CLINICAL RESEARCH PROGRAMME

MIGUEL QUINTELA-FANDINO Acting Programme Director

The Clinical Research Programme (CRP) has two 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 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 CRP is composed of four Clinical Research Units and one supporting Unit. The Breast Cancer Clinical Research Unit has successfully completed the first kinase-based taxonomy of triple-negative breast cancer. The Prostate Cancer Clinical Research Unit, under David Olmos' supervision, has completed its prospective observational PROCURE study involving >1000 patients, whereby different predictive associations are being explored; two major manuscripts regarding the role of germline and somatic variants in response to antiandrogens or conventional chemotherapy have already been published thanks to this effort. 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 and novel treatment approaches, an exciting novel line of research based on the *ex vivo* expansion of natural killer cells is currently ongoing. Finally, the Molecular Diagnostics Unit, led by Luis Lombardía, has continued to provide support to hospitals in the diagnosis of haematological malignancies.

Several contracts with 'Big Pharma' were signed during 2018 in order to progress in the development of cancer immunotherapies (Lung Cancer Unit). The Prostate Cancer Unit was awarded with a Department of Defense Grant in 2018. These achievements highlight the relevance of the translational research activities conducted by the CRP during 2018; we hope to further enhance these activities through future alliances with tertiary hospitals and medical societies over the next few years.

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

BREAST CANCER JUNIOR CLINICAL RESEARCH UNIT

Miguel Quintela-Fandino
Junior Clinical Research Unit Head

Staff Scientists
María José Bueno, Silvana A. Mouron

Clinical Research Fellow
Juan V. Apala

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.
→ Breast cancer functional taxonomy: by using a systems biology approach, we are clustering the disease into subtypes defined by biologic features that constitute therapeutic targets.
→ Study of the mechanisms of resistance against targeted therapies.

"In 2018, the BCCRU completed the first study elaborating a kinase-based taxonomy of triple-negative breast cancer. This will enable therapeutic and biomarker-based precision-medicine initiatives."

Graduate Students
Elena Arconada (until November), Sara Fernández, José Luis Ruiz (since November)

Technicians
Verónica Jiménez, Manuel Muñoz

Titulado Superior (Advanced Degree)

RESEARCH HIGHLIGHTS

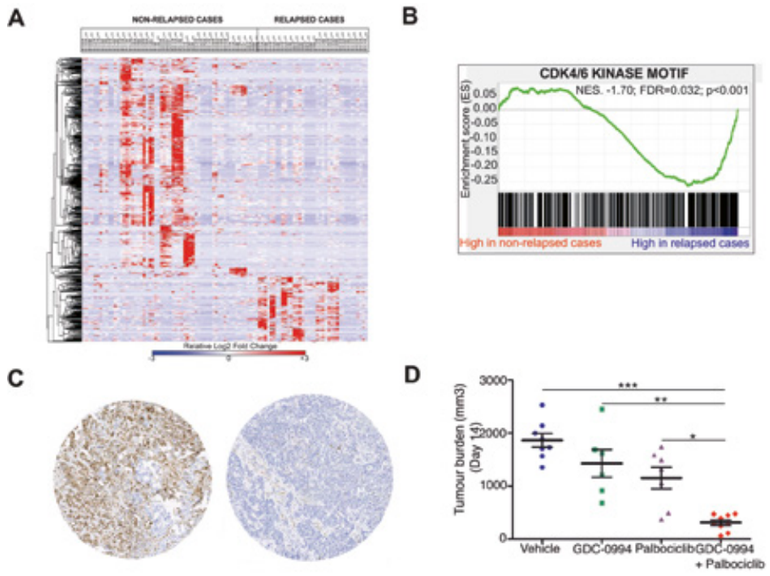

Figure (**A**) Phosphorylation profiles of whole tumours from TNBC with and without relapse were obtained by mass spectrometry. (**B**) Kinases with increased function accounting for such profiles were solved through an in-house algorithm ("Kinase-set enrichment analysis" or KSEAS"). (**C**) This information was then translated to immunohistochemistry; the left sample shows a patient with hyperactivated ERK, compared to a hypoactive one. (**D**) Combined treatment with agents targeting the 2 top-hits (CDK4/6 plus ERK inhibitors) in TNBC xenografts achieved a synergistic effect.

This year, we completed the first phosphoproteomic taxonomy of triple-negative breast cancer (TNBC), the most deadly subtype of this disease. Next-generation sequencing studies have failed in the task of finding simple biomarkers for complex phenotypic traits, such as response or resistance to therapeutic agents or disease course outside the context of penetrant oncogenic-addiction drivers. Rather, the TNBC phenotype traits are the result of multiple contributing low-penetrance mutations. We have found that different clusters of mutations collapse into discrete patterns of activation of the proteome in the form of protein phosphorylation, and that such patterns are driven by a small number of hyperactive/hypoactive kinases. Specifically, we found 6 kinases that, when all of them are "switched off", patients are long-term disease-free after >10 years. However, when 1 or more of those kinases are "on", the risk of relapse increases 10-fold. More importantly, all 6 kinases are actionable and we have found profound synergy in all 2-by-2 combinations in preclinical models.

On the clinical side of our activities, during 2018, we completed 2 clinical trials that were launched based on our research. Specifically, one of the trials explored the reversal of immune-tolerance induced by chronic hypoxia observed after prolonged exposure to antiangiogenics. A second trial explored the reversal of the metabolic switch of tumours experiencing vascular normalisation in response to antiangiogenics. Both trials implement targeted agents (a PD-L1 inhibitor or a mitochondrial inhibitor, respectively) directed against the 2 main regulatory nodes in each of the 2 major patterns of angiogenesis inhibitor escape identified during the period 2015-2017. ■

PUBLICATIONS

- Zagorac I, Fernandez-Gaitero S, Penning R, Post H, Bueno MJ, Mouron S, Manso L, Morente MM, Alonso S, Serrra V, Muñoz J, Gomez-Lopez G, Lopez-Acosta JF, Jimenez-Renard V, Gris-Oliver A, Al-Shahrour F, Piñeiro-Yañez E, Montoya Suarez JL, Apala JV, Moreno-Torres A, Colomer R, Dopazo A, Heck AJR, Altelaar M, Quintela-Fandino M (2018). In vivo phosphoproteomics reveals kinase activity profiles that predict treatment outcome in triple-negative breast cancer. *Nat Commun* 9, 3501-15.
- Alvarez-Fernandez M, Sanz-Flores M, Sanz-Castillo B, Salazar-Roa M, Partida D, Zapatero-Solana E, Ali HR, Manchado E, Lowe S, VanArsdale T, Shields D, Caldas C, Quintela-Fandino M, Malumbres M (2018). Therapeutic relevance of the PP2A-B55 inhibitory kinase MASTL/Greatwall in breast cancer. *Cell Death Differ* 25, 828-40.

PROSTATE CANCER JUNIOR CLINICAL RESEARCH UNIT

David Olmos
Junior Clinical Research Unit Head

Clinical Investigator
Elena Castro

Clinical Research Fellow
Rebeca Lozano

Post-doctoral Fellow
Isabel Aragón

Graduate Students
Ylenia Cendón, Lorena Magraner, Paz Nombela

Technician
Carles Moreno (since May)

Visiting Scientists
Teresa Garcés (*Instituto de Investigación Biomédica de Málaga*), Gala Grau (until March, *Instituto de Investigación Biomédica de Málaga*), Ana M. Gutiérrez-Pecharoman (*Universidad de Móstoles*, Madrid), Fernando López-Campos (until Nov., *Hospital Ramón y Cajal*, Madrid), M. Isabel Pacheco (*Instituto de Investigación Biomédica de Málaga*), Leticia Rivera (*Instituto de Investigación Biomédica de Málaga*), Benjamín Olmos (until Sept., *Hospital Universitario Virgen de La Victoria*, Málaga)

OVERVIEW

Prostate cancer remains a major health burden as over a million men around the world are annually diagnosed with prostate cancer. Up to 30% of them may develop metastatic prostate cancer, which is the advanced form of this disease, once it has spread outside the prostate and is no longer curable. This metastatic stage causes about 6,000 deaths every year in Spain alone, whilst in the US over 30,000 men succumb to the disease every year. In recent years, different subtypes of prostate cancer have been identified based on different genomic profiles. We believe that a better understanding of cancer biology, as well as an improved human prostate cancer taxonomy linked to clinical outcomes, could lead to improved patient outcomes through the application of tailored treatment strategies as opposed to the current one-fits-all approach. As an example of one of these clusters, 20-25% of all metastatic prostate cancers have aberrations in DNA repair genes; about half of these aberrations may correspond to inherited mutations.

RESEARCH HIGHLIGHTS

During 2018, our Group made significant progress in many projects. We finalised the primary analyses of our PROREPAIR-B study and have also completed recruitment in 3 additional studies from our PROCURE platform of prospective biomarker studies: PROSTAC, PROSABI & PRORADIUM. Over 1,000 men with metastatic castration-resistant prostate cancer (mCRPC) have been enrolled in these studies to this day. Our clinical CNIO-IBIMA unit has been consolidated as 1 of the top prostate cancer clinical trials units in Europe, playing a leading role in several early and pivotal clinical trials. We have also reported our first investigator-initiated phase II study. At the preclinical level, we have made some advances in the characterisation of the biological role in prostate cancer of different DDR defects, including Atm loss. We have also made progress in developing representative *in vivo* models of metastatic prostate cancer and patient derived-xenografts.

PROREPAIR-B study. The primary analyses of the PROREPAIR-B study will be published early in 2019 in the *Journal of Clinical Oncology*. The highlights of this study can be briefly summarised as follows: up to 16% of mCRPC may carry germline pathogenic mutations in DDR genes; a *BRCA2* germline mutation has twice the risk of progressing to castration-resistant disease and of death from prostate cancer. We have just started analysing the prevalence of somatic aberrations in DDR genes in this study as part of a DoD IMPACT award. In addition, we are completing the genomic and transcriptomic characterisation of a large collection of *BRCA2* mutated prostate cancers in order to identify secondary events that may contribute to the poor prognosis of the affected men.

AR gain and mCRPC treatment selection. As part of an ongoing international collaboration with Dr Attard's lab (UCL, London) and Dr di Giorgi's team (IRCSS, Meldola), we have determined that mCRPC, having a normal number of copies of the androgen receptor (AR) gene in ctDNA, have a lower risk of disease progression and a higher life expectancy when they are treated with abiraterone/enzalutamide, with a 50% improvement compared to docetaxel. On the other hand, the patients with more copies of the AR gene respond slightly better to docetaxel.

SWITCH phase II study. This study, recently published in the *British Journal of Cancer*, demonstrated that the simple change of the supporting steroid, switched from prednisone to dexamethasone, while maintaining abiraterone, helps to re-induce the response to abiraterone in about 4 out of every 10 patients progressing by PSA criteria. This response does not occur in patients with AR gain detected in plasma ctDNA, while patients with AR normal status benefit the most. ■

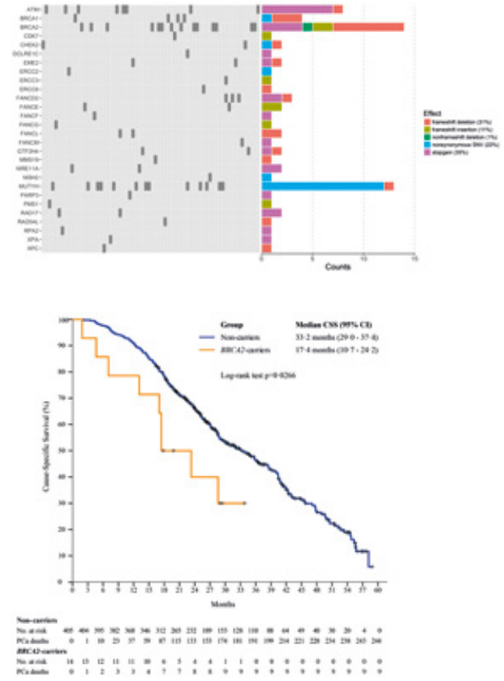

Figure PROREPAIR-B study. Distribution of the pathogenic mutations in DDR genes identified in the study (top). Kaplan-Meier curves for cause-specific survival from diagnosis of mCRPC: *BRCA2* mutant vs noncarriers (bottom).

PUBLICATIONS

- Smith MR *et al.* (2018). Apalutamide treatment and metastasis-free survival in prostate ancer. *New Engl J Med* 378, 1408-1418.
- Lawrence MG *et al.* (incl. Lopez-Campos F, Castro E) (2018). Patient-derived models of abiraterone and enzalutamide resistant prostate cancer reveal sensitivity to ribosome-directed therapy. *Eur Urol* 74, 562-572.
- Lorente D, Olmos D *et al.* (2018). Circulating tumour cell increase as a biomarker of disease progression in metastatic castration-resistant prostate cancer patients with low baseline CTC counts. *Ann Oncol* 29, 1554-1560.
- Mikropoulos C *et al.* (incl. Castro E) (2018). Prostate-specific antigen velocity in a prospective prostate cancer screening study of men with genetic predisposition. *Br J Cancer* 118, 266-276.
- Romero-Laorden N *et al.* (incl. Castro E, Olmos D) (2018). Phase II pilot study of the prednisone to dexamethasone switch in metastatic castration-resistant prostate cancer (mCRPC) patients with limited progression on abiraterone plus prednisone (SWITCH study). *Br J Cancer* 119, 1052-1059.
- Cassinello J *et al.* (incl. Olmos D) (2018). SEOM clinical guidelines for the treatment of metastatic prostate cancer (2017). *Clin Transl Oncol* 20, 57-68.

AWARDS AND RECOGNITION

- Member of the Board of Directors, European Organization for Research and Treatment of Cancer (EORTC).
- Impact Award (Partnering PI). US Department of Defense, Congressionally Directed Medical Research Programs.
- Faculty Board Member, EORTC-EC-CO-AACR-ESMO Methods in Clinical Cancer Research Workshop, Zeist, The Netherlands.
- Elena Castro: Faculty Board Member, ESMO Preceptorships in Prostate Cancer.
- Rebeca Lozano was awarded the ' Merit Award', American Society of Clinical Oncology GU Cancers Symposium, San Francisco; 'Best Communication' Award, 2018 SEOM annual Meeting; and the *Rio Hortega* Fellowship 2018, *Instituto de Salud Carlos III*, Spain.

MOLECULAR DIAGNOSTICS UNIT

Luis Lombardía
Unit Head

Technician
Diana Romero

OVERVIEW

The main objectives of the Molecular Diagnostics Unit (MDU) are directed towards offering quality molecular tests for patients with cancer in order to support the current clinical services and diagnostic laboratories in hospitals of the Spanish National Health System (NHS). In this regard, the Unit provides a wide range of highly sensitive molecular assays to determine changes in the sequence or expression levels of key genes involved in cancer, and to enable the detection of Minimal Residual Disease in patients showing clinical remission as well as to follow-up on their response to therapy. Likewise, MDU is also devoted to implementing recent up-to-date cancer diagnostics solutions, not only to support the NHS but also to assist the Clinical Research Units and Research Groups at the CNIO. In addition, MDU collaborates with international and national groups dedicated to standardising and improving molecular diagnostics tests in cancer, and participates in teaching as well as in educational programmes for clinical post-residents, undergraduate and graduate students.

"In this new era of precision medicine in cancer, Molecular Diagnostics is playing a fundamental role as demonstrated by the increasing variety of assays requested by haemato-oncologists throughout 2018."

CORE UNIT HIGHLIGHTS

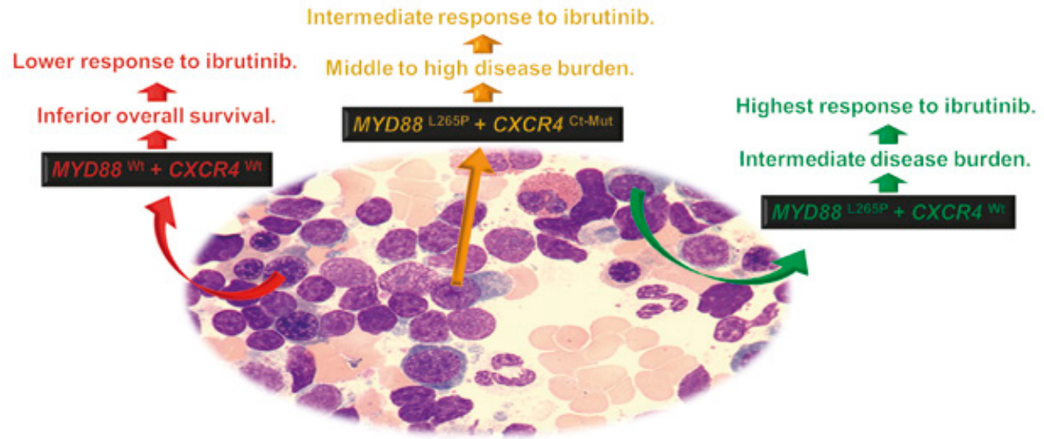

Figure Molecular testing of *MYD88* and *CXCR4* genes in plasmacytoid lymphocytes allows for different prognostic and/or therapeutic options for patients with Waldenström's Macroglobulinemia. (Wt: Wild Type; L265P: Leucine to Proline substitution at position 265; Ct-Mut: C-terminus nonsense/frameshift mutations).

During 2018, we have added and/or expanded 3 diagnostics tests.

First of all, the detection of the fusion gene *BCL1-IgH* by PCR was added to our list of services. Although the genetic translocation t(11;14)(q13; q32) is present in other lymphoproliferative diseases, it occurs mainly in mantle cell lymphomas (50-70%), which are more aggressive and have, in general, a worse prognosis than other low-grade B-cell lymphomas. This assay will be used not only to diagnose patients with a suspected mantle cell lymphoma, but also to monitor and evaluate recurrences of the disease.

We have also complemented the *MYD88* gene testing of patients with Lymphoplasmacytic Lymphoma/Waldenström's Macroglobulinemia (LPL/WM), by implementing a test that enables the detection, by Sanger sequencing, of nonsense and frameshift mutations in the *CXCR4* gene. The protein coded by this gene activates the AKT1/MAPK pathways in B-lineage cells and facilitates cell migration. Mutations in *CXCRA*, commonly found in association with *MYD88 L265P* mutation, are associated with primary resistance and initial lack of response to BTK, PI3K, and mTOR inhibitors. Thus, this assay will be used to aid in the prognosis and therapeutic management of LPL/WM patients (FIGURE).

Additionally, we directed our efforts towards improving the clinical utility of molecular testing based on the *BRAF* gene. In this regard, to complement the detection of the recurrent V600 mutation of BRAF in melanoma patients, we extended the analysis by bi-directional sequencing of exon 11 to enable the management of patients with lung cancer. Mutations in exon 11 are regularly found in lung tumours that are wild type for EGFR, KRAS, ALK, and other driver alterations. Moreover, these patients, with decreased sensitivity to gefitinib, responded to dasatinib with no additional treatment for several years.

Finally, during 2018, in the framework of our training policy, we hosted one medical resident and 2 undergraduate students. ■

AWARDS AND RECOGNITION

- Member of the Committee for Ethical Research *(CEI; Comité de Ética de la Investigación), Instituto de Salud Carlos III*, Madrid, Spain.

H12O-CNIO HAEMATOLOGICAL MALIGNANCIES CLINICAL RESEARCH UNIT

Joaquín Martínez-López
Clinical Research Unit Head

Staff Scientists
Lucía V. Fernández, Miguel Gallardo

Clinical Scientists
Rosa Ayala, Teresa Cedena, Maria Calbacho, Javier de la Serna, Carlos Grande, Ana Jiménez, Pilar Martínez, Inmaculada Rapado, Antonia Rodríquez, Ricardo Sánchez, Beatriz Sanchez-Vega (until October)

Post-Doctoral Fellows
Almudena García, Alejandra Leivas, María Linares, Antonio Valeri

Graduate Students
Sergio Algar (since November), Isabel Cuenca, Jessica Encinas (since November), Elena Maroto, Rebeca Mateos (February-October), M. Luz Morales, Alejandra Ortíz, Alba Rodríguez, Yanira Ruíz, Laura Sánchez

Technicians
Pedro Aguilar (since March), Adrián Fernández (since June), Irene García, Vanesa Garrido, Alexandra Juárez, Laura Moreno, Esther Onecha

Students in Practice
Laura Carrasco (since November, UAH), Cristina Crespo (since November, UCM)

OVERVIEW

The Haematological Malignancies Laboratory focuses on investigating novel drivers, biomarkers, diagnostic tools and therapeutic targets and approaches in haematological neoplasms such as myeloma and acute myeloid leukaemia.

Five main lines define our research project:

- → Generation of mouse models focused on the molecule hnRNP K, a novel driver of lymphoma and leukaemia.
- → Development of novel diagnostic and follow-up tools, such as minimal residual disease analysis in acute myeloid leukaemia (AML).
- → Screening of novel drivers, biomarkers and therapeutic targets by next-generation-sequencing (NGS, e.g. exome sequencing of amyloidosis).
- → Innovation of immunotherapy approaches. Generation of NK CARs and *in vitro/in vivo* validation.
- → Novel therapeutic approaches. Screening of novel compounds (e.g. hnRNP K inhibitors) and pre-clinical trials of new drugs or drug combinations.

"We have developed a strategy to identify undetectable levels of minimal residual disease using an NGS method, thereby improving the capacity to predict AML outcome over the current technical approaches."

RESEARCH HIGHLIGHTS

Minimal residual disease monitoring in acute myeloid leukaemia

Assessment of minimal residual disease (MRD) is critical for monitoring patients in morphological remission as well as to inform decisions about further therapy.

We designed and validated a high-throughput sequencing method for MRD assessment of cell clonotypes with 4 typical AML. Our analysis showed better sensitivities (10^{-4} for SNVs and 10^{-5} for InDels) than current methods or other novel techniques such as dPCR: the sensitivity of dPCR for InDels was similar to that reported in a previously published study (10^{-2}). It should be noted that the prediction of survival and progression of AML using MRD-NGS was improved over the other methodologies employed.

In conclusion, we have optimised a new targeted sequencing method with high sensitivity for MRD evaluation and applicability for a high percentage of AML patients, thereby improving the capacity to predict AML outcome over MFC or qPCR in our cohort (work published in *Haematologica*).

Novel therapeutic combination for primary myelofibrosis

Ruxolitinib is the frontline non-palliative treatment for myelofibrosis; however, a significant number of patients lose or present suboptimal response, are resistant, or have unacceptable toxicity. We found that the combination of ruxolitinib and nilotinib had a synergistic effect against myelofibrosis. Moreover, the addition of prednisone to the ruxolitinib/nilotinib combination improved the synergistic effect in all myelofibrosis samples studied.

We provide evidence that the ruxolitinib/nilotinib/prednisone combination is a potential therapy for myelofibrosis, possibly through the anti-fibrotic effect of nilotinib, the immunomodulatory effects of ruxolitinib and prednisone, and the anti-proliferative effect of ruxolitinib. This combination will be further investigated in a phase Ib/II clinical trial in myelofibrosis (work accepted with major changes for publication in *Haematologica*).

Novel therapeutic combination for acute myeloid leukaemia

Different tyrosine kinase inhibitors have been used in FLT3 targeting, but their effects are limited by drug resistance. In order to rationally combine them with other agents, we explored the alternative pathways activated during the development of resistance.

Differentially phosphorylated proteins and resistance mechanisms after tyrosine kinase inhibitor resistance were identified. Efficacy and safety of rational combinational therapies were assayed *in vitro*, *ex vivo* and *in vivo*.

The results suggested activation of the MEK pathway, therefore, we characterised the effect of the MEK inhibitor trametinib *in vitro* and *ex vivo*. Trametinib exerted strong synergy with the tyrosine kinase inhibitor midostaurin, inhibiting different FLT3 downstream pathways.

Our data provide preclinical evidence that combining a tyrosine kinase inhibitor, such as midostaurin, with a MEK inhibitor, such as trametinib, is a rational and efficacious treatment regimen for a wide range of acute myeloid leukaemias (work under review in the *Journal of Experimental & Clinical Cancer Research*).

DNA methylation mutations predict azacitidine response in myelodysplastic syndromes

Alterations in DNA methylation are involved in the pathogenesis of myelodysplastic syndromes (MDS), however, whether they can also influence their response to azacitidine/decitabine treatment has not been clearly elucidated.

We analysed frequently mutated regions in 34 genes that are likely candidates to be involved in the pathogenesis of MDS. We have found that the profile of several gene mutations identified at diagnosis may represent a useful predictive biomarker of the response to azacitidine therapy. Meta-analysis identified the *TET2* gene as the strongest biomarker of treatment success. Additionally, the presence of mutations in the DNA methylation pathway and the number of driver mutations are predictors of response to hypomethylating agents in patients with MDS (work published in *Oncotarget*). ■

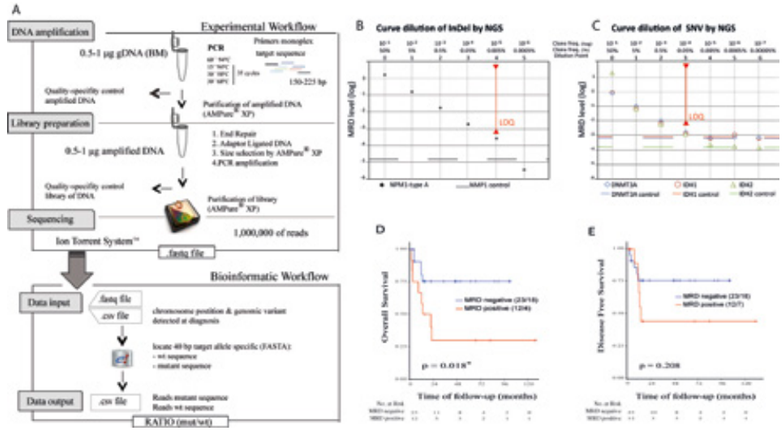

Figure NSG method for MRD detection. (**A**) Workflow of NGS-MRD method. (**B**) InDels calibration curve of MRD in serial dilutions. Top, 10-fold dilution curve for the assessment of sensitivity of sequencing in InDels. (**C**) SNV calibration curve of MRD in serial dilutions. Top, 10-fold dilution curve for the assessment of sensitivity of sequencing in SNV. (**D**) Prognosis analysis of OS in AML patients stratified according to MRD levels by Kaplan-Meier conventional methods. (**E**) Prognosis analysis of DFS in AML patients stratified according to MRD levels by conventional methods. Kaplan-Meier plots.

PUBLICATIONS

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- Arenas Cortés A, Ayala Díaz R, Hernández-Campo P, Gorrochategui J, Primo D, Robles A, Morales ML, Ballesteros J, Rapado I, Gallardo M*, Linares M*, Martínez-López J* (2018). Ruxolitinib in combination with prednisone and nilotinib exhibit synergistic effects in human cells lines and primary cells from myeloproliferative neoplasms. *Haematologica*. PMID: 30026573.
- Misiewicz-Krzeminska I, Corchete LA, Rojas EA, Martínez-López J, García-Sanz R, Oriol A, Bladé J, Lahuerta JJ, Miguel JS, Mateos MV, Gutiérrez NC (2018). A novel nano-immunoassay method for quantification of proteins from CD138-purified myeloma cells: biological and clinical utility. *Haematologica* 103, 880-889.
- Onecha E, Linares M, Rapado I, Ruiz-Heredia Y, Martinez-Sanchez P, Cedena T, Pratcorona M, Perez Oteyza J, Herrera P, Barragan E, Montesinos P, Garcia Vela JA, Magro E, Anguita E, Figuera A, Riaza R, Martinez-Barranco P, Sanchez-Vega B, Nomdedeu J, Gallardo M, Martinez-Lopez J, Ayala R (2018). A novel deep targeted sequencing method for minimal residual disease monitoring in acute myeloid leukemia. *Haematologica*. PMID: 30093399.
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- Fernández L, Leivas A, Valentín J, Escudero A, Corral D, de Paz R, Vela M, Bueno D, Rodríguez R, Torres JM, Díaz-Almirón M, López-Collazo E, Martinez-Lopez J, Pérez-Martínez A (2018). How do we manufacture clinical-grade interleukin-15-stimulated natural killer cell products for cancer treatment? *Transfusion* 58, 1340-1347.
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Selected publications at other insitutions

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AWARDS AND RECOGNITION

Joaquín Martínez-López:

- *Proyectos de Investigación en Salud (PI) 2018* Health Research Project.
- *Proyectos de Desarrollo Tecnológico en Salud (DTS) 2018* Healthcare Technology Development Project.

Miguel Gallardo:

- *Proyectos de Investigación en Salud (PI) 2018* Health Research Project.

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, Santiago Ponce, M. Carmen Riesco

Clinical Investigator
M. Teresa Muñoz (since October)

Post-Doctoral Fellows
Pablo Gella (until May), María Pedraza (until April), Beatriz Soldevilla

Graduate Students
Carlos Carretero, Santiago García, Ángela Marrugal, Ángel Nuñez (since September), Laura Ojeda, Álvaro Quintanal (until February), Javier Ramos, Beatriz Rubio (since September), Patricia Yagüe

Technicians
M. Cristina Cirauqui (since July), Patricia Cozar, M. José Durán (until June), Aicha El Bakkali (until August), Mirella Gallego (since February), Laura García, Beatriz Gil, Rocío Suárez

Students in Practice
Ana González (until July), Alba Santos (since November)

OVERVIEW

Lung cancer continues to be the most frequent cause of cancer-related deaths worldwide. Our Unit focuses on the study of lung cancer, from fundamental research proposals to other more clinically oriented ones that are closer to solving the problems of lung cancer patients. The two main research areas of our Unit involve: 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 contributed to elucidating the molecular determinants of EGFR or FGFR oncogenicity and have discovered biomarkers that may guide the efficacy of inhibitors of those receptors 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 taking 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.

"Our Unit has significantly contributed to the development of novel biomarkers that have impacted the currently availble selection of targeted therapies (e.g. EGFR mutation in the clinic) and novel inmunotherapeutics (e.g. tumour mutational burden). We have led randomised clinical trials with novel agents (e.g. erlotinib, afatinib, Nivolumab, M7824) as well as combinations of checkpoint inhibitors (e.g. Ipilimumab plus Nivolumab, chemotherapy plus Pembrolizumab, Durvalumab following chemoradiation) in lung cancer that have impacted clinical practice worldwide."

RESEARCH HIGHLIGHTS

Biomarker discovery and implementation

The Group has deciphered the biological role of FGFR1 and FGFR4 in non-small cell lung cancer (NSCLC) and has developed new biomarkers with a predictive role for anti-FGFR therapy in NSCLC. Currently, we are validating the results on a series of well characterised PDX models, generating a diagnostic kit and carrying out the technical validation of the biomarker, as well as planning a phase II trial proposal with an FGFR inhibitor in NSCLC patients with high expression of the novel biomarker.

The Group has also validated an NGS-based algorithm for the determination of genomic aberrations (in tumour tissue but also in cfDNA) that may guide treatment for clinical practice. More recently, we have led the first clinical validation of tumour mutational burden as a predictive biomarker for checkpoint inhibitors in lung cancer, and particularly, for Ipilimumab plus Nivolumab.

Early clinical trials

Our Group has significantly expanded its activities regarding the testing of new molecules and combinations in solid tumours, particularly in the field of immune-based approaches; in 2018, we participated in more than 35 projects in this research area. Recently, our Group provided feasibility and encouraging initial data on the anti-tumour activity of M7824, a bifunctional fusion protein targeting PD-L1 and TGF-beta in pretreated NSCLC (response rate in PD-L1 expressing tumours in more than 50% of the cells: 71%). Encouraging tumour-agnostic data of Entrectinib in tumours driven by activated NTRK fusion proteins were presented at the ESMO 2018 Congress (response rate 57.4%; median progression-free survival of 11.4 moths).

Changing standard-of-care treatments in clinical practice

The Lung Cancer Clinical Research Unit has led phase III trials whose results have significantly impacted the clinical practice in the context of stage IV lung cancer with combinations of chemotherapy plus Pembrolizumab or Ipilimumab plus Nivolumab (Hellmann MD *et al.*, *NEJM* 2018; Paz-Ares L *et al.*, *NEJM* 2018). In addition, the Group has actively contributed to the results of a Phase III trial showing a significant improvement in survival for stage III NSCLC patients treated with the anti PD-L1 agent *Durvalumab* following chemoradiation (Antonia S *et al.*, *NEJM* 2018). ■

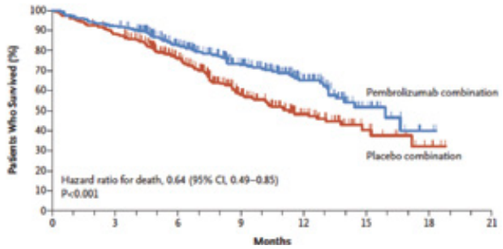

Figure 1 Results of the KeyNote 407 randomised clinical trial, showing an improvement in overall survival (OS) of chemotherapy plus Pembrolizumab, as compared to chemotherapy alone (HR 0.64; p<0.001) in stage IV Non-Small-Cell Lung Cancer (NSCLC) patients.

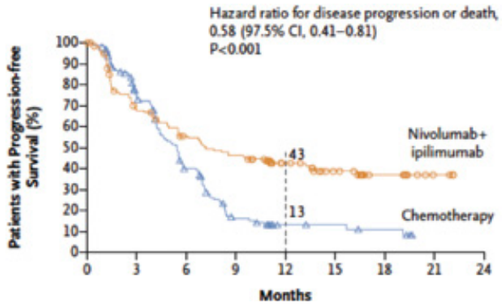

Figure 2 Results of the Checkmate 227 randomised clinical trial, showing the superiority in progression-free survival (PFS) for the Ipilimumab plus Nivolumab combination, as compared to platinum chemotherapy (HR 0.58; p<0.001) in stage IV NSCLC with high mutational burden (>10 mutations per MB).

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AWARDS AND RECOGNITION

- *Premio* 'Best in Class (BIC)' 2018 *en Investigación en Oncología*, Spain.