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 PROCURR 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.”
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.

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.

This year, we completed the first phosphoproteomics study of whole tumours from TNBC with and without relapse and normal tissues obtained from the same patients was used to study the expression of whole tumours from TNBC with and without relapse obtained by mass spectrometry. (C) Kinase activity profiles were solved through an in-house algorithm (“Kinase-set enrichment analysis” or KSEA). (D) Combined treatment with agents targeting the 2 top hits (CDK4/6 plus ERR inhibitors) in TNBC xenografts achieved a synergistic effect.

RESEARCH HIGHLIGHTS

• 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.

From 2015 to 2017, we conducted 4 phase 2 clinical trials that were launched based on our research. 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, 6 kinases are actionable and we have found profound synergy in all 2-by-2 combinations in preclinical models.

This information was then translated to immunohistochemistry: the left sample shows a patient with hyperactivated ERK, compared to a hypoactive one. Combined treatment with agents targeting the 2 top hits (CDK4/6 plus ERR inhibitors) in TNBC xenografts achieved a synergistic effect.

Figure (A) Phosphorylation profiles of whole tumours from TNBC with and without relapse were obtained by mass spectrometry. (B) Kinase activity profiles were solved through an in-house algorithm (“Kinase-set enrichment analysis” or KSEA). (C) Combined treatment with agents targeting the 2 top hits (CDK4/6 plus ERR inhibitors) in TNBC xenografts achieved a synergistic effect.
PROSTATE CANCER JUNIOR CLINICAL RESEARCH UNIT

David Olmos
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Clinical Investigator
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Rebecca Lozano

Gala Grau (until March, Instituto de Investigación Biomédica de Málaga), Letizia Rivera (Instituto de Investigación Biomédica de Málaga), Benjamin Olmos (until Sept., Hospital Universitario Virgen de La Victoria, Málaga), Madrid, M. Isabel Pacheco (Instituto de Investigación Biomédica de Málaga), Letizia Rivera (Instituto de Investigación Biomédica de Málaga), Elena Castro: Faculty Board Member, EORTC-EC-CD-AACI-ESMO Methodologies in Clinical Cancer Research Workshop, Zeist, The Netherlands.

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 (IBCSS, Mendoza), 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.

Figures: 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).

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, PROSKARI & PROBRADUIM. 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.

PUBLICATIONS


AWARDS AND RECOGNITION

• Member of the Board of Directors, European Organization for Research and Treatment of Cancer (EORTC).

• Impact Award Partnering (1), U.S. Department of Defense, Compassionally Directed Medical Research Programs.

• Faculty Board Member, EORTC-EC-CD-AACI-ESMO Methodologies in Clinical Cancer Research Workshop, Zeist, The Netherlands.

• Elena Castro: Faculty Board Member, EDP2 Prepositions in Prostate Cancer.

• Rebeca Lozano was awarded the ‘Merit Award’, American Society of Clinical Oncology EU Cancers Symposium, San Francisco; ‘Best Communication’ Award, 2018 SEOM annual Meeting; and the ‘Río Hortega’ Fellowship, 2018, Instituto de Salud Carlos III, Spain.

ANNUAL REPORT 2018
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 CXCR4, 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.

**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.”

**AWARDS AND RECOGNITION**

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

**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).
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).
- 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.”
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 cycle alterations with 4 typical AML. The new method could detect these alterations with high sensitivity in AML and could be implemented as a routine tool in AML centres. Disadvantages of the new method in comparison to 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 non-frontline 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 in vitro and ex vivo. Transdifferentiation of the MEK pathway, therefore, we characterised the effect of the MEK inhibitor trametinib with ruxolitinib and nilotinib had a synergistic effect in vitro and ex vivo.

Our data provide preclinical evidence that combining a tyrosine kinase inhibitor such as trametinib, with a MEK inhibitor, such as ruxolitinib, in a rational and efficacious treatment regimen for a wide range of acute myeloid leukemias (work under review in the Journal of Experimental & Clinical Cancer Research).
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 available selection of targeted therapies (e.g., EGFR mutation in the clinic) and novel immunotherapeutics (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.*"
Biomarker discovery and implementation

The Group has deciphered the biological role of FGFR4 and FGFRB 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 of a series of well-designed preclinical studies, generating a diagnostic assay 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 Pembrolizumab plus Nivolumab.

Early clinical trials

Our Group has significantly expanded its activities regarding early clinical trials, plus Nivolumab.

In the context of stage IV lung cancer with combinations whose results have significantly impacted the clinical practice particularly in the field of immune-based approaches; in 2018, our Group has significantly expanded its activities regarding TranslaTional research.

Translational research highlights

- **Publications**

- **Early clinical trials**
  - 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 Pembrolizumab plus Nivolumab.

- **Selected publications at other institutions**

- **R&D**
  - Ruiz-Pérez insertion resistance in stage IV NSCLC patients treated with the anti-PD-1 agent Durvalumab following chemoradiation (Antonia S et al. 2018).  

- **Clinical practice**
  - Our Group has significantly expanded its activities regarding early clinical trials, plus Nivolumab.

- **Translational research highlights**
  - **Publications**

- **Early clinical trials**