The Clinical Research Programme (CRP) 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 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, 2 functional objectives summarise the CRP’s new operating model: i) generating synergies with ongoing research lines in the basic research programmes; ii) constituting a bi-directional bridge to facilitate closer interactions between the CNIO and tertiary cancer hospitals. There are 4 agreements in place with tertiary hospitals (Hospital 12 de Octubre, Hospital de Málaga, Hospital de Fuenlabrada and Hospital Quirón Pozuelo), where the clinical activity of the CRP’s Clinical Units takes place. These agreements foster the interaction between clinicians and scientists, and allow scientists from all CNIO Programmes to participate in translational research studies. The number of ongoing collaborations between the CRP Units and CNIO Research Groups have increased to 29 projects and 3 coordinated grants, which account for the high translational research activity of the institution. Nine medical oncology residents from different Spanish hospitals completed their 3-month optional stays at CNIO during 2019.

The Breast Cancer Clinical Research Unit, led by Miguel Quintela-Fandino, published a major finding about the role of fatty acid synthase as a key factor eliciting transformation of the breast epithelium. This is of importance for the development of prevention strategies for populations at high risk of developing breast cancer. The Lung Cancer Clinical Research Unit, led by Luis Paz-Ares, completed a pivotal clinical trial to register the immunotherapy combination nivolumab and ipilimumab for lung cancers with high mutational burden, where the risk for disease progression was almost halved compared to standard chemotherapy. The Haematological Malignancies Clinical Research Unit, headed by Joaquín Martínez-López, enhanced our understanding of hnRNP K as a bona fide oncogene. The Prostate Cancer Clinical Research Unit, under David Olmos’s supervision, expanded the PROCURE network and confirmed the role of ATM in hereditary prostate cancer, which led to the publication of a seminal manuscript in the field; the Unit was recently awarded the prestigious ‘Proyectos AECC Grant’ to strengthen this pioneering line of research. Finally, the Molecular Diagnostics Unit, led by Luis Lombardía, continued to provide support to hospitals in the diagnosis of haematological malignancies. With the large number of ongoing translational research collaborations, the arrival of novel immuno-oncology drugs, and the search for novel groups for the CRP, we face an exciting year 2020 for patient-oriented oncology research at CNIO.

“The Clinical Research Programme aims to improve cancer care by developing novel agents and personalising therapeutic approaches on the basis of biomarkers.”
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 they 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 biological features that constitute therapeutic targets.
- Study of the mechanisms of resistance against targeted therapies.

"In 2019, the BCCRU finalised its characterisation of fatty acid synthase as a key mediator of breast epithelium transformation. This opens up an unprecedented opportunity for cancer prevention."

RESEARCH HIGHLIGHTS

Contrary to what was previously known, we have found that fatty acid synthase (FASN, an enzyme with low expression in healthy tissue but high expression in epithelial malignancies) exerts a key role during the early steps of cancer initiation, but not when the cancer is established. In vitro and in vivo models of breast carcinogenesis cannot undergo transformation in the absence of FASN; however, its deletion after the cancer is established has little effect. Mechanistically, this preventive role is independent of its biosynthetic product. FASN consumes acetyl-CoA, which unlocks reductive isocitrate dehydrogenase-dependent carbonylation. This allows the production of the reductive power necessary to quench the reactive oxygen species (ROS) produced during the 2D-to-3D growth transition. This necessary hallmark of cancer is abrogated in the absence of FASN due to intramitochondrial ROS accumulation, which disrupts the mitochondrial respiratory supercomplexes and results in cell death. These findings open up therapeutic opportunities in the preventive phase of breast cancer.

Our previous findings about the metabolic adaptation of tumours in response to metabolic-normalising antiangiogenics were confirmed in a clinical trial, where patients with early HER2-negative breast cancer were treated with bevacizumab alone or bevacizumab plus a mitochondrial inhibitor. The latter patients experienced a 3-fold decrease in the Ki67 replicative fraction.

Finally, we worked on the search for predictive markers of activity of anti-PD-L1 agents. In patients with advanced breast cancer, we found that those with a baseline higher quotient of T-effector/T-memory populations had a higher chance of responding to durvalumab.

**PUBLICATIONS**

During 2019, our Group made significant progress in its multiple projects, with the following highlights:

→ We demonstrated that ATM gene aberrations associate with a higher frequency of advanced disease and metastatic spread. Similarly, in TRAMP® (GEMMs and prostate cancer xenografts), ATM knock-out or silencing translated into increased aggressiveness and a higher rate of metastasis.

→ Previously, we had shown that germline BRCA2 mutations are associated with worse prognosis in different stages of prostate cancer and may be a predictors of response to certain standard therapies. In 2019 we demonstrated that somatic RARB2 and RBR co-deletion as well as MYC amplification occur more frequently in gBRCA2 mutant tumours. The addition of these events progressively increases the risk of metastasis and death from prostate cancer in both sporadic and gBRCA2 mutant associated prostate cancer.

→ We established a methodology for developing prostate cancer metastatic xenograft models using both cell lines and patient derived xenografts, in which we can trace biomarkers of treatment sensitivity and resistance (using circulating tumour cells -CTCs- and metastasis). As proof of concept, we identified a biomarker of both docetaxel efficacy and resistance. First, we observed an early (post-24h) increase of pHH3 (mitosis arrest) in CTs derived from tumours that responded to docetaxel and then, an increase in the aneuploidy index in tumours that did not respond to docetaxel. The early increase of pHH3 in CTs was associated with docetaxel response in advanced prostate cancer patients undergoing treatment with docetaxel.

**PROSTATE CANCER JUNIOR CLINICAL RESEARCH UNIT**

**Research Highlights**

During 2019, we added one new molecular diagnostic test based on bi-directional Sanger sequencing. This assay detects activating mutations in exons 4, 5, 6, and 8 of the runt-related transcription factor 1 (RUNX1), a key regulator gene required for the differentiation of myeloid progenitor cells to granulocytes. The RUNX1 gene is the most frequent target for chromosomal translocations associated with human leukaemias. However, loss-of-function RUNX1 mutations have also been identified in patients with de novo AML. These alterations could coexist, or be mutually exclusive, with mutations in other genes included in the assays already available in our portfolio (e.g., FLT3, TP53, IDH1, IDH2, TET2, NPM1, CEBPA, etc.). Consequently, even if patients with RUNX1 mutations were classified as high-risk group due to adverse prognostic outcomes (i.e., shorter relapse-free survival), depending on coexisting detected mutations, they could benefit from specific targeted therapies (FIGURE).

Additionally, MDU started several project collaborations with the CNIO Microenvironment and Metastasis Group. Thus, for the purpose of evaluating the clinical utility of liquid biopsies for molecular testing, we designed allelic discrimination assays that make qRT-PCR detection of BRAF V600E and GNAQ Q209L/P mutations possible in different biological fluids (plasma, circulating exosomes, lymphatic fluids) from patients with, respectively, cutaneous or uveal melanomas. Another collaboration with this Group aims to analyse, by whole exome sequencing, primary tumours and lymph nodes from melanoma patients to understand the genomic evolution of nodule metastasising tumours. The objective is to establish a mutational signature that could help to determine risk groups that have residual disease using plasma samples and/or post-lymphadenectomy lymphatic fluids.

Finally, our Unit remains committed towards its policy of training and mentoring students, technicians and medical residents in our techniques and methods.
translated research

Overview

Haematological malignancies include a myriad of heterogeneous diseases with high frequency, such as non-Hodgkin’s lymphoma, or with high mortality rates, such as multiple myeloma and acute myeloid leukaemia. In the laboratory, we dissect the biology of haematological cancers by investigating: (i) novel diagnosis and prognosis biomarkers; (ii) innovative tools and techniques to identify and monitor the disease; (iii) original therapeutic targets and treatments against haematological malignancies; and (iv) how to decipher the biological processes underlying the diseases and characterise the drivers, oncogenes and tumour suppressors.

The following main lines of research define our laboratory:

- Role of hnRNP K, a novel driver of lymphoma and leukaemia in tumorigenesis.
- Molecular fingerprint in clonal evolution, heterogeneity and drug resistance.
- Liquid biopsy and next-generation sequencing.
- Immunotherapy: NK-CAR and T-CAR in haematological and paediatric cancers.

“We have identified a novel master regulator of cancer, hnRNP K, a tumour suppressor now characterised as an oncogene. Lymphoma patients might benefit from more personalised therapies based on targeting hnRNP K.”
**ANNUAL REPORT 2019**

Implications of monoallelic TP53 mutations for the clinical outcome of hNRNP K in B-cell lymphomas

Heterogeneous nuclear ribonucleoprotein K (hNRNP K) is an RNA-binding protein that is aberrantly expressed in cancer. We, and others, have previously shown that reduced hNRNP K is oncogenic potential stems from its ability to post-transcriptionally and translationally regulate MYC. Our findings indicate that hNRNP K is a bona fide oncogene when overexpressed and represents a novel mechanism for c-Myc activation in the absence of MYC lesions (work published in JNCI).

**Hierarchies of TP53 alterations in multiple myeloma cell fitness**

Recently, bi-allelic (“double hit”) TP53 inactivation, occurring in 2% to 4% of newly diagnosed multiple myeloma (MM) patients, was identified as an unique high-risk feature of MM, being associated with a median survival of <2 years. The implications of monoallelic TP53 mutations for the clinical outcome remain controversial, but clonal selection and evolution is a common feature of myeloma progression, and patients with TP53 wild-type (WT) or monoallelic inactivation may present a double hit on relapse. Here, we addressed the hypothesis that sequential acquisition of TP53 hits lead to a gain in the proliferative fitness of MM cancer cells, inducing the expansion and domination of the affected clones within a patient’s bone marrow. To test this hypothesis, we established fluorescence-matched isogenic AMO-1 MM sublines with WT, mono-, and bi-allelic TP53 alterations, and co-cultivated these cells in different in vitro competition assays. In our model, we were able to observe clonal evolution and estimate competitive advantages of both mono- and bi-allelic TP53 variants. Strikingly, we demonstrated that subclones with TP53 double hits outcompete and overgrow other TP53 variants. Reflecting these results, in combination with publically available data sets, confirmed single- and double-hits myeloma to be significantly enriched in patients who relapsed (work published in Blood).

**Mitochondrial activity plays a key role in multiple myeloma**

Mitochondria control several key biological pathways involving cell proliferation and apoptosis. Many studies have implicated a functional role for mitochondria in tumour formation and development; however, their impact on the pathogenesis of multiple myeloma (MM) remains largely unexplored. We investigated the impact of mitochondrial load and activity on the progression and relapse of MM. RNAseq data from 770 newly diagnosed patients with MM revealed overexpression of mitochondrial activity-related genes correlating with poor outcome. The expression of mitochondrial genes and proteins were elevated in patients who relapsed with bortezomib compared with previous stages, concomitant with an increase in mitochondrial activity. In proteasome inhibitor-relapsed MM patients, an elevation in c-Myc and CD38 expression, both involved in metabolic activation, could explain the consequent increase in mitochondrial activation triggered by proteasome inhibitor treatment. In vitro and in vivo studies with primary MM cells and the JNJ3-Luc-GFP cell line showed the efficacy of the mitochondrial inhibitor tegifericycline, alone and in combination with the frontline treatment bortezomib, reversing the bortezomib resistance induced by mitochondrial activation. Our findings provide a strong rationale for investigating tegifericycline and other mitochondrial inhibitors in combination with current MM therapies (work under review in Blood).

**Publications**

- Puig N et al. (incl. Martinez-Lopez J) (2019). Fluorescence anisotropy (FA) binding curves. (Left) and murine (right) cells.
- Puig N et al. (incl. Martinez-Lopez J) (2019). Fluorescence anisotropy (FA) binding curves. (Left) and murine (right) cells.
- Puig N et al. (incl. Martinez-Lopez J) (2019). Fluorescence anisotropy (FA) binding curves. (Left) and murine (right) cells.
- Puig N et al. (incl. Martinez-Lopez J) (2019). Fluorescence anisotropy (FA) binding curves. (Left) and murine (right) cells.
- Puig N et al. (incl. Martinez-Lopez J) (2019). Fluorescence anisotropy (FA) binding curves. (Left) and murine (right) cells.
- Puig N et al. (incl. Martinez-Lopez J) (2019). Fluorescence anisotropy (FA) binding curves. (Left) and murine (right) cells.
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, always aiming to solve the problems of lung cancer patients. We are particularly interested in two research areas: (i) the identification of new molecular biomarkers for diagnostic, prognostic and predictive purposes; and (ii) the development of novel treatment strategies, including 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. We have developed an extensive platform of patient-derived xenografts of non-small-cell lung cancers to test new therapeutic strategies. Finally, our Unit has extensive experience in taking new drugs to the clinic, as well as in conducting practice-changing phase II/III trials in the fields of personalised cancer care 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 as well as combinations of targeted therapies (e.g. Ramucirumab plus Erlotinib) or checkpoint inhibitors (e.g. chemotherapy plus Pembrolizumab or Nivolumab plus Ipilimumab) in lung cancer that have impacted clinical practice worldwide.”
We currently own an extensive PDX platform that has led to deciphering the role of the tyrosine kinase receptors FGFR1 and FGFR4 in non-small cell lung cancer (NSCLC) and to develop new biomarkers with a predictive role for anti-FGFR therapy in NSCLC (Quintanal-Villalonga et al., JTO 2019). In addition, this PDX platform has contributed to the discovery of novel therapeutic targets, such as Ysc (Garmendia I et al., AJCCM 2019) or Notch (Bousquet Mur E et al., JCI 2019), as well as to the development of novel therapies based on targeting the cardiokinin-like cytokine factor 1 (CLCF1) -cilary neuroendocrine factor receptor (CNTFRe) signalling axis (Kim JW et al., Nut Med 2019).

We performed a harmonisation study to determine tumour mutational burden (TMB) in a clinically well-annealed cohort of NSCLC patients. We calculated the TMB of these samples (n=100) with 3 in-house NGS-based panels and correlated it with the TMB obtained with the gold-standard method (Foundation One CDx), demonstrating a strong correlation among them. Additionally, we adjusted the cut-offs for each of the in-house panels (Garrido-Martín EM et al., IJSMO 2019). We performed multivariate molecular and immune profiling of NSCLC tumours (n=200 early stage), after which a genomic and transcriptomic study of the tumours was carried out. This allowed us to describe novel subgroups of tumours based on multiparametric signatures with putative predictive value as biomarkers of response to therapy with immune checkpoint inhibitors.

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 2019, we participated in more than 40 projects in this research area, including 9 new trials. Recently, we published a phase II trial of Lurbenecitin, a novel transcription inhibitor, in small cell lung cancer. The results showed encouraging activity in the second-third line setting (Response rate: 34%), particularly in patients with sensitive relapse (median survival: 11.8 months) (Trigo JM et al., Lancet 2020). Based on encouraging activity, the drug is now being tested in a phase III registration study.

Changing standard-of-care in treatment practices in clinical trials

The Lung Cancer Research Unit has led phase III trials whose results have significantly impacted the clinical practice in the stage IV lung cancer, such as the combination of chemotherapy plus Durvalumab in small cell lung cancer (SCLC) patients (Paz-Ares L et al., Lancet 2019). The novel regimen was shown to significantly improve survival when compared to standard-of-care, including a significant improvement in survival (HR 0.73, p<0.0047; median survival 13 vs 10.3 months). In addition, the mature results of the Checkmate 227 trials validated the Ipilimumab-Nivolumab combination strategy, impacting on the proportion of long-term survivors of stage IV NSCLC patients, regardless of the expression of PD-L1 in their tumours (Hellmann M et al., NEJM 2018, Hellmann M et al., NEJM 2019).

First-line afatinib for advanced EGFRm+ NSCLC in patients with the presence of actionable defects in the LUSL 5, 6 and 7 trials. Lung Cancer 52, 10-18.


First-line pembrolizumab plus chemotherapy in patients with previously untreated advanced non-small cell lung cancer: a global, randomised, open-label, phase I/IIb trial. Lancet Oncol 21, 1072-1086.

First-line afatinib plus pembrolizumab in patients with previously treated advanced non-small cell lung cancer: an international, multicentre, randomised, open-label, phase IIIb trial. Lancet Oncol 21, 970-980.