The Clinical Research Programme (CRP) aims to translate advances in cancer research into the prevention, diagnosis, and treatment of patients. The major goals of the CRP are the conduction of early clinical trials with novel drugs, the discovery of biomarkers of drug action and disease outcome, the implementation of a strategy for personalised medicine, and the launching of a training programme in drug development.

The CRP is composed of 5 Clinical Research Units (CRU) and 1 support Unit. The Gastrointestinal Cancer CRU, led by Manuel Hidalgo until December 2016, studies novel therapeutics and personalised medicine in pancreatic cancer. Miguel Quintela-Fandino leads the Breast Cancer CRU that works on the development of kinase and angiogenesis inhibitors in breast cancer, as well as on the understanding of the molecular taxonomy and metabolic vulnerabilities of this disease. The Prostate Cancer CRU, led by David Olmos, explores novel therapeutics and biomarkers of the disease, with a particular interest in understanding DNA damage repair deficiency mechanisms in prostate cancer. The Lung Cancer CRU, headed by Luis Paz-Ares, and the Haematological Malignancies CRU, led by Joaquin Martinez-Lopez – both established as part of an agreement with the Hospital Universitario 12 de Octubre – focus on molecular and preclinical studies in non-small cell lung cancer and in multiple myeloma, respectively. The Molecular Diagnostics Unit, led by Luis Lombardía, provides support to medical professionals of the National Health system and the CRP through the provision of a wide variety of molecular tests that determine alterations in biomarkers involved in cancer. In 2016, the Programme continued the expansion of its clinical trials activities in collaboration with several hospitals in Spain.

“The Clinical Research Programme focuses on developing novel and more effective treatments against cancer.”
OVERVIEW

The Gastrointestinal (GI) Cancer Clinical Research Unit focuses on the clinical development of novel therapeutics for patients with cancers of the gastrointestinal tract as well as personalised medicine approaches for these patients. The work of the Group combines the preclinical assessment of novel anticancer agents in ‘Avatar’ mouse models with the design, conduction, and analysis of clinical trials with novel anticancer agents in patients with gastrointestinal tumours. Over the last few years the Group has implemented a growing portfolio of clinical trials with new agents spanning a broad range of mechanisms of action.

Key to the work is the development and characterisation of Avatar mouse models for drug screening, biomarker development, and personalised medicine. The Group has developed and has characterised the largest collection of these models in pancreatic cancer. Avatar models are used in 3 critical applications: (i) the screening of new anticancer agents, (ii) conduction of co-clinical trials, in which ongoing clinical trials are performed in parallel with studies using Avatar models of the same cancer type in order to elucidate mechanisms of action and biomarkers of drug response/resistance; and (iii) finally, the Avatar models for personalised cancer treatment integrated with next generation sequencing.

Key to the work is the development and characterisation of Avatar mouse models for drug screening, biomarker development, and personalised medicine. The Group has developed and has characterised the largest collection of these models in pancreatic cancer. Avatar models are used in 3 critical applications: (i) the screening of new anticancer agents, (ii) conduction of co-clinical trials, in which ongoing clinical trials are performed in parallel with studies using Avatar models of the same cancer type in order to elucidate mechanisms of action and biomarkers of drug response/resistance; and (iii) finally, the Avatar models for personalised cancer treatment integrated with next generation sequencing.

• PUBLICATIONS
In the field of functional taxonomy, we have completed our study in triple-negative breast cancer. We have interrogated the disease from the bimodal relapse pattern point of view, and performed a phosphoproteomic screening that would reduce the countless patterns of genomic, epigenomic and transcriptomic aberrations into a discrete number of patterns of hardwired signalling pathways. We found 6 kinases whose hyperactivity accounted for 94% of the relapsed cases. These kinases were grouped into a maximum number of 34 patterns, the largest of which (25%) was virtually associated with cure. This taxonomy was also useful because all the kinases in the final ‘relapse signature’ were also targetable nodes.

Regarding the study of targeted therapies, we have observed that the generally assumed hypothesis of vascular normalisation upon exposure to antiangiogenics is not always true. In fact, resistance against antiangiogenics can originate after a vascular normalising or ‘abnormalising’ response. Whether a tumour experiences the former or the latter depends on the tumour type and the type of agent. What is quite important from the clinical point of view is that we can track, individually, whether a tumour experiences a normalising or an abnormalising response after less than 2 weeks of exposure to the agent, using a non-invasive imaging test with 18F-fluoromisonidazole. This has been demonstrated in animals and in patients. The applicability of this finding lies in the fact that we have also unravelled the mechanisms of resistance depending on whether the tumour reacts with normalisation or abnormalisation against antiangiogenics: in the first case, the tumour switches from glycolytic to mitochondrial metabolism, which is reversible by mitochondrial inhibitors. In the latter, the tumour experiences an immune-switch. Since both mechanisms are targetable, we can now individually track which pathway a tumour is undergoing upon exposure to antiangiogenics and tailor which synergistic agent that patient would need.
An adequate biological knowledge and reliable biomarkers to deleterious mutations are linked to poor outcomes. Currently, we has established that some alterations, e.g. germline and mCRPC, respectively. Seminal work from our Group, and others, repair defects have been identified in about 5% and 25% of early PrCa therapy leading to resistance to current treatment options, DNA.

In addition to AR aberrations following androgen-deprivation predisposition to develop aggressive mCRPC could lead to the early identification of PrCa patients with greater succumb after the acquisition of a castration-resistant status. Despite advances in diagnosis and early-disease treatment, up 30% of PrCa patients will develop metastasis at some point leading cause of cancer mortality among men in Western countries. Prostate cancer (PrCa) is the most common cancer and the 2nd

OVERVIEW

Prostate cancer (PrCa) is the most common cancer and the 2nd leading cause of cancer mortality among men in Western countries. Despite advances in diagnosis and early-disease treatment, up to 30% of PrCa patients will develop metastasis at some point and succumb after the acquisition of a castration-resistant status (mCRPrCa). The early identification of PrCa patients with greater predisposition to develop aggressive mCRPC could lead to the development of novel treatment strategies and improved outcomes. In addition to AR aberrations following androgen-deprivation therapy leading to resistance to current treatment options, DNA repair defects have been identified in about 5% and 25% of early PrCa and mCRPC, respectively. Seminal work from our Group, and others, has established that some alterations, e.g. germline BRCA1/BRCA2 deleterious mutations, are linked to poor outcomes. Currently, we lack the adequate biological knowledge and reliable biomarkers to select the right treatment for the right patient at the right time.

RESEARCH HIGHLIGHTS

PROCURE biomarkers platform

This network was started by our Group in 2013; it currently has 5 ongoing prospective studies (PROREPAIR, PROSTAC, PROSABI, PROSENZA, PRORADIAM) in mCRPC in 63 participating centres with over 900 enrolled patients.

PROREPAIR study

This is a prospective multicentre cohort study involving 50 Spanish centres within the PROCURE network. By April 2016, 432 mCRPC patients were enrolled to evaluate the prevalence and impact of DNA repair germline mutations in mCRPC survival and the response to systemic treatments for mCRPC. Germline mutations were analysed in the following genes: ATM, ATR, RAD51, BRCA2, BRCA1, BRIPI, CHK2, GEN1, MLH1, MRE11A, MSH2, MSH6, NBN, PALB2, PMS2, RAD51C, RAD51D and XRCC2. Current results suggest that up to 12% of the patients in this series harbour a germline deleterious mutation. Analyses of the clinical impact of germline and somatic mutations in outcomes are still undergoing. BRCARAD and BRCAPROS studies, although in a retrospective fashion, will address similar questions at an early prostate cancer stage.

SWITCH Phase II study

In 2016, we also completed the enrolment and follow-up of our first clinical trial, Phase II pilot study of the prednisone to dexamethasone switch in mCRPC patients with progression on abiraterone and prednisione1, aimed at analysing the role of certain steroids in the resistance and response to novel androgen-synthesis inhibitors in 28 patients. A simple change in prednison to dexamethasone rescued the sensitivity to abiraterone and prolonged the time benefiting from this treatment in 40% of the patients; such responses could be linked to AR mutations detected in cDNA.

Biological characterisation of BRCA2 and ATM mutated tumours

Initial results from human tumour characterisation and mouse models conducted by our Group support that BRCA2 germline and/or somatic alterations may occur early in cancer progression, and that ATM aberrations will favour cancer progression and early intratumour heterogeneity.

1. Nuria Romero (since September), Elena Castro (since March), Fernando Lázaro, Maria L. Pacheco, Leticia Romera (since May).
**OVERVIEW**

The Molecular Diagnostics Unit (MDU) is mainly dedicated to developing, implementing, standardising and making available a wide variety of highly sensitive and specific molecular diagnostics assays that are scarcely available in the Hospitals of the Spanish National Health System. MDU’s portfolio of genetic tests enables the determination of alterations in the sequence or expression levels of key genes involved in cancer. In turn, these assays can be used for the early diagnosis of neoplasias, the detection of minimal residual disease in patients showing clinical remission, for monitoring the response to therapy in patients, as well as for facilitating decision-making amongst different treatment options. Furthermore, 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 portfolio. Finally, MDU is very much committed to disseminating knowledge in the field of molecular diagnostics by hosting and mentoring biomedical students.

“**In this transition phase of precision medicine, MDU is increasingly focused on the implementation of assays for the detection of biomarker alterations that could grant a more selective diagnosis for cancer patients.**”

**RESEARCH HIGHLIGHTS**

**Strengthening our support**

During 2016, our catalogue has grown with the addition of a new molecular diagnostics test based on the detection, by bi-directional Sanger sequencing, of mutations in exons 4 and 5 of the MYD88 gene. Waldenström’s macroglobulinemia (WM) is a rare form of blood cancer that is characterised by an excess of malignant white blood cells (lymphoplasmacytic cells) in the bone marrow. It has been shown that WM is the result of a multistep transformation process that accumulates sequential oncogenic alterations. The most prominent is the L265P somatic activating mutation in the MYD88 gene (present in 90% of WM). Hence, its detection would enable us to differentiate WM (but also diffuse large B-cell vitreoretinal lymphoma or marginal zone lymphomas) from indolent B-cell or other chronic lymphoproliferative disorders.

Additionally, because identification of several gene alterations involved in the onset of myeloproliferative neoplasms (MPNs) has revealed the huge complexity of these diseases and has challenged their accurate differential diagnosis, we started working on the implementation and validation of a new assay that will enable us to detect mutations in the TET2 gene, this will complement the diagnosis of MPNs patients. Mutations in this tumour suppressor gene (present in 13% of MPNs) lead to genomic instability via epigenetic modifications and foster cancer progression. Recent studies have revealed that the order in which these mutations are acquired is critical. Thus, patients with early mutations in TET2 were more likely to have better prognosis compared to patients who had previous mutations in others genes linked to MPNs (FIGURE).

Lastly, we have completed the initial experimental phase of a clinical trial sub project, FRAGANCE, led by the CNIO Gastrointestinal Cancer Clinical Research Unit, which is geared towards precision medicine for fragile patients with advanced pancreatic cancer.

**Tutoring**

MDU has also upheld its policy regarding training programmes in 2016 by welcoming one medical resident and one undergraduate student.
RESEARCH UNIT
H12O-CNIO HAEMATOLOGICAL MALIGNANCIES CLINICAL RESEARCH UNIT

ANNUAL REPORT 2016

Vice-Direction of translational research

In vitro research

Molecular research of haematological cancer on 3 main objectives:

1. To translate preclinical findings to the mechanisms of resistance to anticancer drugs.
2. To determine new genomic and proteomic biomarkers for a better diagnosis of these haematological diseases.
3. To study immune response to cancer cells using activated and expanded NK cells.

Clinical Research Unit Head

Joaquín Martínez-López

Clinical Research Unit

Finalised the role of stringent complete response in elderly patients with newly diagnosed MM.

SELECTED PUBLICATIONS AT OTHER INSTITUTIONS


PUBLICATIONS

Finally, we redefined the role of stringent complete response by next generation sequencing in amyloidosis. We have not found any recurrent mutation.

We reported a phase I clinical trial based on an innovative cell therapy approach using activated and expanded NK cells in Multiple Myeloma (MM). The results of exploring this approach in phase II and III clinical trials are promising.

We published the first report of exosome sequencing in amyloidosis. We have not found any recurrent mutation.

We contribute towards redefining the role of stringent complete response by next generation sequencing in amyloidosis.

Figure Phenotyping of multiple myeloma macrophages. (A) from Bone Marrow (BM) patient samples. (A) Multi-colored staining of BM aspirates containing particles from active disease MM patients, as indicated. Upper panels represent pan-macrophage views, whereas bottom panels are magnified ones. Nuclear-6-diamino-2-phenylindole appears in blue in all cases. (B) Plot showing the mean fluorescence intensity for each marker in CD163+ tumour associated macrophages (TAM; n = 10 cases). Cells > 25 arbitrary units (a.u.) are considered positive, relative to negative control. Scale bars as indicated.

The most relevant achievements of our Group in 2016 were:

We reported a phase I clinical trial based on an innovative cell therapy approach using activated and expanded NK cells for Multiple Myeloma (MM). The results of exploring this approach in phase II and III clinical trials are promising.

We published the first report of exosome sequencing in amyloidosis. We have not found any recurrent mutation.

Finally, we redefined the role of stringent complete response by next generation sequencing in Multiple Myeloma.

OBERVIEW

The Haematological Malignancies Clinical Research Unit focuses on 3 main objectives:

1. Molecular research of haematological cancer: the study of cancer-induced changes at the proteomic and genomic levels. We aim to:
   - Identify new genomic and proteomic biomarkers for a better diagnosis of these haematological diseases.
   - Identify new molecular alterations as predictors of response to treatment, e.g. to study minimal residual disease.
   - Study immune mechanisms of cancer control, with a special focus on NK cells.

2. In vitro research:
   - Establish the effect of new anticancer molecules in vitro models of the disease.
   - Determine the mechanisms of resistance to anticancer drugs.

3. Clinical research: translate preclinical findings to the patients through a phase I clinical trial unit.

WE CONtribute towards redefining the role of stringent complete response criteria for Multiple Myeloma (MM) through the usage of new molecular techniques. In 2016, we published the first reports sequencing complete exomes of amyloidosis. Finally, we reported a new cell therapy approach based on infusion of NK cells in MM.
Our Group combines basic preclinical studies with clinical and translational research, mainly in lung cancer and other solid tumours. In summary, the main research areas of our Group focus on 2 modalities: (1) the identification of new molecular biomarkers that can be used in the clinic for diagnostic, prognostic, predictive and pharmacogenic purposes; and (2) developing novel treatment strategies. For example, we have comprehensively profiled bronchoalveolar lavage (BLA) fluids of COPD and lung cancer patients, showing a differential miRNA, protein and inflammatory cytokine expression between both diseases and different subtypes of lung cancer. On the other hand, we have developed a patient-derived xenograft (PDX) platform of non-small-cell lung cancers to test new drugs/targets. We are also developing PDXs of small-cell lung cancers. Finally, our Group has extensive experience in the development of new drugs, as well as in conducting practice-changing phase II/III trials in the fields of precision oncology and immuno-oncology.

**NEW DEVELOPMENT AND EARLY CLINICAL TRIALS**

Our Group has been actively involved in pharmacogenomic, pharmacokinetic, translational and clinical studies with novel antitumour agents in several types of solid tumours, particularly lung cancer. Our principal translational research area has been immunotherapy and immune-based early clinical trials. As a first relevant example we can mention the CheckMate CA 209-032 trial testing nivolumab +/- ipilimumab in recurrent or extensive-stage small-cell lung cancer, which was fully recruited in 2016, with a substantial contribution by investigators from our Group. These important data have been recently published in The Lancet Oncology. In addition, Luis Paz-Ares is the principal investigator of a phase 1 trial (JIVD) testing a novel combination of pembrolizumab plus ramucirumab in different types of solid tumours. Encouraging preliminary clinical data were presented at ASCO 2016 in the cohort of non-small-cell lung cancer, showing a response rate of 35% and 7-months of progression-free survival in pretreated patients. Finally, a first-in-human trial with a novel T-cell bispecific antibody targeting carcinoembryonic antigen (CEA) expressed on tumour cells and CDS-5 on T-cells was initiated and is actively recruiting patients.

**RESEARCH HIGHLIGHTS**

**New drug development and early clinical trials**

Our Group has also made a substantial contribution in conducting pivotal trials with immune checkpoint inhibitors. In particular, an important phase III trial, led by Dr Paz-Ares (the international principal investigator), with pembrolizumab in completely resected non-small cell lung cancer patients is actively recruiting participants. Furthermore, the first randomised trial comparing second-generation (afatinib) versus first generation (gefitinib) tyrosine-kinase inhibitors in patients with EGFR-mutant lung cancers, also internationally led by Dr Paz-Ares, was completed in 2016 and its results were recently published in The Lancet Oncology.

**Conducting practice changing randomised controlled trials**

IL-11 and CCL-1 have been proposed as novel diagnostic biomarkers of lung adenocarcinoma in bronchoalveolar lavage fluid. This finding has potential implications in early lung cancer diagnosis. Moreover, different members of our Group contributed towards providing further insights into the role of PD-L1 expression and other potential immune biomarkers for the benefit of immune checkpoint inhibitors.