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) 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; and ii) 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 Fuenlabrada). These agreements foster the interaction between clinicians and scientists and allow scientists from all CNIO Programmes to participate in translational research studies. The ongoing collaborations between CRP Units and Groups from other CNIO Programmes now represent 16 projects and 3 coordinated grants, which accounts for the high translational research activity of the institution. Twelve medical oncology residents from different Spanish hospitals completed their 3-month optional stays at the CNIO during 2021.

Although clinical activity was hampered considerably in 2021 due to COVID-19, the clinical groups managed to produce highly impactful research. The Breast Cancer Clinical Research Unit, led by Miguel Quintela-Fandino, provided proof-of-concept about the right niches in which NGS profiling is indicated for patients with advanced breast cancer. The Lung Cancer Clinical Research Unit, led by Luis Paz-Ares, completed a chemo-immunotherapy registration trial in non-small-cell lung cancer, the most common subtype of lung cancer, and its results were recently published in The Lancet Oncology. The Haematological Malignancies Clinical Research Unit, headed by Joaquín Martínez López, developed a CAR-T therapy against multiple myeloma, a highly unmet clinical need. The Prostate Cancer Clinical Research Unit (PCCRU), headed by David Olmos, continued working on the development of novel therapies for prostate cancer, building a nomogram that can accurately predict benefit from abiraterone in advanced cases. Additionally, they fine-tuned the technology for disease monitoring with non-invasive techniques in peripheral blood: circulating tumour cells and cell-free DNA. Finally, the Molecular Diagnostics Unit, headed by Luis Lombardía, continued to provide support to hospitals in the diagnosis of different malignancies, performing >1000 diagnoses in 2021. In 2022, we expect major changes in the Programme: 1 Junior Group (PCCRU) is leaving the CNIO and moving to Instituto i +12 because of the completion of the junior scientist’s career development at the CNIO, and the active search for novel candidates to expand our activities is ongoing. ■

“The Clinical Research Programme aims to improve cancer care by developing novel agents and personalising therapeutic approaches on the basis of novel biomarkers.”
This year, we established a collection of 24 patient-derived tumoroids from breast cancer patients. We call a tumoroid a mix of a patient-derived organoid (a well-established model for cancer research, which perpetuates the tumour material from a given patient, preserving its mutations and general features, and is highly reliable for drug screening and predictive purposes) and the patient’s own immune cells. This sophisticated model allows us not only to screen conventional drugs, but also to understand their impact on the ability of the immune system to reject the tumour, a feature that is absent in common patient-derived mouse models of cancer. Tumoroids enable us to improve our understanding of immunotherapy and to better understand the impact of other drugs on the immune system, allowing for personalisation of synergistic treatment combinations. This collection is expanding, and we expect this to be the core of our research in the coming years.

Next-generation sequencing panels have become widely used tools to try to allocate and individualise treatments in advanced breast cancer. However, considerable controversies exist regarding their usefulness and the specific niches in which they should be used. We issued practical guidelines for the indication of these tests in advanced breast cancer on the basis of our results with 140 patients analysed using NGS and followed-up for clinical outcomes, proving that there are very few clinical niches that justify the use of these panels for now.

Finally, we completed our work regarding predictive factors of sensitivity to paclitaxel in early breast cancer from the perspective of phosphor-proteomics. ACDK4-Filamin A axis that converges in the regulatory machinery of tubulin acetylation is responsible for making cancer cells sensitive to this drug. This pair of markers is highly accurate in predicting sensitivity in the clinical setting. 

**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 mechanistic and clinical trials.

Our current research areas are:
- Studying the implications of hypoxia for immunotherapies.
- Understanding the individual factors regulating the response to immunotherapy in breast cancer, taking advantage of an advanced, personalised “tumoroid” platform.
- Tackling the mechanisms of resistance against novel therapies in advanced breast cancer.
- Incorporating our findings into concept-driven clinical trials.

**PUBLICATIONS**


**PATENT**

- Quintana-Fandino MA, P27 single-nucle- otide polymorphism as a predictor of benefit of hormonal therapy alone or in combination with CDK4 inhibitors in breast cancer. EP2020081533

**AWARDS AND RECOGNITION**

- CNIO and Applied Biological Materials Inc. (ABM) license agreement for the commercial use of HCT-PAK-knockout cells (developed in our laboratory) for non-human research purposes. December 10, 2021.
OVERVIEW

Prostate cancer 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 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 9 years, our group has made significant contributions in:

→ 1) Establishing and developing several biomarkers based on the concept of liquid biopsy;

→ ii) Understanding the implication of gene alterations leading to DNA repair deficiency in this disease.

→ iii) Developing new treatments for prostate cancer.

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 Lancet Oncology, The Journal of Clinical Oncology, European Urology, Annals of Oncology, and many others.

• PUBLICATIONS

The activity of the Molecular Diagnostics Unit (MDU) is primarily aimed at providing an array of reliable and time/cost-efficient molecular diagnostic assays to help our National Health System’s clinicians make early diagnosis, detect possible relapses, and/or monitor the response to therapy in patients with different cancers. Therefore, we devote effort to strengthening, updating and expanding the assays that are currently offered by implementing the latest tests available, or by upgrading the most established ones. Likewise, the Unit also provides support to the research needs of CNIO’s Clinical Research Units and Research Groups by checking their samples for alterations in the biomarkers included in our catalogue. Furthermore, MDU collaborates with several international and national organisations focused on the standardisation and improvement of molecular diagnostics in cancer. Finally, the MDU is also involved in disseminating knowledge in the field of molecular diagnostics by instructing biomedical students in our techniques and methods.

“Our last 15 years, MDU has supported over 300 clinicians by providing nearly 7000 specific and sensitive assays, with the aim of improving the diagnosis, prognosis, and response to therapy of more than 3000 cancer patients.”

Extending our portfolio

During 2021, we expanded our offer of assays by adding a new one that will enable the detection of activating mutations in exons 14 and 17 of the CSF3R gene encoding the receptor for colony-stimulating factor 3, a cytokine that controls the production, differentiation, and function of granulocytes. Alterations in CSF3R, commonly found in patients with chronic neutrophilic leukaemia (CNL) or some atypical chronic myeloid leukaemia (aCML), have been reported as useful prognostic and predictive markers, since patients with altered CSF3R showed an aggressive course of CNL and some sensitivity to ruxolitinib, a nonselective JAK inhibitor.

We also increased the detection coverage of a test implemented more than 10 years ago. This test uses qRT-PCR to detect BCL2-IgH fusion gene variants necessary for the diagnosis of follicular lymphoma and some cases of large B-cell lymphomas, as well as to monitor for minimal residual disease after treatment. The former assay detected only 50-60% of the cases with the MBR (major breakpoint region) variant and 5-10% with the mcr (minor cluster region) variant. However, recent findings have revealed new variants that had not been previously used to evaluate patients with follicular lymphoma. As a result, with the current test, we have improved our capability to notify those patients with follicular lymphoma sharing 3 MBR or ICR variants (10-15% of) and 5 mcr (15-20%) variants (FIGURE 1).

Finally, we also started a pilot study to evaluate the feasibility of implementing a test using Next Generation Sequencing technology that will enable us to analyse the mutational status of the IGHV (immunoglobulin heavy chain variable region) gene; this analysis is crucial for the prognosis and response to therapy of patients with chronic lymphocytic leukaemia.

Training

During the first semester of 2021, MDU hosted 2 undergraduate students who carried out their end-of-degree projects.
Haematology represents one of the most “hot topics” areas in cancer of the last 5 years, due to society’s growing interest in the immunology that drives one of the biggest discoveries of the 21st century, immunotherapy. In addition, haematology has been gaining traction because of the interest in applying peripheral blood analysis to determine the diagnosis and prognosis of multiple cancers and diseases, and in emerging promising cutting-edge technology such as liquid biopsy, a method currently used to measure minimal residual disease (MRD).

The Haematological Malignancies Clinical Research Unit focuses on 2 research areas: 1) novel immunotherapies against cancer and, more specifically, NK-CAR technology; and 2) the development and improvement of liquid biopsy protocols through next-generation-sequencing.

Moreover, our group investigates the molecular mechanisms of haematological malignancies and then uses the identified molecules and markers (e.g., PIEZO1, HNRNPK) to develop mouse models of the disease that could be exploited therapeutically.

“Our results showed that it was feasible to genetically modify Natural Killer (NK) cells from patients and to safely express CAR-NKG2D, as well as the efficacy of these cells against multiple myeloma.”

RESEARCH HIGHLIGHTS

NKG2D-CAR-transduced natural killer cells efficiently target multiple myeloma

CAR-T-cell therapy is the most common genetically modified cell-based immunotherapy. However, CAR-T therapy usually has high toxicity. In contrast, CAR-NK cells may exert less toxicity. To explore this, we analysed the antitumour activity of activated and expanded NK cells (NKAES) and CD45RA- T cells from multiple myeloma (MM) patients that were engineered to express an NKG2D-based CAR. Although memory T cells were more stably transduced, CAR-NKAES cells exhibited greater in vitro cytotoxicity against MM cells, while showing minimal activity against healthy cells. In vivo, CAR-NKAES cells mediated highly efficient abrogation of MM growth, with 25% of the treated mice remaining disease free. Overall, these results demonstrate that it is feasible to modify autologous NKAES cells from MM patients. They also show that it is possible to genetically modify NK cells from patients and to safely express CAR-NKG2D, as well as the efficacy of these cells against multiple myeloma.

Making clinical decisions based on measurable residual disease (MRD) improves the outcome in multiple myeloma

The use of MRD results to make clinical decisions in MM has been unexplored to date. In our study of 400 MM patients,
Implications of increased mitochondrial content in multiple myeloma

Many studies over the last 20 years have investigated the role of mitochondrial alterations in carcinogenesis. However, the status of the mitochondria in MM and its implication on the pathogenesis of the disease remain unclear. Our results showed increased mitochondrial (DNA content, gene expression, and activity) during the progression of MM. Mechanistically, OXPHOS metabolism was raised by regulation of increased MYC. We tested the efficacy of the mitochondrial inhibitor citicline, to overcome MM proliferation. In vivo and in vitro therapeutic targeting using the mitochondrial inhibitorciticline showed promising efficacy and cytotoxicity in monotherapy and in combination with the MM frontline treatment bortezomib. Thus, we identified new vulnerability in multiple myeloma and provide a novel alternative for MYC inhibition by targeting mitochondrial activity, as an indirect mechanism to avoid MM proliferation. 

**FIGURE 1**

NKG2D-CAR-transduced NKEs exhibit potent efficacy in vivo. (A) Imaging of tumor burden monitored by bioluminescence at the indicated time points in MM mice. NKE-CAR-transduced, and CAR-NK-transduced mice (all at 73 mm 2 and 3 from the NKE group were accidentally interchanged). (B) Quantification of the bioluminescence signal in 7 mice. NKE-CAR-transduced mice, CAR-NK-transduced mice, CD45RA− T-cell-treated mice, MM mice, NKAE-cell-treated mice, CAR-NKAE-cell-treated mice at the indicated time points. (C) Kaplan-Meier survival curves.

**FIGURE 2**

Kaplan-Meier curves showing the impact of making clinical decisions based on MRD. (A) MRD from the first MRD data point, comparing patients who were on therapy based on MRD with those for whom no change in therapy was made. (B) MRD-negative patients: treatment discontinuation (maintenance or transpl) vs. no change in therapy. (C) MRD-positive patients: beginning a new therapy or intensifying therapy vs. no change in therapy.

**Group Original Articles**

Clinical 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, always aiming to solve the problems of lung cancer patients. We are particularly interested in two 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 (PDXs) and organoids (PDOs) 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.

Biomarker discovery and implementation

We own an extensive patient-derived xenograft (PDX) platform of 50 non-small cell lung cancer (NSCLC) models that are comprehensively characterised at the histological, genomic, transcriptomic and proteomic levels, and that have contributed to the discovery of relevant findings. For example, using an EGFR+ NSCLC PDX-bearing huPBMC-driven humanized NSG mouse model, we have demonstrated the nontoxic broad antitumour activity of a humanized EGFR-targeted 4-1BB-agonistic trimerbody (4-1BBN/CEGFR) against EGFR+ tumours (Compte M et al., Clin Cancer Res 2021). Our platform has been expanding in number and histology types (including small cell lung cancer [SCLC] and mesothelioma), as well as cell source (tumours but also SCLC circulating tumour cells), and it includes PDXs and patient-derived organoids. We have also successfully developed a number of huPDX models.
Early clinical trials

Our Group has significantly expanded its activities regarding the field of immune checkpoint and targeted therapies, and in 2021, we participated in more than 129 research projects in this area, including 32 new trials. We reported data from the novel combinations ramucirumab plus pembrolizumab (Herbst RS, ...), ramucirumab plus osimertinib (Yu HA, ...), and necitumumab plus chemotherapy for advanced NSCLC: The CASPIAN Results. In this phase I clinical trials demonstrated that both combinations have encouraging safety and antitumour activity in advanced, treatment-naive NSCLC or T790M-positive EGFR-mutant NSCLC, respectively. We also reported patient outcomes from a global phase II basket study of entrectinib for ROS1 fusion-positive NSCLC and NTRK fusion-positive solid tumours. Findings were consistent with the favourable safety profile of entrectinib and confirmed the benefit-risk profile of this treatment, indicating minimal overall treatment burden.

could potentially predict response to immunotherapy (Ramos-Cardenas LC, ...). In addition, we have comprehensively characterized the immune and treatment landscape of a cohort composed of 120 resected tumour samples from limited-stage SCLC patients. These studies have led to the categorisation of specific subgroups of SCLC, which will allow us to understand individual immune escape mechanisms and to propose optimal individualisation treatment strategies (manuscript in preparation).

Changeing standard-of-care treatments in clinical practice

The Lung Cancer Clinical Research Unit has led phase III trials whose results have significantly impacted clinical practice in the context of advanced NSCLC, such as the combination of durvalumab plus etoposide with either cisplatin or carboplatin in extensive small cell lung cancer (ES-SCLC) patients (Goldman JW, ...). Lancet Oncol 2021. The unblinded results of the CASPIAN trial reported that first-line durvalumab plus platinum-etoposide showed sustained overall survival improvement versus platinum-etoposide, but the addition of tremelimumab to durvalumab plus platinum-etoposide did not significantly improve outcomes versus platinum-etoposide. In addition, we investigated whether adding a limited course (2 cycles) of chemotherapy to first-line tremelimumab plus platinum-etoposide could potentially improve overall survival compared to chemotherapy alone and had a favourable benefit-risk profile. ■

SELECTED PUBLICATIONS

• Paiz-Ares L et al. (2021). First-line nimotuzumab plus platinum-splitted combination with cisplatin plus platinum-etoposide (HE: D75 [95% CI 0.62-0.81]) in treatment-naive patients with extensive-stage small-cell lung cancer (ES-SCLC).


• PATENTS

• Paiz-Ares L, Martínez Torrecilla A, ... (2021). Interleukin-11 receptor alpha subunit (IL-11Ra) using recombinant and purified forms, their applications. PCT/ES2021/050074. PCT application.


• OTHER INSTITUTIONS


