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

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) the development of novel agents; 2) the 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 new operating model: a) generating synergies with ongoing research lines in the basic research programmes; and b) constituting a bi-directional bridge to facilitate interaction 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 La Paz, and Hospital de Fuenlabrada). These agreements foster the interaction between clinicians and scientists and enable scientists from all CNIO Programmes to participate in translational research studies. The ongoing collaborations between CRP Units and CNIO Groups from other Programmes now represent 18 projects and 4 coordinated grants, which account for the CNIO’s high translational research activity. During 2022, 9 medical oncology residents from different Spanish hospitals completed their optional training visits (3-month stays) at the CNIO.

Although clinical activity was hampered considerably in 2022 due to COVID-19, the clinical groups have managed to produce highly impactful research. The Breast Cancer Clinical Research Unit, led by Miguel Quintela-Fandino, found the first specific, predictive, and explainable predictive factors for paclitaxel. The Lung Cancer Clinical Research Unit, led by Luis Paz-Ares, contributed to several immunotherapy registration trials for new standards of care in lung cancer. 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 Molecular Diagnostics Unit, headed by Luis Lombardía, continues to provide support to hospitals in the diagnosis of different malignancies, performing >1000 diagnosis this year. Also in 2022, the Junior Prostate Cancer Clinical Research Unit ended its stay with us because of the completion of the Junior Group Leader’s career development plan at the CNIO, and we started a process of recruiting additional Groups for the Clinical Research Programme. The selection process is now complete and we are excited to announce the incorporation of 2 new Senior Groups: the Hospital 12 de Octubre-CNIO Cancer Immunotherapy Clinical Research Unit, led by Dr Luis Álvarez-Vallina; and the Hospital La Paz-CNIO Paediatric Cancer Clinical Research Unit, headed by Dr Antonio Pérez-Martínez. These groups will cover 2 unmet needs at the CNIO: the development of novel cancer immunotherapy agents, as well as research in childhood cancer.
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.

Our current research areas aim to:

- Study the implications of hypoxia for immunotherapies.
- Understand the individual factors regulating the response to immunotherapy in breast cancer, taking advantage of an advanced, personalised “tumouroid” platform.
- Tackle the mechanisms of resistance against novel therapies in advanced breast cancer.
- Incorporate our findings into concept-driven clinical trials.

“At the Breast Cancer Clinical Research Unit, we are focused on individualising therapy for advanced breast cancer.”
We have established a collection of 35 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 cells derived from the immune system. 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 personalised synergistic treatment combinations. This collection is expanding, and we plan to this be the core of our research in the coming years.

A critical problem in hormone-positive breast cancer is the development of clonal heterogeneity. Tumours, after progression on aromatase plus CDK4/6 inhibitors, develop many different mutations to circumvent drug exposure, impacting the duration of response to subsequent treatments. Our preliminary data suggest that different tumour sub-compartments harbour different sets of mutations, and even selecting a “right” therapeutic choice is insufficient for eradicating a whole tumour. We are now undertaking an approach based on mutational signatures that are pervasive across different clonotypes that are pervasive across different clones and that may allow for selecting therapies that kill broader tumour compartments than therapies selected according to traditional point mutations. This is being tested in patient tumors.

We finalised our work regarding predictive factors of sensitivity to paclitaxel in early breast cancer from the perspective of phosphoproteomics. A CDK4-Filamin A axis that converges in the regulatory machinery of tubulin acetylation is responsible for turning cancer cells sensitive to this drug. This pair of markers is highly accurate in predicting sensitivity in the clinical setting.

### Figures

**Figure 1** Fluorescently labelled paclitaxel was added to live cultures of MDA-MB-231 WT, CDK4 or FLNA cells. MDA-MB-231 CDK4 cells with Filamin A knockdown were added to the experiment as well. The greener the signal, the higher the amount of paclitaxel bound to microtubules. One can appreciate how both CDK4- and filamin A-overexpressing cell lines display both earlier and higher paclitaxel binding. Scale bar: 75 micrometres. The chart on the right-hand side depicts the signal (in fluorescent surface units) for paclitaxel accumulation over a 48-hour time interval, displaying a clearer increase in the 2 overexpressing transfectants (CDK4 and FLNA) compared to the parental cell line, and a reversion of the phenotype by Filamin A knockdown in MDA-MB-231 CDK4 ΔFLNA cells. General methodology for patient-derived organoid generation.

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**PUBLICATIONS**

- **β of PI3K/AKT and CDK4/6 in breast cancer.** Clin Cancer Res 28, 2257-69.
- **β of PI3K/AKT and CDK4/6 in breast cancer.** Mol Cancer 16, 148-165.
- **A Phase I study investigating AZD8186, a potent and selective inhibitor of CDK4 and 6, in patients with advanced solid tumors.** Oncologist 27, 2587-97.
- **A clinicogenomic approach identifies co-amplification of MYC and CDK4 in triple-negative breast cancer.** EMBO Mol Med 14, e5152.
- **A clinically compatible drug-screening platform based on organotypic cultures identifies vulnerabilities to prevent and treat breast cancer.** EMBO Rep 14, e44652.

**INTELLECTUAL PROPERTY REGISTRATION**


**Awards and Recognition**

- Finalist, Preparado de Investigación en Salud, Instituto de Salud Carlos III (IS-CIII), Spain.
OVERVIEW

The Molecular Diagnostics Unit (MDU) is primarily engaged in providing support to oncologists, haematologists and pathologists of our National Health System, by offering quality molecular tests for cancer patients. In this regard, the Unit has developed a catalogue with a broad variety of sensitive and specific assays to determine changes in sequences or expression levels of crucial genes that are involved in cancer, and that help to monitor minimal residual disease in patients showing clinical remission as well as to follow-up on their response to therapy. Consequently, MDU is also committed to implementing novel diagnostic solutions, not only to improve clinical practice but also to resolve periodic inquiries from CNIO’s Research Units and Groups. MDU also forms part of several international and national groups aimed at normalising and improving molecular tests in cancer. Finally, an essential part of our mission is to contribute to academic programmes by hosting clinical post-residents and pre/post graduate students.

“The ongoing accumulation and combination of actionable biomarkers included in molecular diagnostics tests is bringing us closer to precision medicine, especially for haematological tumours.”

During 2022, our catalogue grew with the addition of a new assay, which will enable the detection, through bi-directional Sanger sequencing, of structural alterations in exon 3 of the β-catenin gene, CTNNB1. High frequencies of CTNNB1 activating mutations and in-frame deletions have been spotted in 3% of all cancers, including melanoma, lung, endometrium, colon, kidney, and ovarian tumours. Since they have been associated with altered sensitivity to specific drugs, their analysis can be useful as a predictive marker by suggesting different therapy options.

We also improved the clinical utility of KRAS gene testing by supplementing the detection of the recurrent mutations already implemented in exons 2 and 3 to exon 4. The extended assay is intended to enable clinicians to manage their patients with colorectal, pancreatic, or lung adenocarcinomas, since somatic mutations in exon 4 have been linked to a better prognosis, and they can also be used as an inclusion criterion to enrol patients in active or forthcoming clinical trials.

Additionally, in the context of our partnership with GBMH (Grupo de Biología Molecular y Hematología), we are participating in the development of comprehensive national guidelines for the management of patients with different haematological cancers. Our initial contribution was to complete a list of diagnostic, prognostic, and predictive markers that should be systematically analysed using Next Gene Sequencing (NGS) in order to manage patients with acute myeloid leukaemia (AML). To evaluate the clinical and analytical utility of this diagnostic tool, the next step will be to design a panel containing at least the markers required for the analysis, and then to establish the feasibility of using RNAseq technology to be able to analyse simultaneously both single and fusion genes (FIGURE 1).

Finally, during 2022, in the framework of our training policy, we hosted a medical resident, an undergraduate student, and 2 future technicians in anatomical pathology.
Haematological clinical research has traditionally focused on haematological malignancies, aplasia, and syndromes. Now, with advances in immunotherapy, haematologists play a key role in research on novel immunotherapeutic approaches, the role of the immune response to tumours, or the role of infection and inflammation in cancer.

In the Haematological Malignancies Clinical Research Unit at CNIO we investigate:

- Traditional haematological neoplasms (leukaemia, myeloma, lymphoma): new diagnostic approaches, biomarkers, and treatments.
- Aplastic haematological malignancies such as bone marrow failures: new drivers and molecular mechanisms.
- Novel diagnosis and tumour burden monitoring: liquid biopsy and minimal residual disease.
- Role of inflammation and infection in haematological neoplasms.
- Novel immunotherapeutic approaches in haematological malignancies: NK-CARs, BITES.
- Traditional immunotherapeutic approaches in haematological malignancies and paediatric cancers: T-CARs and immune checkpoints inhibitors.

“Teclistamab, a bispecific anti-CD3 and anti-BCMA monoclonal antibody, marker of myeloma cells, has demonstrated a high rate of deep and durable response in relapsed multiple myeloma patients.”
Teclistamab in relapsed or refractory multiple myeloma

Teclistamab is a bispecific anti-CD3 and anti-BCMA monoclonal antibody. We recently published, in collaboration with other groups in the consortium, a novel clinical trial in multiple myeloma-relapsed patients. Our results showed a high rate of durable and deep response in the patients studied, with toxicity (grade 1 and 2) consistent with T-cell reeducation.

Tisagenlecleucel trials in B-cell lymphomas

Tisagenlecleucel is an autologous anti-CD19 chimeric antigen receptor (CAR) T-cell therapy approved for different B-cell lymphomas. In 2022 we published, in collaboration with other groups in the consortium, an article in the New England Journal of Medicine describing the results of second-line tisagenlecleucel in aggressive B-cell lymphoma. Our results showed that tisagenlecleucel was not superior to standard salvage therapy in this trial.

However, in another clinical trial, the ELARA phase 2 multinational trial against follicular lymphoma, we reported its safety and effectiveness in high-risk patients with relapsed follicular lymphoma. This work was recently published in Nature Medicine.

Infection prediction in multiple myeloma

Infections are among the most common complications in multiple myeloma, in association with morbidity and mortality. We analysed the clinical variables of 4 clinical trials of the Spanish Myeloma Group with n=1,347 patients. We discovered that an increased risk of severe infection correlates with serum albumin, ECOG, gender, and -1q42 type multiple myeloma. These simple variables led to the stratification into low, intermediate, and high risk of severe infection. Patients with intermediate and high risk could be candidates for prophylactic antibiotic therapy. This work was published in Blood Cancer Journal.
Lung cancer continues to be the most frequent cause of cancer-related deaths worldwide. Our Unit focuses on the study of lung cancer, with a pragmatic orientation, 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 developing 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 2/3 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 immunotherapies and other agents as monotherapies or in combination (e.g., chemotherapy plus durvalumab in SCLC or chemotherapy plus nivolumab and ipilimumab in NSCLC) in lung cancer that have impacted clinical practice worldwide.

**Overview**

**Biomarker discovery and implementation**

We own an extensive patient-derived xenograft (PDX) platform of 50 non-small cell lung cancer (NSCLC) and 7 small cell lung cancer (SCLC) 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, 2 NSCLC PDX models with high and low expression levels of EGFR contributed to demonstrate that cetuximab-functionalised gold nanoparticles can be used for selective drug delivery in mitochondria-targeted cancer therapy (González-Rubio S et al., *Nanoscale*, 2022). In addition, SCLC PDXs were used to confirm YB1 as a new druggable oncogenic target in SCLC. Pharmacologic blockade with the novel YB1 inhibitor CH6953755 or dasatinib induced marked antitumour activity in organoid models and cell- and patient-derived xenografts (Redin E et al., *J Thorac Oncol*, 2022). Our platforms are expanding in numbers and histologies (NSCLC, SCLC and mesothelioma as well), cell source (tumours but also circulating tumour cells), and include PDX and patient-derived organoids. We have also successfully developed a number of huPDX models.

We have comprehensively characterised the molecular and immune features of a cohort of 18 early-stage, clinically annotated, large cell carcinoma (LCC) cases by genomic and immune-targeted sequencing panels, along with immunohistochemistry of immune cell populations (FIGURE 1). Unbiased clustering defined 2 novel subgroups of LCC that allowed us to identify a set of biomarkers that could potentially predict response to immunotherapy in the least studied form of NSCLC (Ramos-Paradas J.,... Páz-Ares L., *J Clin Med*, 2022). In addition, we performed a multiparametric characterisation of a cohort composed of 120 resected tumour samples from limited-stage
SCLC patients. Samples were described by immunohistochemistry, RNA-seq targeted panel and cancer genome-related exome, genetic sequencing, and spatiotemporal cascading. We found a novel classification of early-stage SCLC with potential clinical impact in both prognosis and immunotherapy response (manuscript in preparation).

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 and targeted therapies; in 2022, we participated in more than 150 projects in this research area, including 85 new trials. We reported data from a multicenter, international, phase 2 study in which trastuzumab deruxtecan was administered to patients with metastatic HER2-mutant NSCLC that was refractory to standard therapy. Trastuzumab deruxtecan showed durable antitumour activity, and the observed toxicity effects were generally consistent with those in previously reported studies (Li et al., Paz-Ares L., Nat Eng J Med, 2022).

We also evaluated the efficacy and safety of pralsetinib in patients with RET fusion-positive solid tumours. Our pan-cancer phase 1/2 clinical trial showed pralsetinib as a potential well-tolerated treatment option with robust, rapid and durable antitumour activity in these patients (Subbiah V., Paz-Ares L., Nat Med, 2022).

Changing standard-of-care treatments in clinical practice
The Lung Cancer Clinical Research Unit has led phase 3 trials whose results have significantly impacted clinical practice in the context of stage IV lung cancer, such as the combination of first-line nivolumab plus ipilimumab in advanced NSCLC (Paz-Ares L et al., J Thorac Oncol, 2022). With the updated results from the randomised, open-label, phase 3 CheckMate 227 Part 1 trial, we showed that at more than 4 years’ minimum follow-up, with all the patients off immunotherapy treatment for at least 2 years, first-line nivolumab plus ipilimumab continued to demonstrate durable long-term efficacy. We also assessed pembrolizumab as adjuvant therapy for completely resected stage IB-IIIA NSCLC (O’Brien M, Paz-Ares L et al., Lancet Oncol, 2022). In this randomised, three-blind, phase 3 trial we found that pembrolizumab significantly improved disease-free survival compared with placebo and was not associated with new safety signals (FIGURE 2). Pembrolizumab is potentially a new front-line treatment option for stage IB-IIIA NSCLC after complete resection and, when recommended, adjuvant chemotherapy, regardless of PD-L1 expression.


Our Unit focuses on understanding the molecular and cellular mechanisms of cancer immune escape in order to design next-generation cancer immunotherapies. For example, we have developed a novel strategy based on the secretion of bispecific T cell-engaging antibodies by engineered human T (STAb-T) cells, which has been shown to be effective in solid and haematological malignancies and is currently being tested in clinical trials. The Cancer Immunotherapy Clinical Research Unit has several research areas of interest: 1) reactivation of T cell acute lymphoblastic leukemia with engineered and unmodified bystander T cells, present at the tumour site, might lead to a significant boost in antitumour T cell responses (FIGURE 1). During 2022, we demonstrated the remarkable therapeutic impact in preclinical models of haematological cancers (B cell leukaemia, T cell leukaemia and multiple myeloma), with a cell product (STAb-T19) currently in a phase I, first-in-human clinical trial in patients with B cell malignancies. Throughout this period, the implementation of this strategy in solid tumours, as well as the design of dual targeting strategies, has been considerably improved.

**OVERVIEW**

Our Unit is committed to introducing new immuno-oncology drugs and adopting cell therapies in the clinic, to provide high-quality personalised treatments.