The Clinical Research Programme (CRP) has 2 main goals: 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) developing novel agents; 2) studying 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: a) generating synergies with ongoing research lines in the basic research programmes; and b) constituting a bi-directional bridge to facilitate closer interactions between the CNIO and tertiary cancer hospitals. The clinical activity of the CRP’s Clinical Units takes place through agreements with tertiary hospitals (Hospital 12 de Octubre, Hospital de Málaga and Hospital de Puente Alto). These agreements foster the interaction between clinicians and scientists and enable scientists from all CNIO Programmes to participate in translational research studies. The number of ongoing collaborations between units of the CRP and research groups of other CNIO Programmes have increased to 26 projects and 3 coordinated grants, which translates into the high translational research activity of the institution. Eleven residents in medical oncology from different Spanish hospitals completed their 3-month optional visiting stays at CNIO during 2020.

Although clinical activity was hampered considerably this year due to COVID-19, the clinical groups managed to produce highly impactful research. The Breast Cancer Clinical Research Unit, led by Miguel Quintela-Fandino, validated for the first time in a clinical trial the preclinical observation of synergy between mitochondrial inhibitors and antiangiogenics. The Lung Cancer Clinical Research Unit, led by Luis Paz-Ares, brought novel approaches for the treatment of small-cell lung cancer to the clinic: chemo-immunotherapy with durvalumab in first-line treatment, and lurbinectedin for pre-treated patients, 2 unmet clinical needs. These findings were published in separate papers in *The Lancet*, and supported FDA and EMA approval, respectively. The Haematological Malignancies Clinical Research Unit, headed by Joaquín Martínez-López, made relevant contributions towards the understanding of COVID-19 in haematological malignancies. The Prostate Cancer Clinical Research Unit (PCCRU), headed by David Olmos, significantly contributed to the development of the first 2 targeted therapies for metastatic prostate cancer: olaparib in patients with HRRm prostate cancer, recently approved by FDA and EMA, and ipatasertib in PTEN null prostate cancer, both findings were published in top journals such as the *New England Journal of Medicine* and *The Lancet*. Additionally, they described that poor outcomes within a significant proportion of BRCA2 mutant prostate cancers are linked to the co-deletion of BRCA2 and RB1 genes; this codeletion identifies a subgroup of particularly aggressive prostate cancers at diagnosis that may benefit from novel treatment approaches. The PCCRU was awarded one of the two CRIS Cancer Grants for Junior Researchers endowed with 1.25 million euro. Finally, the Molecular Diagnostics Unit, headed by Luis Lombardía, continued to provide support to hospitals in the diagnosis of different malignancies, performing >1000 diagnoses this year. With the large number of ongoing translational research collaborations, the arrival of novel immune-oncology drugs, and the search for novel groups for the CRP, we face an exciting year 2021 for patient-oriented oncology research at CNIO.

“The Clinical Research Programme aims to improve cancer care by developing novel agents and by personalising therapeutic approaches on the basis of novel biomarkers.”
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 correlative studies and clinical trials. Specifically, our research areas cover the:

- Study of the mechanisms of resistance against CDK inhibitors.
- Role of FGFRI in cancer progression and therapeutic resistance in hormone-positive cancer.
- Study of the implications of hypoxia in response to immunotherapy.

“We have provided clinical proof-of-concept of the synergy between antiangiogenics and mitochondrial inhibitors in breast cancer, which we previously described in animal models.”

Research highlights

The following highlights some of the achievements of the Breast Cancer Clinical Research Unit during 2020:

- We demonstrated that PD-L1 inhibitors in combination with antiangiogenics in advanced breast cancer restrict their activity to those cases in which antiangiogenics normalise tumour hypoxia and vasculature.
- We confirmed our preclinical findings regarding acquired resistance against antiangiogenics: in those cases where antiangiogenics normalise tumour hypoxia and vasculature, a targetable mitochondrial switch takes place. Consequently, mito-inhibitors were found to synergise with antiangiogenics. We confirmed the findings in a clinical trial in early breast cancer.
- In parallel, in the preclinical setting, we are solving the mechanism of escape against antiangiogenics that increases vascular abnormality and tumour hypoxia. Preliminary data suggested that hypoxic areas are excluded from the antitumor immune response. Diverse therapeutic avenues are being explored to induce immune re-infiltration in hypoxic areas.
- We characterised FGFRI as a driver of acquired resistance to combined treatment with hormonal and CDK inhibitors. We found that in FGFRI-amplified or overexpressed tumour models, the triple combination of FGFRI, CDK4/6 and ER blockade is the only one able to completely suppress RB phosphorylation.
- Completion of the first phosphoproteomic screening in a randomised clinical trial in early breast cancer treated with paclitaxel monotherapy revealed that elevated P70S6K phosphorylation.
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Prostate cancer (PrCa) is the most common cancer diagnosis in men and, despite its potential to be cured in almost 90% of early stages, its metastatic spread causes about 6,000 deaths every year in Spain alone, whilst in the US over 30,000 men succumb to the disease each year.

During the last few years, our Group has focused precisely on the concept of liquid biopsy. And many others.

Our work has been widely recognised with several highly cited publications in top journals in our field, including The New England Journal of Medicine, The Journal of Clinical Oncology, Annals of Oncology, and many others.

Our research efforts, especially in the clinic, had to be slowed down as doctors in our teams and other associated researchers had to focus on delivering patient care. Still, we also tried to contribute through international collaborations to understanding the role of TMPRSS2, a key AR-regulated gene, which could be involved in SARS-CoV-2’s entry into the cell.

During 2020, our Group was recognised with the 1st CRIS Excellence in Research Award. This award will help, among other aspects, to continue our work on understanding gene alterations that could be synergistic with DNA repair defects promoting oncogenesis and prostate cancer progression, and therefore to be exploited as potential new targets.

For example, during the 2020 American Society of Clinical Oncology meeting, we presented the results of the PROPEPAIR-A study, in which we reported that BRCA2 defects are frequently associated to RBB loss and/or MYC amplification, and that their combination is associated to poor outcomes. We also advanced in our understanding of ATM defects in prostate cancer. As part of the thesis project of our student Ylenia Cendón, we established that ATM may contribute or not to cancer oncogenesis and progression depending on the genetic background (i.e., it may be synergistic in an RBB suppression or MYC overexpression context, and the opposite in a PTEN loss context).

In addition, the group actively participated in several clinical trials, and this included our participation in the steering committees of large phase II trials. We particularly contributed to the approval of olaparib as the first targeted treatment for precision medicine in metastatic prostate cancer.

Like everyone around the world, we were also affected by the Covid-19 pandemic. Our research efforts, especially in the clinic, had to be slowed down as doctors in our teams and other associated researchers had to focus on delivering patient care.

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During the last few years, our Group has focused precisely on the development of new methods to identify and treat the most aggressive and lethal forms of prostate cancer, in order to accelerate precision medicine for the disease. In particular, over the last 8 years, our group has made significant contributions in:

- Understanding the implication of gene alterations leading to DNA repair deficiency in this disease.
- Developing new treatments for prostate cancer.
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Broadening our genetic testing catalogue

During 2020, we implemented 3 new molecular diagnostic tests based on bi-directional Sanger sequencing. These tests will allow us to provide more comprehensive molecular diagnostics of some haematological malignancies like myeloproliferative neoplasms (MPN), myelodysplastic syndromes (MDS), chronic myelomonocytic leukaemia (CMML) and acute myeloid leukaemia (AML), for which we were already offering a sizable panel of clinically applicable markers (FIGURE).

The first 2 assays aim to detect mutations in the \textit{ASXL1} (additional sex combs-like 1) gene that plays a role in both embryonic development and chromatin remodelling, and in the \textit{SF3B1} (splicing factor 3b subunit 1) gene, which encodes a catalytic core component of the RNA splicing machinery and is involved in transcription and mRNA processing. Somatic mutations in \textit{ASXL1} and \textit{SF3B1} genes have been observed, among many other cancers, mostly in MDS, CMML and AML. Mutually exclusive, \textit{ASXL1} mutations are prognostic of high-risk MDSs, acute transformation in CMML, and shorter overall survival of patients with AML, while \textit{SF3B1} structural alterations are predictive of a longer overall survival of MDS patients but shorter overall survival in \textit{de novo} AML and chronic lymphocytic leukaemia (CLL) patients.

The third test developed seeks to detect mutations in the \textit{SETBP1} (SET-binding protein 1) oncogene, encoding a binding partner for the multi-function SET oncoprotein involved in apoptosis, transcription and nucleosome assembly. \textit{SETBP1} overexpression is associated with a worse overall survival of elderly AML patients. Furthermore, somatic gain of function mutations of \textit{SETBP1} are associated with myeloid leukemic transformation and convey poor prognosis of MDS and CMML patients.

Tutoring

In 2020 we hosted, in the framework of our training policy, one medical resident.
Recently, the field of haematology has been gaining traction in cancer research, not only for the study of critical disease affecting human health, but also for its implications in solid cancers and the applicability of haematological tools to other areas.

In terms of cancer implications, immune cells play a remarkable role in metastasis, inflammation, and immune surveillance. In fact, this concept triggered the development of the most promising cancer therapy of the 21st century, immunotherapy.

Moreover, the haematology research area has been developing cutting-edge applications such as liquid biopsy, an easy peripheral blood/plasma analysis that can anticipate the appearance of disease, or the emergence of tumour clones in relapsed patients.

The following main lines of research define our laboratory:

→ Liquid biopsy, minimal residual disease, and next-generation sequencing.
→ Immunotherapy: NK/T-CAR, BITES and immune checkpoints in haematological and paediatric cancers.
→ Role of hnRNP K, master regulator of tumorigenesis.
→ Viral infection and cancer.

“We improved our in-house deep-sequencing analysis for measurable residual disease (MRD), revealing its value in decision-making for clinical trials and as a prognosis marker.”

Joaquín Martínez-López
Clinical Research Unit Head

Staff Scientists
Santiago Barrio, Lucía V. Fernández, Miguel Galindo,

Maria Linares
Clinical Investigators
Rosa Ayala, María Calbacho, Gonzalo Carreño, Pilar Carreras (since October), Teresa Cerdeña,

H12O-CNIO HAEMATOLOGICAL MALIGNANCIES CLINICAL RESEARCH UNIT

Overview

Francisco Javier De La Serna, Ana Jiménez, Pilar Martínez, Inmaculada Repado, Antònia Rodríguez, Ricardo Sánchez

Graduate Students
Pedro Aguilar, Noemi Alvarez, Eva Castañer, Laura Cendón (since February), Isabel Cuerva, Jessica Encinas, Roberto García (since October), Elena Merino, Michael Qi Hong (since August), Alexandra Ortiz, Alba Rodríguez, Laura Sánchez

Post-Doctoral Fellows
Almudena García, Alejandra Lenguí, Yanto Ruiz-Hornedo, Antonio Valens, María Velasco-estévez (since February) (PEJ, CAM)

Researchers
Víctor de Esteban, Joaquin Canete (since Employment Plan, Community of Madrid)

Technicians
Natalia Sofia Benamech (since August), Adrián Fernández, Yantou González, Ailene Gómez, M. Luz Morales, Laura Moreno, Miguel Ángel Navarro, Laura Rullan

Master’s Students
María Balaguer (until May) (Universidad de Barcelona, Spain)

Graduate Students
Pedro Aguilar, Noemí Álvarez, Eva Castañer, Laura Cendón (since February), Isabel Cuerva, Jessica Encinas, Roberto García (since October), Elena Merino, Michael Qi Hong (since August), Alexandra Ortiz, Alba Rodríguez, Laura Sánchez

Technicians
Natalia Sofia Benamech (since August), Adrián Fernández, Yantou González, Ailene Gómez, M. Luz Morales, Laura Moreno, Laura Rullan

Visiting Scientists
María Hernández (since March) (IBSAL, Salamanca, Spain), Alfonso Navarro (since February) (IDIPAZ, Madrid, Spain)
Next-generation sequencing of measurable residual disease in multiple myeloma by immunoglobulin repertoire analysis

Measurable residual disease (MRD) is a poor study variable in routine practice. The most common measure of MRD was developed through low cytometry, of which our laboratory has conducted previous studies in multiple myeloma (MM) and other haematological malignancies. However, preliminary next-generation sequencing data from MRD studies show an increase in sensitivity, specificity, and applicability. In 2020, we measured MRD by next-generation sequencing of immunoglobulin genes with a sensitivity of 10−6. Here we present our single-institution experience assessing MRD in 234 MM patients, both newly diagnosed (159) and relapsed (75). We describe the impact of depth, duration, and direction of response on prognosis. Those patients achieving MRD negativity at 10−6, as well as those with progressively higher progression-free survival (PFS). In the MM diagnosis cohort, 40% of the patients achieved MRD negativity at 10−6 vs <10−5 (PFS: 87 months vs 32 months, P < 0.001). In the MM relapsed cohort, 36% achieved MRD negativity at 10−6 and 47% at 10−5. Median PFS was superior for the cohort achieving MRD at 10−6 vs 10−5 (PFS: 42 months vs 17 months, P = 0.03). Serial MRD monitoring identified 3 categories of MM patients at diagnosis: (A) patients with MRD 10−6 negative samples, (B) patients with detectable but continuously declining clonal numbers, and (C) patients with stable or increasing clonal number (aLoG). PFS was superior in groups A and B vs C (P = 0.01).

This work validates the importance of MRD evaluation as part of clinical care, both as an important prognostic marker at diagnosis and at relapse in multiple myeloma disease. Our data support its use as an endpoint in future clinical trials as well as for clinical decision-making. (Work published in Blood Advances).

Impact of prolonged maintenance of the immunomodulatory drug lenalidomide in multiple myeloma

Lenalidomide is an immunomodulatory drug approved for maintenance treatment in newly diagnosed multiple myeloma, and it has been shown to improve progression-free survival (PFS) and, in several studies, overall survival. Nevertheless, the impact of prolonged treatment with lenalidomide on the kinetics of minimal residual disease (MRD) and its prognostic impact have not been studied in depth. To obtain better knowledge in this regard, we retrospectively analysed 139 patients who received lenalidomide maintenance in real-world clinical practice and whose MRD levels were observed during the treatment period by multiparametric flow cytometry or next-generation sequencing with a sensitivity of at least 10−6. Lenalidomide maintenance correlated with an increased depth of the disease response, with 38% of patients achieving continuous residual disease during lenalidomide treatment, compared with 34.3% of patients who were MRD positive after induction treatment achieved MRD-negative status during maintenance and ultimately had improved PFS. Sequential MRD assessments identified patients with progressively decreasing MRD levels who also had better PFS outcomes, with patients not showing a decreasing pattern of MRD.

These results support the role of maintenance therapy, not only to sustain, but also to increase the depth of disease response with a PFS benefit. In addition, MRD monitoring during maintenance identifies patients with better prognosis and may help in their clinical management. (Work published in Blood Advances).

RESEARCH HIGHLIGHTS


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• Barrio García S., Ayala Díaz RM, Martínez López J et al. (2020). Impact of prolonged maintenance with detectable but continuously declining clonal numbers, and (C) patients with stable or increasing clonal number (aLoG). PFS was superior in groups A and B vs C (P < 0.001).
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 2 research areas: the identification of new molecular biomarkers for diagnostic, prognostic, and predictive purposes; and 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 continued to develop an extensive platform of patient-derived xenografts of non-small-cell and 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 contributed remarkably to the development of predictive biomarkers that have impacted the currently available selection of targeted therapies (e.g., EGFR mutation, NTRK rearrangements) and novel immunotherapeutics (e.g., tumour mutational burden in the clinic). We have led controlled clinical trials with novel agents as well as combinations of targeted therapies (e.g., ramucirumab plus pembrolizumab) or checkpoint inhibitors (e.g., chemotherapy plus nivolumab plus ipilimumab) in lung cancer that have impacted clinical practice worldwide.

**RESEARCH HIGHLIGHTS**

**Biomarker discovery and implementation**

We currently own an extensive patient-derived xenograft (PDX) platform that has led to the deciphering of the role of the tyrosine kinase receptors, FGFR1 and FGFR4, and the adhesion molecule N-cadherin in non-small cell lung cancer (NSCLC), and to develop new biomarkers with a predictive role for anti-FGFR therapy in NSCLC (Quintanal-Villalonga A et al., *EBioMedicine* 2020). In this study, only co-expression of FGFR1 and/or FGFR4 with N-cadherin in different lung cancer patient cohorts inferred a poorer outcome. Treatment of high FGFR1- and/or FGFR4-expressing lung cancer cell lines and PDXs with selective FGFR inhibitors showed high efficacy, but only in models with high FGFR1/4 and N-cadherin expression. We therefore provide in vitro and in vivo evidence showing that expression of the adhesion molecule N-cadherin is key for the oncogenic role of FGFR1/4 in NSCLC. In addition, our data show that the complementary determination of N-cadherin and FGFR1/4 expression may further optimise patient selection for anti-FGFR therapy efficacy. Moreover, our PDX platform has also contributed to discovering Notch as a novel therapeutic target in lung adenocarcinoma osimertinib-treated patients after disease progression (Bousquet Mur E et al., *JCI* 2020), as well as to test novel combination therapies, including a novel neutralising anti-HER3 antibody, to overcome resistance to EGFR targeted therapies (Romanello D. et al., *Cancers (Basel)* 2020).

In 2020, we performed a harmonisation study to determine the tumour mutational burden (TMB) in a clinically well-annotated cohort of 96 resected patients with NSCLC. We therefore provide in vitro and in vivo evidence showing that expression of the adhesion molecule N-cadherin is key for the oncogenic role of FGFR1/4 in NSCLC. In addition, our data show that the complementary determination of N-cadherin and FGFR1/4 expression may further optimise patient selection for anti-FGFR therapy efficacy. Moreover, our PDX platform has also contributed to discovering Notch as a novel therapeutic target in lung adenocarcinoma osimertinib-treated patients after disease progression (Bousquet Mur E et al., *JCI* 2020), as well as to test novel combination therapies, including a novel neutralising anti-HER3 antibody, to overcome resistance to EGFR targeted therapies (Romanello D. et al., *Cancers (Basel)* 2020).

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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 2020, we participated in more than 45 projects in this research area, including 10 new projects. We reported data from novel combinations of first-line ramucirumab plus pembrolizumab (Herbst RS, Paz-Ares L, JTO 2020), and second-line ramucirumab plus osimertinib (Yu HA, Paz-Ares L, Clin Cancer Res 2020). Recently, we tested the safety and efficacy of bintrafusp alfa, a first-in-class bifunctional fusion protein composed of the extracellular domain of the transforming growth factor β (TGF-β) receptor II (a TGF-β-“trap”) fused to a human immunoglobulin G1 antibody blocking programmed death-ligand 1 (PD-L1) in patients with advanced NSCLC (Paz-Ares L, JTO 2020). In addition, we published a phase II trial of lurbinectedin, a novel transcription inhibitor, in small cell lung cancer with encouraging activity on the second-third line setting (Trigo J, Paz-Ares L, Lancet 2020). At present, combination studies with immunotherapies and atezolizumab are ongoing. Bintrafusp and lurbinectedin are now being tested in ongoing bintrafusp alfa and lurbinectedin trials.

Changing standardized care treatments in clinical practice

The Lung Cancer Clinical Research Unit haled phase III trials whose results have significantly impacted clinical practice in the combination of pembrolizumab plus chemotherapy in NSCLC patients (Paz-Ares L et al., JTO 2020). In the protocol-specified final analysis of KEYNOTE-407, this combination continued to exhibit a clinically meaningful improvement in survival (OS), progression-free survival (PFS), second PFS (PFS-2), overall response rate (ORR), and duration of response (DOR), compared with placebo plus carboplatin-paclitaxel/nab-paclitaxel in patients with previously untreated metastatic squamous NSCLC. In addition, the exploratory results of the PACIFIC trial of outcomes by tumour cell (TC) programed death-ligand 1 (PD-L1) expression showed that PFS benefit with durvalumab was observed across all subgroups, and OS benefit across all but TC <1%, for which limitations and wide HR CI preclude robust conclusions (Paz-Ares L et al., Ann Oncol 2020). Finally, the WILA trial showed the superiority (including prolonged survival) of a short course of chemotherapy plus pemipilumab and nivolumab, compared to chemotherapy alone in advanced NSCLC.