing hyaluronidase: from bench to patients
MARCH			
06/03/2017	Sara Priego Moreno	Institute of Cancer and Genomic Sciences, University of Birmingham, UK	Roles of Ubiquitin and SUMO during eukaryotic chromosome replication
13/03/2017	Pere Roca-Cusachs	Institute for Bioengineering of Catalonia IBE, Barcelona, Spain	Sensing matrix rigidity: transducing mechanical signals from integrins to the nucleus
27/03/2017	Scott Williams	University of North Carolina at Chapel Hill, US	Spindle orientation in stratified epithelial development, stem cells and cancer
30/03/2017	Ricardo Baptista	Gene and Cell Therapy Catapult, UK	Development of cost efficient platforms for the industrial manufacture of pluripotent stem cell-derived products for cell therapy: cell expansion is the starting point
APRIL			
06/04/2017	María Pilar Alcolea	Wellcome Trust-MRC Stem Cell Institute, University of Cambridge, UK	Oesophageal stem cell plasticity; relevance for tumour development
18/04/2017	Ana O’Loughlen	Queen Mary University of London, UK	The chromobox protein CBX7 as a master regulator of pluripotency and senescence
20/04/2017	Lars Fugger	University of Oxford, Weatherall Institute of Molecular Medicine, Oxford, U.K.	Functional genomics in autoimmune diseases
20/04/2017	Lisa Torp Jensen	Aarhus University, Clinical Immunology, Aarhus, Denmark	Steps towards a mouse model for celiac disease
24/04/2017	Antoni Celià-Terrassa	Princeton University, Princeton, US	Mechanisms regulating stem cell properties and immune interplay of tumor- and metastasis-initiating cells
MAY			
25/05/2017	Isabel Beerman	National Institute on Aging, Baltimore, US	Aging of the Hematopoietic Stem Cell Compartment
26/05/2017	Santiago Zelenay	Cancer Inflammation and Immunity Cancer Research UK Manchester Institute, The University of Manchester, UK	Manipulating inflammation to raise cancer immunogenicity
30/05/2017	Hana Algül	Technical University München, Germany	Insight into the role of non-receptor tyrosine phosphatases in pancreatic cancer

JUNE			
01/06/2017	Miguel A. Tam	BioLegend, San Francisco, US	Versatile tools for Magnetic Cell Separation & Antigen-Specific T-Cell (Tetramer) Research
06/06/2017	Víctor Pérez García	Mathematical Oncology Laboratory, University of Castilla La Mancha, Ciudad Real, Spain	Mathematics against cancer: Examples in Neuro-Oncology
15/06/2017	Oritz Uziel	Laboratory for Telomeres and Telomerase Research, The Felsenstein Medical Research Center, Rabin Medical Center and Tel Aviv University, Israel	The shuttle of cancer cells derived exosomal hTERT mRNA induces phenotypic changes in the recipient fibroblast cells
JULY			
10/07/2017	Ana I. Vazquez	Michigan State University, US	Beyond genomics, Multi-layer omics data from cancer for outcome predictions
12/07/2017	Wolfgang Weninger	Centenary Institute, Camperdown. Australia	Function and development of perivascular macrophages
14/07/2017	Bruno Conti	The Scripps Research Institute, La Jolla, US	Core Body Temperature and Aging
24/07/2017	Raul Rabadan	Columbia University College of Physicians and Surgeons, New York, US	Heterogeneity and Evolution of Glioblastomas
28/07/2017	Anabel Rojas González	Cabimer, Andalusian Center for Molecular Biology and Regenerative Medicine, Sevilla, Spain	GATA transcription factors in pancreas development and disease
31/07/2017	Antonio García Herreros	IMIM Parc de Salut Mar, Barcelona, Spain	Snail, epithelial to mesenchymal transition and fibroblast activation
SEPTEMBER			
07/09/2017	Axel Behrens	The Francis Crick Institute, London, UK	Unraveling cancer cell heterogeneity
15/09/2017	Jack Welch	Center for Global Health, US National Cancer Institute, Bethesda, US	NCI Global Health approaches and trending topics in cancer
20/09/2017	Slyvia Knapp	CeMM, Viena, Austria	Regulatory role of innate immunity in homeostasis and disease
OCTOBER			
03/10/2017	David Gallego Ortega and Fatima Valdes Mora	Kinghorn Cancer Centre at the Garvan Institute of Medical Research in Sydney, Australia	Understanding the link between cancer and immune system using massively-parallel single-cell RNAseq technologies and “Histone Variants, Epigenetics and Cancer”
05/10/2017	Luis Arnes	Columbia University Medical Center, New York, US	Positionally conserved lncRNAs are necessary for the specification of pancreatic lineages in development and disease
16/10/2017	Gaetano Gargiulo	Max-Delbrück Center for Molecular Medicine (MDC), Berlin, Germany	Polycomb Control of Cell Identity and Tumor Suppression: Implications for Therapy
20/10/2017	Mario Pende	The Institut Necker-Enfants Malades (INEM), Paris, France	Growth control by mTOR/S6K and Hippo/YAP master regulators in Tuberous Sclerosis
NOVEMBER			
07/11/2017	Rui Benedito	CNIC, Madrid, Spain	Using multispectral and combinatorial genetic mosaics to understand angiogenesis in development and disease

24/11/2017	Stefano Stella	The Novo Nordisk Foundation Center for Protein Research University of Copenhagen, Denmark	CRISPR-Cpf1, the new blade for genome manipulation
30/11/2017	Marta Shahbazi	University of Cambridge, UK	The making of an epithelium: a stem cell perspective
DECEMBER			
12/12/2017	Silvestre Vicent Cambra	CIMA - University of Navarra, Spain	Killing two birds with one stone: integrating gene-expression approaches to identify KRAS oncogene vulnerabilities across tumors
13/12/2017	Carson Thoreen	Yale University School of Medicine, New Haven, US	Translational control of cell growth by the mTOR signaling pathway
18/12/2017	Isidro Cortes Ciriano	Biomedical Informatics, Harvard Medical School, US	Comprehensive analysis of chromothripsis in 2,658 human cancers using whole-genome sequencing
19/12/2017	Alba Gutiérrez Sacristán	Universitat Pompeu Fabra, IMIM (Hospital del Mar Medical Research Institute), Barcelona, Spain	A bioinformatics approach to the study of comorbidity
CNIO-WOMEN IN SCIENCE (WISE) SEMINARS			
10/01/2017	María Angeles Durán	Sociologists. Ad Honorem Professor, Center for Human and Social Sciences (CSIC)	Medicine, health organization and care system. The changing boundaries
31/01/2017	Belén Yuste and Sonnia L. Rivas-Caballero	Rocaviva Eventos, Madrid, Spain	Marie Skłodowska-Curie: Ella Misma
28/02/2017	Teresa Jurado and Mariano Nieto Navarro	PPIINA (Plataforma por Permisos Iguales e Intransferibles de Nacimiento y Adopción), Madrid, Spain	How the current design of parental leaves is hampering the professional development of women. The ‘PLENTy of rights’ proposal
07/03/2017	María Ruiz and Clara Sanchis	María Ruiz stage director -Clara Sanchis actress	“Una habitación propia”
25/04/2017	Ana Botella	Política y ex-alcaldesa de Madrid/ Politician and ex-Mayor of Madrid	Experiencias de una mujer perteneciente a la generación de españolas que nacemos cuando todavía no existe una igualdad jurídica entre mujeres y hombres
23/05/2017	Natalia Flores Sanz	Ex-jugadora Selección Española Fútbol Sala/Ex player of Spanish National indoor football team. Directora programa Mujer y Deporte/Director of Woman and Sport programme del Consejo Superior de Deporte	Situación del deporte femenino en España. Experiencia personal
19/09/2017	Katharina Miller	Founder Partner 3CCompliance, Madrid, Spain	Leave your comfort zone and be disruptive!
17/10/2017	Ana Requena	Journalist and columnist at “El Diario”	Micromachismos, el machismo cotidiano que condiciona la vida de las mujeres
12/12/2017	Margarita de Cos	Head Major Donors Relations WWF Spain and CEO of RAINSOFT SL, Madrid, Spain	El futuro de la mujer en nuestras manos

SCIENTIFIC OUTREACH EVENTS

WORLD CANCER RESEARCH DAY: ‘PRESENT AND FUTURE OF CANCER RESEARCH’
ENCUENTRO EXCLUSIVO CON EL NOBEL DR HARALD ZUR HAUSEN
25 SEPTEMBER 2017

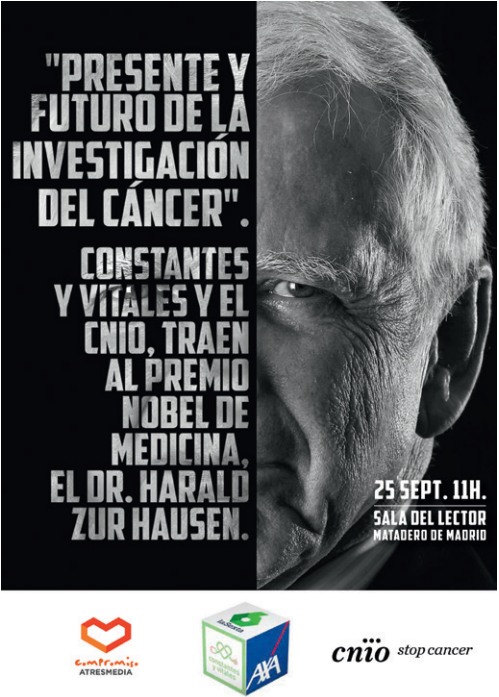
To honour World Cancer Research Day, we organised, together with AXA Foundation and *Constantes y Vitales*, the event entitled ‘Present and Future of Cancer Research’; our renowned keynote speaker was Prof. Harald zur Hausen, Nobel Prize winner in Medicine in 2008. Following the talk (Perspectives for Prevention of Cancers and Chronic Diseases), Prof. Harald zur Hausen joined a panel discussion on cancer research that also included: Maria A. Blasco, CNIO Director, Ángela Nieto, from the Neuroscience Institute of Alicante, and Pilar Garrido, Chief of Section of the Medical Oncology service at the *Ramón y Cajal* Hospital. The panel was moderated by *laSexta* journalist, Mamen Mendizábal.

ORGANISERS

- *Constantes y Vitales*
- Spanish National Cancer Research Centre (CNIO)
- *Fundación AXA*

SPEAKERS

- Dr. Harald Zur Hausen
- Dr. Maria A. Blasco
- Dr. Ángela Nieto
- Dr. Pilar Garrido



RESEARCHERS' NIGHT
29 SEPTEMBER 2017

This year, the CNIO participated in Researchers’ Night; an activity aimed at bringing researchers closer to the general public and concerned families in order to give them the opportunity to learn more about what researchers do for society. Each year, more than 300 European cities participate in parallel in what is ultimately a great night for science. During the activities – promoted by the European Commission and coordinated by the Madrid Regional Government and the madri+d Foundation – a total of 240 people came to the Spanish National Cancer Research Centre (CNIO) to attend Researchers’ Night (September 29, 2017) to learn about cancer research. The activities were entirely organised and held thanks to the voluntary efforts of 60 researchers. The guests were provided with the opportunity to meet researchers in an interactive and entertaining way, including welcome talks and short talks, hands-on experiments, view of a virtual tour through the facilities via a video project recorded by scientists from CNIO ‘CNIO for Kids’, and a speed dating session with the researchers.



OPEN DOORS DAY: INVESTIGATING TO DISARM CANCER
6-19 NOVEMBER, 2017

The CNIO also dedicates considerable efforts to bringing science and society closer together; one of these endeavours is its collaboration with the madri+d research network for the organisation of the Madrid Science Week (XVII *Semana de la Ciencia*, 6-19 November 2017). In 2017, 94 people participated in the guided visit to the Centre’s facilities.



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 2017, more than 490 people participated in such guided visits; most of them were ESO and *Bachillerato* student groups, but also professionals in the health sector.

ADMINISTRATION

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Minister of Economy, Industry and Competitiveness
Ministro de Economía, Industria y Competitividad

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Secretariat of State for Research, Development and Innovation of the Spanish Ministry of Economy, Industry and Competitiveness
Secretaria de Estado de Investigación, Desarrollo e Innovación del Ministerio de Economía, Industria y Competitividad

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

→ Appointed Members

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

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Director of the Department of National Affairs of the Cabinet of the Presidency of the Government
Directora del Departamento de Asuntos Nacionales del Gabinete de la Presidencia del Gobierno

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

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

- **Luis Ángel León Mateos**
Director of Health Research Planning and Promotion of the Galician Health Service (*SERGAS*)
Director del Área de Planificación y Promoción de la Investigación Sanitaria del Servicio Gallego de Salud de la Xunta de Galicia (SERGAS)

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

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

→ Elected Members

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

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

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

→ Secretary

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

→ Legal Advisor

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

* In accordance with the Spanish Transparency Legislation (Spanish Royal Decree 451/2012, of March 5), the following information is hereby provided:

- At the close of the financial year, the accumulated remuneration received by the Top Management of the Foundation - the CNIO’s Director plus the Managing Director - has amounted to a total of 268,287 Euros. This amount was received as base salary, seniority, as well as position salary supplements.
- Members of the CNIO Board of Trustees are not remunerated.

SCIENTIFIC ADVISORY BOARD

- **Mariann Bienz, PhD, FRS, FMedSci (Chair)**
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Medical Research Council Laboratory of Molecular Biology
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Biomedicum, University of Helsinki
Helsinki, Finland
- **Genevieve Almouzni, PhD**
Director, *Institut Curie* Research Centre
Head of Nuclear Dynamics & Genome Plasticity Unit
Institut Curie, Paris, France
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New Haven, USA

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Oregon Health & Science University
Portland, USA
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Research Professor of Microbiology at the University of Washington
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Memorial Sloan-Kettering Cancer Center
New York, USA
- **Ángela Nieto, PhD**
Full Professor and Head of the Developmental Neurobiology Unit
Neuroscience Institute of Alicante (CSIC-UMH)
Alicante, Spain

- **Josep Tabernero, MD PhD**
Director, Vall d’Hebron Institute of Oncology (VHIO)
Head, Medical Oncology Department of Vall d’Hebron University Hospital
Barcelona, Spain
- **Alfred Wittinghofer, PhD**
Emeritus Group Leader
Department of Structural Biology
Max Planck Institute for Molecular Physiology (MPI)
Dortmund, Germany
- **Ada E. Yonath, PhD**
Director, the Helen and Milton A. Kimmelman Center for Biomolecular Structure and Assembly
Martin S. and Helen Kimmel Professor of Structural Biology
Weizmann Institute of Science
Rehovot, Israel

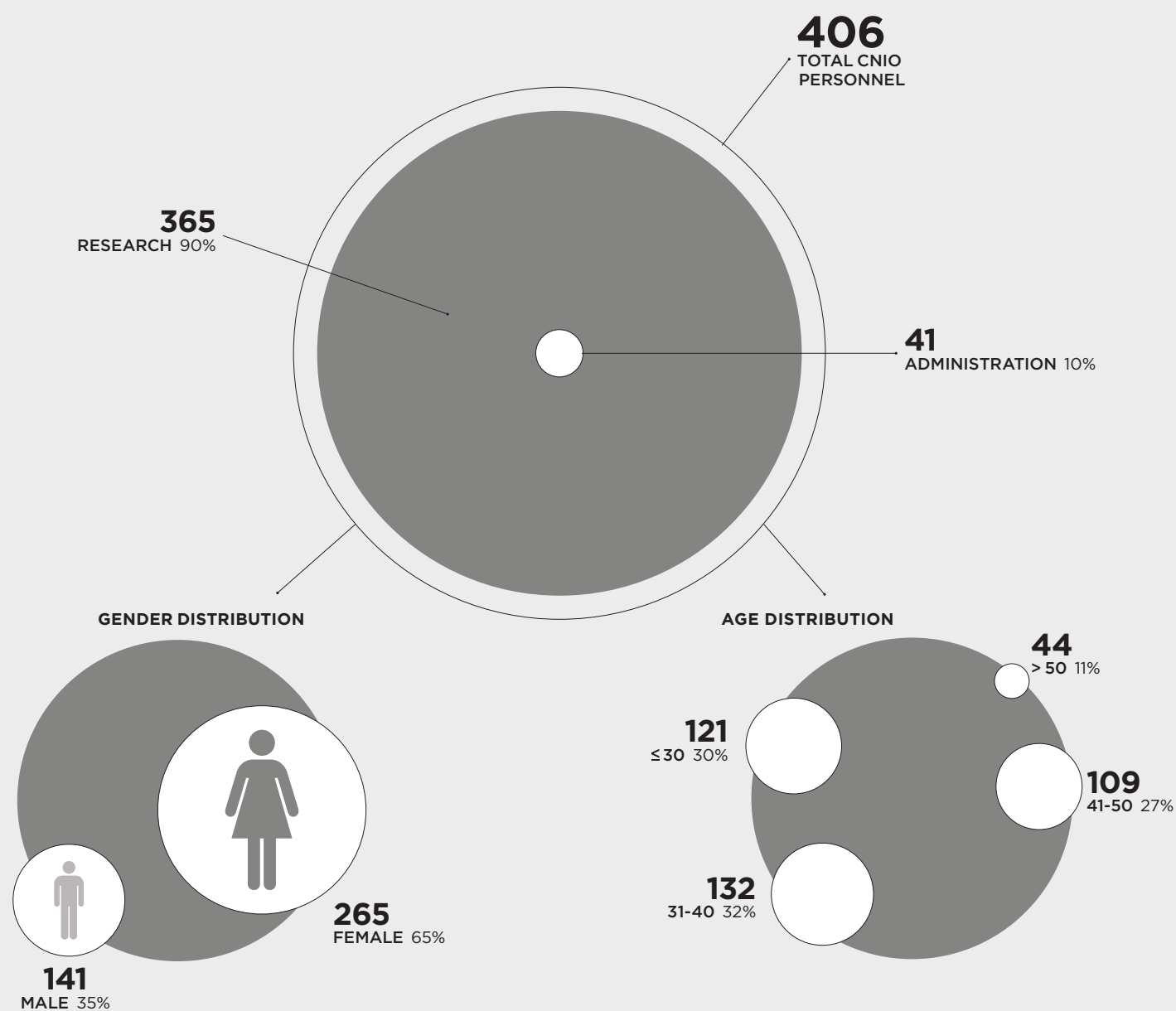
MANAGEMENT

DIRECTOR	Blasco, Maria A.		
	SECRETARIATE	Alcamí, María Jesús	
VICE-DIRECTOR	Fernández-Capetillo, Óscar		
DIRECTOR'S OFFICE	Peláez, Fernando Director		
COMMUNICATION	Noriega, Nuria Head	De Martos, Cristina (Communications Officer)	Camacho, Pablo (Science Outreach Officer) (since May)
INNOVATION	Fernández-Capetillo, Óscar Director		
	TECHNOLOGY TRANSFER & VALORISATION OFFICE	Sanz, Anabel Director	Marín, M. Cruz (Technology Transfer Manager) Manzano, Rocío*
INTERNATIONAL AFFAIRS	Pola, Carolina Director		
SCIENTIFIC MANAGEMENT	Barthelemy, Isabel Director		
	PROJECTS & CONSORTIA	Liébanes, M. Dolores Head Ares, Raquel	Merino, Ana Vergés, Leyre
	EDUCATION & TRAINING PROGRAMMES	Molina, Juan Ramón (until June)	Del Codo, Almudena*
	SCIENTIFIC EVENTS	Moro, Mercedes Head	
	SCIENTIFIC PUBLISHING	Cerdá, Sonia	
	LIBRARY & ARCHIVES	López, Victoria	
	SECRETARIATE (COMMUNICATION, INNOVATION, SCIENTIFIC MANAGEMENT)	Rodríguez, M. Carmen	




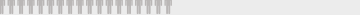
MANAGING DIRECTOR	Arroyo, Juan		
	SECRETARIATE	Ámez, María del Mar	
MANAGING DIRECTOR'S OFFICE	Fernández-Vera, José Ignacio Director (since October)		
SAP	Ferrer, Alfonso Head		
FINANCE & ADMINISTRATION	Fontaneda, Manuela Director		
	PURCHASING	Álamo, Pedro Head Baviano, Marta García-Andrade, Javier (until April)	Novillo, Angélica De Luna, Almudena* Luongo, Victoria Eloina*
	HUMAN RESOURCES	Pérez, José Lorenzo Head Bardají, Paz	Carbonel, David Marco de la Cal, Claudia (since March)
	ECONOMIC MANAGEMENT	Salido, M. Isabel Head Galindo, José Antonio García, Juan J.	Rodríguez, M. José Fernández, Rut*
	AUDIT	García-Risco, Silvia Head Moreno, Ana María Head	Hernando, M. Elena Doyagüez, Laura*
INFRASTRUCTURE MANAGEMENT	de Dios, Luis Javier Director		
	MAINTENANCE	Vacant	
	PREVENTION & BIOSECURITY	Cespón, Constantino Head	Bertol, Narciso
	INFORMATION TECHNOLOGIES	Fernández, José Luis Head	de Miguel, Marcos
EXTRAMURAL CLINICAL RESEARCH	López, Antonio Director (since December)		

* Plan de Empleo Joven (Youth Employment Plan) (until December)

CNIO PERSONNEL 2017







GENDER DISTRIBUTION IN SENIOR ACADEMIC AND MANAGEMENT POSITIONS

GROUP LEADERS, HEADS OF CLINICAL RESEARCH UNIT/SECTION	FEMALE MALE	33% 67%	10 20	
HEADS OF UNIT/BIOBANK	FEMALE MALE	56% 44%	10 8	
SCIENTIFIC DIRECTION: DIRECTORS, HEADS OF AREA	FEMALE MALE	46% 54%	6 7	
MANAGEMENT: DIRECTORS, HEADS OF AREA	FEMALE MALE	31% 69%	4 9	

SCIENTIFIC PERSONNEL 2017

TOTAL SCIENTIFIC PERSONNEL **365**

DISTRIBUTION BY PROGRAMMES

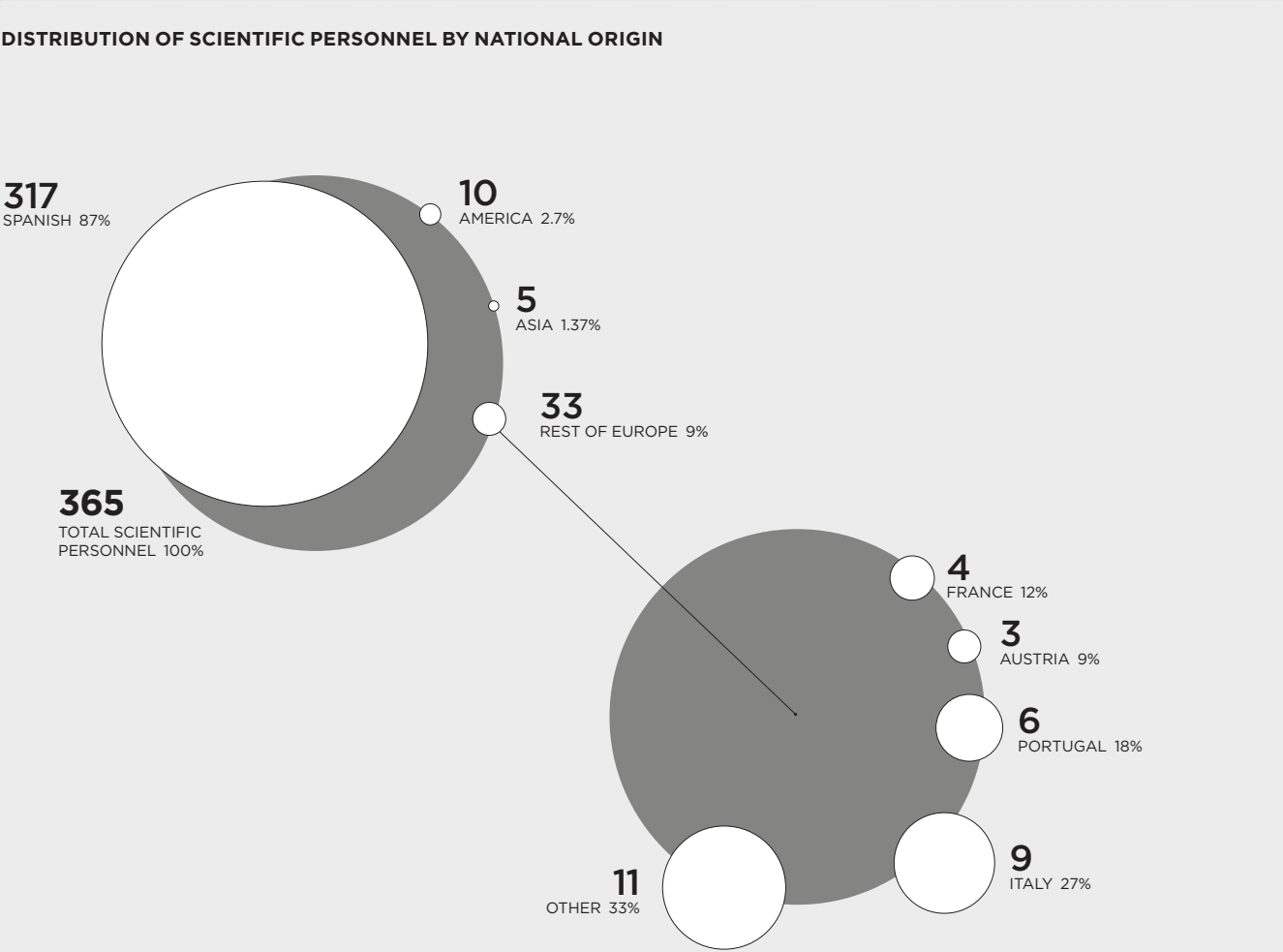
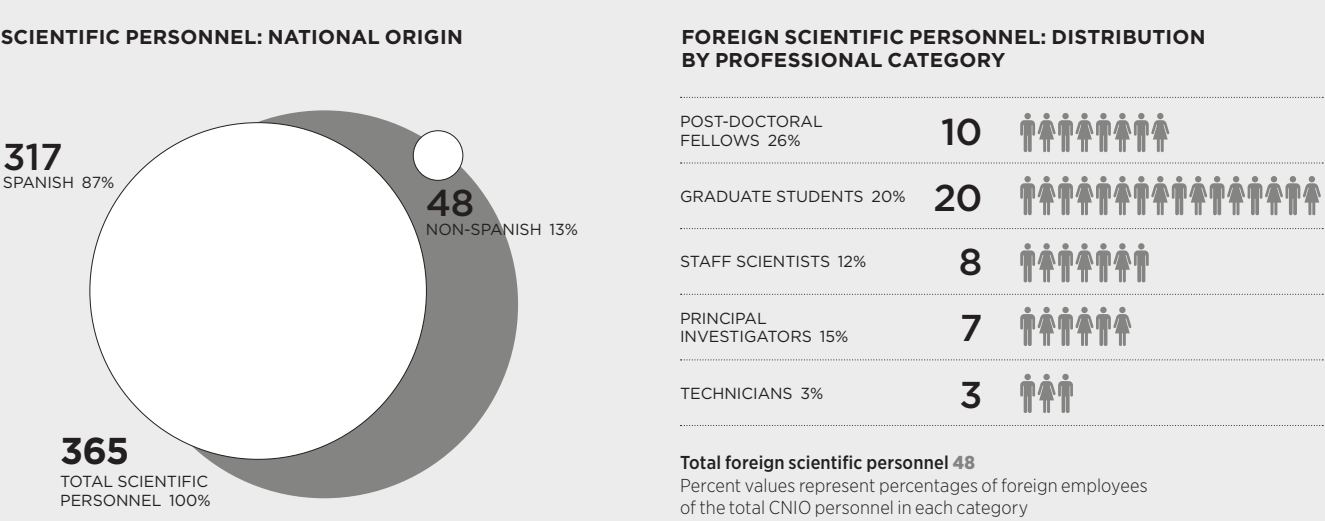
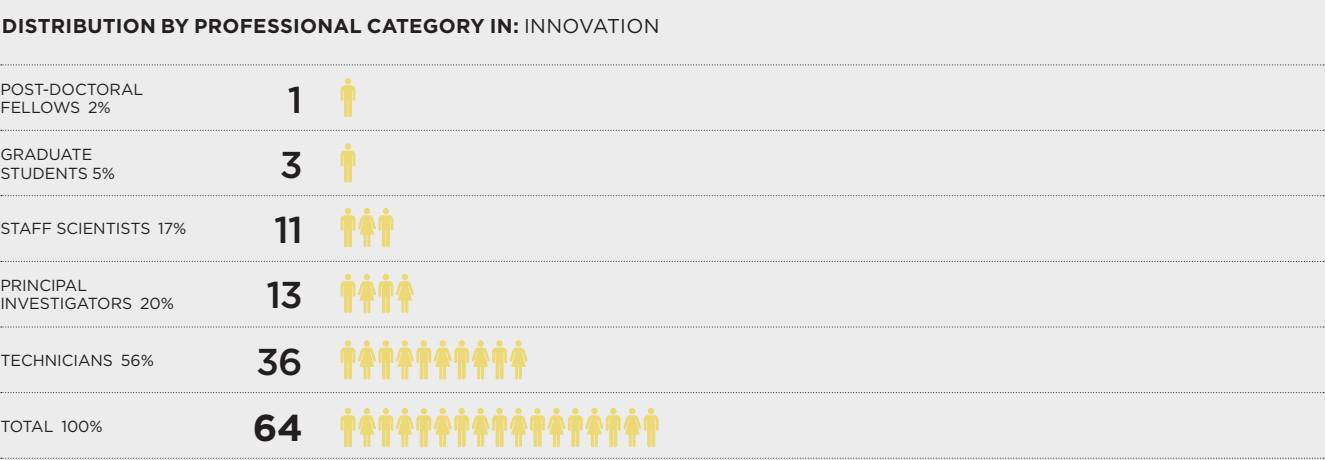
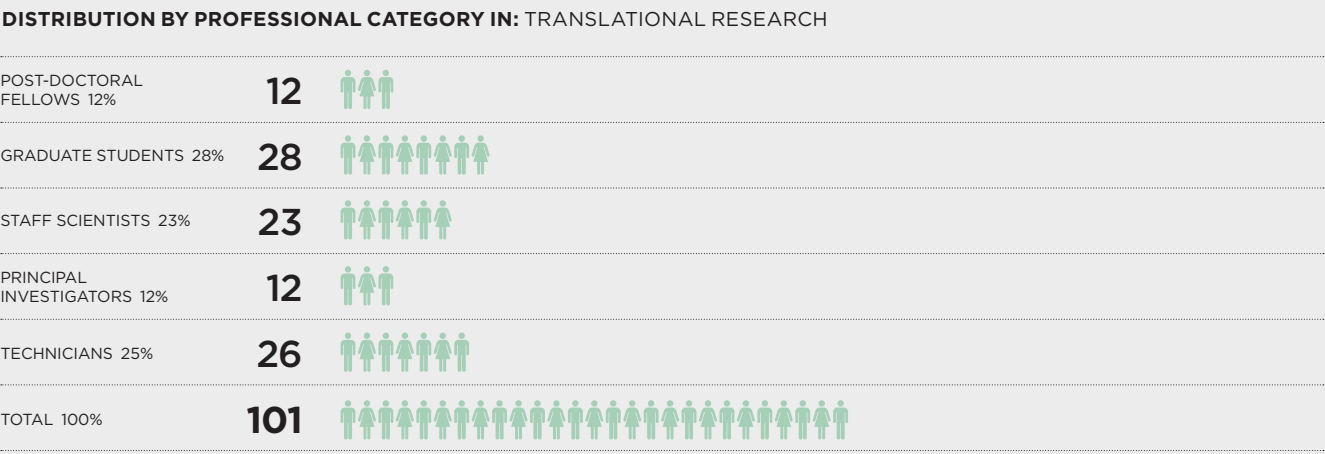
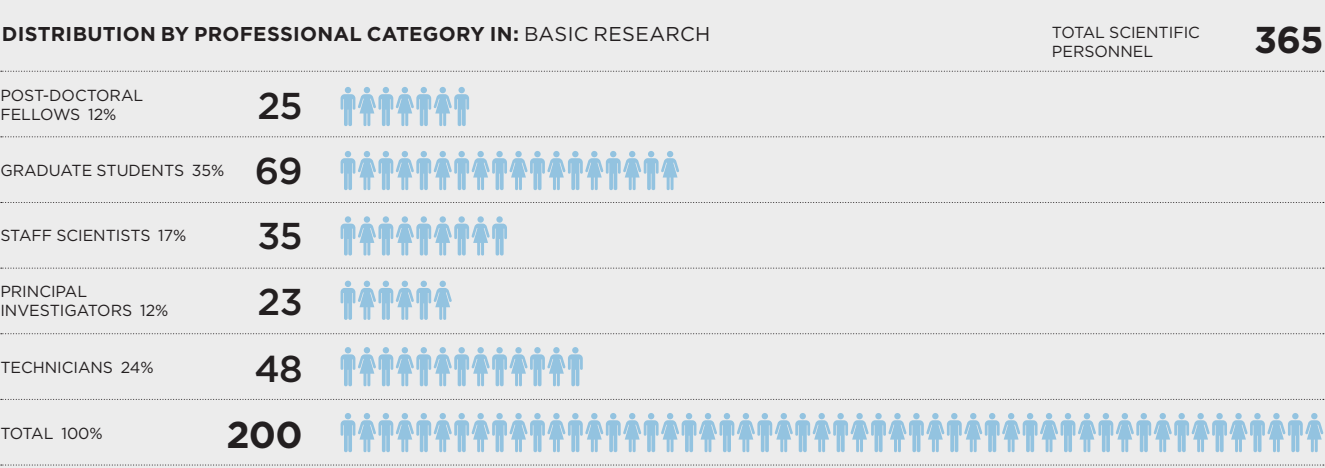
STRUCTURAL BIOLOGY 11%	41	
BIOTECHNOLOGY 10%	35	
CANCER CELL BIOLOGY 11%	40	
HUMAN CANCER GENETICS 10%	37	
CLINICAL RESEARCH 18%	64	
MOLECULAR ONCOLOGY 33%	119	
EXPERIMENTAL THERAPEUTICS 8%	29	

DISTRIBUTION BY PROFESSIONAL CATEGORY

POST-DOCTORAL FELLOWS 11%	38	
GRADUATE STUDENTS 27%	100	
STAFF SCIENTISTS 19%	69	
PRINCIPAL INVESTIGATORS 13%	48	
TECHNICIANS 30%	110	

GENDER DISTRIBUTION BY PROFESSIONAL CATEGORY


POST-DOCTORAL FELLOWS	FEMALE	58%	22	
	MALE	42%	16	
GRADUATE STUDENTS	FEMALE	67%	67	
	MALE	33%	33	
STAFF SCIENTISTS	FEMALE	74%	51	
	MALE	26%	18	
PRINCIPAL INVESTIGATORS	FEMALE	38%	18	
	MALE	62%	30	
TECHNICIANS	FEMALE	74%	81	
	MALE	26%	29	
TOTAL SCIENTIFIC PERSONNEL	FEMALE		239	
	MALE		126	



PRIVATE SPONSORS

“We take this opportunity to express our thanks and appreciation to all our sponsors and donors for the generous support that we received from them in 2017. 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 accredited 'Severo Ochoa' Centres of Excellence. In 2017, a new doctoral Fellowship Programme of "la Caixa" Foundation, named INPhINIT, was established with the aim of recruiting outstanding international students; in addition, a new call was launched to carry out a doctorate at Spanish universities and research centres. The *Fundación "la Caixa"* also helps finance our most prominent international conferences, the CNIO-"la Caixa" Foundation Frontiers Meetings.

 *Fundación CRIS* is dedicated to the promotion and development of research with the aim of eliminating the serious health threat of cancer. *Fundación CRIS* generously supports 3 research groups at the CNIO: the Prostate Cancer Clinical Research Unit (CRU), headed by David Olmos; the Breast Cancer CRU, headed by Miguel Quintela; and the H120-CNIO Haematological Malignancies CRU, led by Joaquín Martínez-López. These Groups focus on the translation of advances in cancer research into improvements in patient care.

 The *Fundación Marcelino Botín* and the *Banco Santander* are committed to supporting scientific research and knowledge transfer from academia to the market through science programmes; this transfer is regarded as one of the main driving forces for Spain's economic and social development. These 2 well-recognised organisations collaborate with the CNIO in this regard by supporting the research groups led by Manuel Serrano, Maria A. Blasco and Óscar Fernández-Capetillo.

 *The Fundación Banco Santander* funds the Banco Santander Foundation – CNIO Fellowships for Young Researchers. These fellowships have the aim to support highly talented and motivated young scientists who wish to pursue their postdoctoral training at the CNIO. Additionally, thanks to the support of the *Fundación Banco Santander*, a group of 3 young researchers received training on managerial and entrepreneurial skills, in collaboration with the IE Business School.

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

 The *Fundación Jesús Serra-Catalana Occidente* continues to fund the Visiting Scientists Programme that was established to support prestigious international professors for short stays at the CNIO. The recipients of the *Jesús Serra* Foundation's Visiting Scientist Award in 2017 were Raul Rabadan, Professor of Systems Biology and Director of the Columbia University Center for Topology of Cancer Evolution and Heterogeneity (USA), and Professor Wolfgang Weninger, Head of the Department of Dermatology at the Royal Prince Alfred Hospital of Sydney (Australia).

 The *AXA Research Fund (ARF)* – a global initiative of scientific philanthropy run by the insurance group AXA – awarded an AXA-CNIO Endowed Permanent Chair position in Molecular Oncology to Mariano Barbacid as part of its 2011 call.

OTHER SPONSORS

 Our activities are also supported by individual donations – citizens who wish to contribute personally to the battle against cancer – as well as via external fundraising from local associations that are equally dedicated to the battle against cancer. During 2017, our research activities and seminars were supported by: *Fundación Juegaterapia*, *Asociación para la Investigación y Formación Neoplásica (ASIFEN)*, *Fundación Investigación Biomédica Hospital Universitario 12 de Octubre*, *Instrumentos Testo*, *Bristol Myers Squibb*, *Compañía Logística de Hidrocarburos*, *Fundación PFIZER*, *Fressia Group*, *ASISA Vida*, *Fundación Banco Sabadell*, *the U.S. Embassy*, and *the British Embassy*.

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less CANCER
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Lastly, we extend our heartfelt thanks to all 'CNIO Friends' donors, sponsors and benefactors who, thanks to their generous donations to support cancer research at the CNIO, have ensured the continuation of our research endeavours throughout 2017.

CNIO Friends

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CNIO FRIENDS

This year, once again, the ‘CNIO Friends’ philanthropic initiative was a source of immense gratification for our Centre. By December 2017, we had over 950 Friends; their solidarity, support and efforts push us even further, in as far as possible, to advance with our crucial task of advancing cancer research.

Thanks to all of our Friends, we were able to launch two new contracts in 2017 with the aim of attracting research talent that will enable us to commence and/or continue promising new lines of research to help us battle against cancer. One of the beneficiaries is the researcher, Carolina Maestre, from the Cell Division and Cancer Group, who will research the mechanisms of cell division in tumours. The other contract went to the scientist Sebastian Thompson, from the Growth Factors, Nutrients and Cancer Group, who will carry out research in the field of nanotechnology and its application for the treatment of the disease. In Maestre’s own words, “I would like to thank all those who have collaborated in this initiative that is enabling me to continue my scientific career in Spain”. Thompson spotlights the donors: “From the donor’s point of view, seeing that the money is being used for the desired purpose is extremely enriching and necessary.” This year, we have also maintained the collaboration with *Juegaterapia* Foundation to foster research in childhood cancer. This alliance has enabled the researcher Irene Felipe, from the Epithelial Carcinogenesis Group, to continue her research for a second year. Felipe’s work focuses on neuroblastoma, one of the most common infancy tumours in the early years of life and the second most common tumour in children after brain tumours.

In February 2017, within the framework of the ‘CNIO Friends’ initiative, the CNIO published the book *Excelentes* featuring portraits of the Centre’s most influential visitors in recent years. The visual artist Amparo Garrido took care of the artistic representations and the texts were written by the journalist Mónica G. Salomone. Its pages invite the non-specialised public into the world of scientists through some of the ideas that move the world. Ideas from molecular biologists, such as the Nobel Prize-winners, Elizabeth Blackburn, Robert Huber and Paul Nurse; physicists, such as Ignacio Cirac, who is featured on the book cover and entices the readers to open up its pages; the palaeontologist, Juan Luis Arsuaga... as well as experts from other fields, such as the alpinist Edurne Pasaban, and the jurist María Teresa Fernández de la Vega. The book launch presentation was held at *El Corte Inglés* in Preciados with Mago More as the master of ceremonies, and the participation of one of Spain’s most popular humourists, José Mota. The book can be purchased at *El Corte Inglés*, VIPS stores and through the CNIO website; all generated funds go to ‘CNIO Friends’ initiative for cancer research.

“With every little helpful bit, we can achieve a lot!”

Throughout the year, we received many proposals for charity activities from anonymous citizens, town halls, etc. to support our work. This led to, for example, a thousand people turning the streets of *Rojales* in Alicante into a huge human wave to support cancer research during the 4th Women’s Charity Walk organised by the Town Hall. The message was also clear in *Las Pedroñeras*, Cuenca, whose Town Hall held the 2nd Cancer Charity Race, the profits of which also went to CNIO Friends. Many other initiatives were organised across the length and breadth of the country.

For yet another year, companies have also formed part of our Friends community. In 2017, the CLH Group, a leading company for the transportation and storage of oil products in the Spanish market, renewed its commitment and support to cancer research. *Instrumentos Testo, S.A.* a company specialised in portable measuring tools, also joined the charity initiative through the challenge of running 25,000 Km in favour of the CNIO. These accomplishments demonstrate that there are no challenges that cannot be overcome when it comes to research.

CNIO Friends ended the year with an optimistic outlook towards the future, and gave expression to this positivity with the help of two of Spain’s most popular humourists. José Mota and Mago More both allowed their image to be used in a sketch – whose release coincided with Giving Tuesday, the international day of donations – to encourage society to support and encourage our work. In the sketch, they used all of their wit to shout out the message loud and clear: “if many of us provide a bit, we will all do a lot!”

Another form of philanthropy that is progressively increasing in Spain has to do with the donation of legacies and inheritances in favour of research institutions such as the CNIO. In fact, during 2017 we were notified of the execution of 4 new legacies (one more than the previous year), which included deposits, accounts and other monetary assets, as well as real estate. In addition, we have received communications from several people informing us of their intention to include the CNIO in their will. We hope that this trend of favouring the research institutions of this country, and the CNIO in particular, through testamentary dispositions will increase, thereby matching what is already common practice in other countries. In summary, our Friends are an essential driving force in our efforts to find new solutions to fight cancer. Our deepest thanks to them all!

‘CNIO FRIENDS’ POSTDOCTORAL CONTRACTS



The new recipients of the ‘CNIO Friends’ postdoctoral contracts are Carolina Maestre and Sebastian Thompson, from the Cell Division and Cancer Group and Growth Factors, Nutrients and Cancer Group, respectively.

Maestre is an expert on the progression of mitosis – cell division – as a target for the development of new cancer therapeutic drugs. “We have identified a molecule that regulates mitosis and which is involved in the survival of tumour cells during their division; we believe that if we can manage to inhibit its function, we could attack tumour proliferation.”

Thompson’s work focuses on nanoparticles as drug carriers, particularly on their increased chance of reaching tumour cells in the organism. “Thanks to CNIO Friends, we will continue to work on finding the best way of getting as many of these nanoparticles as possible to the tumours; this could pave the way for the application of the latest advances in nanotechnology to the treatment of cancers.”

‘EXCELENTES’: A BOOK OF PORTRAITS ON IDEAS THAT DRIVE THE WORLD



In February 2017, the CNIO published the book *Excelentes*, of which the sales revenues all go to the ‘CNIO Friends’ philanthropic initiative for cancer research. The launch of the book was wonderfully presented by Mago More, the master of ceremonies. The special event was even more momentous thanks

to the participation of CNIO’s director Maria Blasco, the visual artist Amparo Garrido, the science journalist and writer of the protagonists’ stories Monica G. Salomone, and the popular Spanish humourist José Mota, who put the icing on the cake and used his ingenuity and imagination to spread the message.

CNIO OPENS ITS DOORS TO ‘CNIO FRIENDS’



One of the most exiting events hosted in 2017 was the ‘Jornada Amigos del CNIO’ that took place in June and welcomed our donors/CNIO Friends in order to bring them closer to CNIO’s science. Our donors spent an afternoon with us and once again demonstrated their great enthusiasm for being part of the important mission of conquering of cancer.

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

BENEFACTOR FRIENDS/SPONSOR FRIENDS

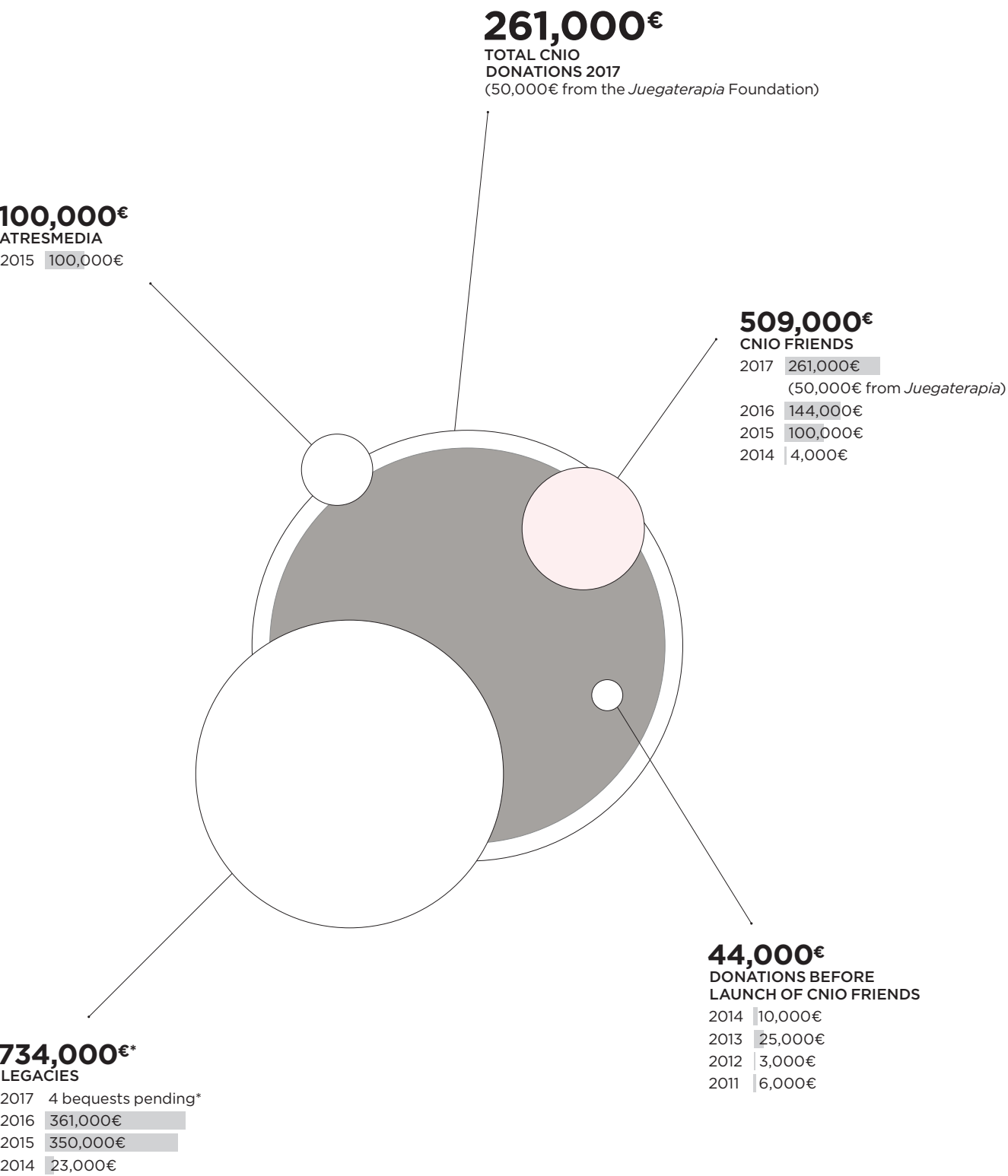
→ Benefactor Friends

· Alfonso Agüera Nieto Santa Ana-Cartagena, Murcia	· <i>IES “San Vicente” de San Vicente del Raspeig</i> San Vicente del Raspeig, Alicante
· Alvaro Gil Conejo Mijas, Málaga	· International Road Technology Consulting S.L Parla, Madrid
· Andrés Sánchez Arranz Madrid, Madrid	· Jaime Escobar Feroselle Cadalso de los Vidrios, Madrid
· <i>Asociación de Afectados de Cáncer-AFECC</i> Llerena, Badajoz	· Jesús Miguel Iglesias Retuerto Valladolid, Valladolid
· <i>Breatel, S.L.U.</i> Madrid, Madrid	· José Limiñana Valero Alicante, Alicante
· <i>Deltaclon, S.L.</i> Madrid, Madrid	· José Luis Catalá López Las Palmas de Gran Canaria, Las Palmas
· Dolors Bosch Güell Olot, Girona	· Juan Félix Ortigosa Córdoba Granollers, Barcelona
· Encarnación Fernández Pérez Madrid, Madrid	· Luis David Sanz Navarro Madrid, Madrid
· Enrique García Díaz Tomares, Sevilla	· Mercedes Cáceres Alonso Madrid, Madrid
· Esther Valdivia Carrión Madrid, Madrid	· María Dolores Díaz Almagro Sevilla, Sevilla
· Fernando Pascual Carreras Madrid, Madrid	· María Jesús Amores Molero Jábaga, Cuenca
· Ferrán Nácher Carull Xativa, Valencia	· María Rodríguez López Celada de los Calderones, Cantabria
· Francisco Javier Gállego Franco Barbastro, Huesca	· Nemesio Carro Carro León, León
· Gema Rubio González Madrid, Madrid	

→ Sponsor Friends

· <i>Asisa Vida Seguros S.A.U.</i> Madrid, Madrid	· Freesia Group Salou, Tarragona
· <i>Asociación de Mujeres Alameda del Tormes por la Igualdad</i> El Barco de Ávila, Ávila	· <i>Fundación Juegaterapia</i> Madrid, Madrid
· <i>Colectivo de afectados “El árbol de la Vida”</i> Las Pedroñeras, Cuenca	· <i>Instrumentos Testo, S.A.</i> Cabrils, Barcelona
· <i>Compañía Logística de Hidrocarburos CLH, S.A.</i>	· María Josefa Azcona Peribañez Madrid, Madrid
	· <i>Petroplast</i> Logroño, La Rioja

DONATIONS TO THE CNIO



* In 2017, the CNIO was notified of 4 legacies; calculation of their value is yet to be determined.

CREATIVE TEAM

In order to pour the Annual Report into a more creative concept, the CNIO works closely with selected professionals in the artistic and creative sectors who ensure delivery of an end product that is attractive in more ways than one. We extend our thanks to the

creative team, the visual artist Amparo Garrido, and the graphic design studio underbau whose invaluable work created the images and design that illustrate this Annual Report.

AMPARO GARRIDO PHOTOGRAPHY



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

the photographic holdings of the Madrid regional authority, the Coca-Cola Foundation, the *Es Baluard* Museum of Modern and Contemporary Art in Palma de Mallorca, and the ‘Types and Trends on the Threshold of the 21st Century’ Alcobendas Collection, among many others. Amparo’s most recent solo exhibitions in Spain were shown at the *Sala Robayera de Miengo*, Cantabria 2017, *Galería Trinta*, Santiago de Compostela 2015, and the *Museo del Romanticismo*, Madrid 2012.

UNDERBAU DESIGN



Underbau is a design studio that emerged in 2008 from professional designers with 15 years of experience in the field of corporate design, publishing and advertising. From the very beginning, the studio has sought to maintain its primary focus on art and culture, working together with Spanish and international bodies such as the *Orquesta y Coro Nacionales de España*, *Instituto Cervantes* and *Museo Thyssen-Bornemisza*. Underbau’s total-design approach puts the emphasis on coherency. To achieve

that, the studio assumes full responsibility for the entire creative process, from the initial concept to the final product.

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