# X SIMPOSIO · SYMPOSIUM | 2025
# BIOPSIA LÍQUIDA · LIQUID BIOPSY

## EL CAMINO A LA ONCOLOGÍA DE PRECISIÓN · THE WAY TO PRECISION MEDICINE

**23, 24 Y 25 DE ENERO 2025** · JANUARY 23rd, 24th and 25th 2025

# BOOK OF ABSTRACTS


**Director científico:**
*Scientific director:*

**Dr. Rafael López López**
Medical Oncology Service
Complexo Hospitalario Universitario de Santiago de Compostela

## List of abstracts

| | |
|---|---|
| **1** | Proteomics of Plasma-Derived Extracellular Vesicles Isolated with ExoGAG for Lung Cancer Biomarkers |
| **2** | OCTOPUS: blood-based signature to predict immunotherapy response in advanced NSCLC patients |
| **3** | Patient-Derived Organoids from Uterine Aspirates of Endometrial Cancer Patients: A Preclinical Model for Testing Novel Therapies. |
| **4** | BlaDimiRplus: A precise, multimodal, urine-based tool for non-invasive prediction of immunotherapy response in bladder cancer |
| **5** | Clinical utility of ctDNA detection in cerebrospinal fluid by NGS for diagnosis of CNS Lymphoma |
| **6** | Exploring the Stool Virome as a Source of Biomarkers for Colorectal Cancer Screening |
| **7** | IDENTIFICATION OF MINIMAL RESIDUAL DISEASE USING ULTRASENSITIVE ctDNA DETECTION TO ANTICIPATE LATE RELAPSE IN EARLY BREAST CANCER |
| **8** | Insights into Carboplatin Resistance in High Grade Serous Ovarian Cancer: A Study Using Ascitic Fluid-Derived Organoids |
| **9** | Novel patient-specific ctDNA-NGS test to predict outcome in real-word follicular lymphoma patients |
| **10** | Testing for ESR1 Mutations to Guide Therapy in Patients with Metastatic Breast Cancer in Lleida |
| **11** | Liquid biopsy as a tool for identifying actionable mutations in metastatic breast cancer: Insights from a clinical cohort |
| **12** | Non-invasive epigenetic biomarkers for detection and prognosis of colorectal cancer |
| **13** | Impact of ctDNA test TAV16 and SMARCA4/ERCC3 co-mutations on relapse and survival dynamics in pancreatic cancer patients |

| | |
|---|---|
| **14** | NSCLC Exosomes: Unlocking Biomarker Potential |
| **15** | Extracellular vesicle DNA methylation signature as a predictor of chemotherapy response in metastatic colorectal cancer |
| **16** | CLINICAL UTILITY OF LIQUID BIOPSY FOR ALK FUSION POSITIVE NON-SMALL CELL LUNG CANCER PATIENTS |
| **17** | Cross-Platform Comparison of Plasma-SeqSensei™ CRC panel with reference Technologies for Detecting RAS/BRAF Mutations in ctDNA and tissue samples. |
| **18** | SOLTI-2102 HOPE Prostate study to assess the feasibility and impact of liquid biopsy-based genomic profiling in metastatic prostate cancer |
| **19** | Therapeutic systems based on nanoemulsions for ovarian cancer treatment |
| **20** | A novel cfDNA methylation signature for the prediction of FOLFOX-based therapy response in advanced colorectal cancer |
| **21** | Predicting BCG Failure in Bladder Cancer Patients: a microRNA-Driven Biomarker Approach |
| **22** | A First-Line Approach: Liquid Biopsy-Guided Osimertinib Therapy for EGFR-Mutated NSCLC |
| **23** | Liquid Biopsy for monitoring response in KRAS-Mutated NSCLC Patients Treated with First-Line Immunotherapy |
| **24** | A tumor-agnostic ctDNA whole-exome sequencing approach enhances minimal residual disease detection in localized colon cancer patients |
| **25** | Assessing Mutational Status in NSCLC through Exosomal Liquid Biopsies |
| **26** | Genomic and Methylomic ctDNA Analysis Reveals Epigenomic Dysregulation as a Key Driver of Therapy Resistance in Metastatic Colorectal Cancer |
| **27** | Liquid biopsy-based epigenetic biomarkers for guiding first-line treatment in metastatic pancreatic cancer |
| **28** | Clinicopathological and Molecular Features of Recurrence Based on Circulating Tumor DNA in Resected stage II-III Colorectal Cancer Patients |

| | |
|---|---|
| **29** | KEAP1 and STK11: emerging biomarkers in the clinical management of refractory non-small cell lung cancer |
| **30** | NON-CODING RNAs AS BIOMARKERS IN THE EARLY BREAST CANCER SETTING |
| **31** | miRNAs in Blood and Urine as Companion Diagnostic Biomarkers in Genitourinary Tumors. |
| **32** | Liquid Biopsy in Bladder Cancer: Uncovering the clinical value of DNA methylation-Based biomarkers to predict Bacillus Calmette-Guérin response |
| **33** | Genomic diversity and BCL9L status in CTC-pools predict overall survival in metastatic colorectal cancer |
| **34** | Liquid Biopsy in Localized Prostate Cancer Patients Undergoing Radiotherapy: Urinary Exosomal miRNAs as Potential Predictive Biomarkers |
| **35** | Non-invasive immunogram to characterize and monitor immune status in Non-Small Cell Lung Cancer patients treated with immunotherapy |
| **36** | ESR1 Mutation Testing in Breast Cancer: Clinical Relevance and Therapeutic Applications |
| **37** | ANALYZING IMMUNOTHERAPY RESPONSES IN UROTHELIAL CANCER USING LEGENDPLEX TECHNOLOGY |
| **38** | Early cancer detection from liquid biopsy using cell-free RNA |
| **39** | Utility of CTC Gene Expression in the Molecular Characterization of Metastatic Recurrence in Breast Cancer |
| **40** | Identification of treatment response biomarkers in tumor-educated platelets from patients with luminal breast cancer |
| **41** | 3D Modelling of CTC Clusters: focusing on immune-cancer cells interaction |
| **42** | Clinical Translational Study in Gastrointestinal Cancer (GI): EMT Status of Circulating Tumor Cells (CTCs) and Clinical Utility |
| **43** | Establishment and Characterization of a CTC-Derived Cell Line from Mouse Blood Reveals Prognostic Gene Signatures in Breast Cancer |

| | |
|---|---|
| **44** | Exploring the Mutational Landscape in Locally Advanced HNSCC Using a Targeted Next-Generation Sequencing |
| **45** | Development of an error corrected deep whole-exome ctDNA sequencing pipeline for evolutionary dynamics analysis in metastatic gastro-oesophageal adenocarcinoma |
| **46** | Development of a Long-term Circulating Tumor Cells Organoid Model Derived from Metastatic Triple Negative Breast Cancer |
| **47** | Comparative Evaluation of Circulating Tumor Cells Enrichment Technologies in Colorectal Cancer |
| **48** | Comparison of CTC isolation technologies in patients with advanced urothelial carcinoma |
| **49** | Identifying biomarkers of senescence after chemotherapy to guide cancer treatment |
| **50** | Characterization of CTCs and CTC-clusters in breast cancer xenografts |

## 1 Proteomics of Plasma-Derived Extracellular Vesicles Isolated with ExoGAG for Lung Cancer Biomarkers

**Poster**

**Rocío Ferreiro Miguéns[1,2] , Víctor Cebey[2,3,4] , Javier Álvarez[2,3,4] , Jorge García[2,3,4] , Miguel Abal[2] , Luis León Mateos[2,3,4]**

*1) NasasBiotech S.L (Rúa da Carreira do Conde, 20, local 3-2, 15702, Santiago de Compostela, A Coruña)*
*2) Translational Medical Oncology (Oncomet), Health Research Institute of Santiago (IDIS), Santiago de Compostela, Spain*
*3) Department of Medical Oncology, Complexo Hospitalario Universitario de Santiago de Compostela (SERGAS), Santiago de Compostela, Spain*
*4) Centro de Investigación Biomédica en Red de Cáncer (CIBERONC), Madrid, Santiago de Compostela, Spain*

Liquid biopsy (LB) is the analysis of tumor cells or tumor cell products in blood or another biofluids. LB includes circulating tumor cells (CTCs), circulating tumor DNA (ctDNA) and extracellular vesicles (EVs) like exosomes, among others. The latter could be a complement and an alternative to traditional sources of tumoral material. Recently, numerous studies shown the potential of EVs for the diagnosis of different diseases, such as lung cancer and other cancer types.

Lung cancer is the main cause of tumor mortality in the world and the second in incidence in industrialized countries. In Spain, 31,282 new cases are detected annually with a mortality of 23,000 people, approximately. Moreover, worldwide, 18,2% of the total deaths from cancer due to lung cancer, 1,793,144 deaths in one year. The main problem is the late diagnosis, when the patients are in advanced stages of the disease, this situation is given by non-specific symptoms in early phases. For these reasons, it is crucial to develop effective screening that could allow to detect these patients when it is possible to increase their life expectancy.

In this context, EVs could be a good tool for early detection as these small vesicles (40-160nm) are very stable and released by tumor cells to biofluids with their cargo (DNA, RNA, proteins, lipids...) protected by a double membrane. These characteristics confer advantages over other tumour material and allow them to be used as a source of biomarkers.

## 2 OCTOPUS: blood-based signature to predict immunotherapy response in advanced NSCLC patients

**Oral Communication**

**Ana Oitabén Fernández[1,2] , Manuel Pino González[1] , Patricia Mondelo Macía[3] , Martín Emilio Lázaro Quintela[1] , Luis Léon Mateos[3] , Irene Alonso Álvarez[1] , Carme García Benito[4] , Jorge García[3] , Laura Juaneda Magdalena Benavides[1] , Ihab Abdulkader Nallib[5] , Laura Muinelo Romay[3] , Mónica Martínez Fernández[1]**

*1) Translational Molecular Oncology Unit. IIS Galicia Sur. SERGAS-UVIGO. Hospital Álvaro Cunqueiro, Vigo, Spain.*
*2) Mobile Genomes Group. Genomes and Disease. CiMUS. University of Santiago de Compostela, Spain.*
*3) Liquid Biopsy Unit. Translational Medical Oncology Group. IDIS. Complexo Hospitalario Universitario de Santiago de Compostela, Spain.*
*4) Medical Oncology Department. Complexo Hospitalario Universitario de Ourense, Spain.*
*5) Pathological Anatomy Department. Complexo Hospitalario Universitario de Santiago de Compostela, Spain.*

**Background:** Advanced NSCLC still represents the leading cause of death. Despite immunotherapy has changed the patient management, the absence of reliable biomarkers to predict response limits effective treatment decisions. Available markers, like PD1/PD-L1 immunohistochemistry or TMB, offer some guidance but are often inadequate and inaccessible, highlighting the need for more precise predictive tools.

**Objective:** We aim to identify genetic alterations that help to predict the response to immunotherapy at first line in advanced NSCLC patients in a minimally invasive way.

**Methodology:** We collected pre-treatment blood samples from patients treated with immunotherapy at first line to detect somatic mutations in circulating tumor DNA (ctDNA), DNA from peripheral blood mononuclear cells (PBMCs) to obtain germline, and DNA from primary tumor biopsies. By using targeted sequencing, we detected somatic mutations in ctDNA and compared them with the primary tumor profiles. We also evaluated the ability of blood-based tumor mutational burden (bTMB) to predict response to the treatment. We calculated a mutational signature to predict response, based on an adjusted linear model and assessed the potential of incorporating different clinical features into the signature, in order to select the best combination of biomarkers.

**Results:** Our analysis identified a baseline mutational profile of immunotherapy response in blood with high concordance in tissue. We also identified a signature composed of 10 genes with ability to discriminate between responder and non-responder patients with an AUC=0.94 (100% precision; 88% recall) and with an AUC=0.90 (100% precision; 66% recall) for durable clinical benefit (response >6 months). These data support the significant predictive power of the proposed signature, even for long-term response. Besides, the signature presents higher predictive capacity when compared with current biomarkers (bTMB and PD-L1).

**Conclusions:** We proposed a mutational signature based on 10 genes to predict response to immunotherapy of advanced NSCLC patients at the time of the diagnosis and through the use of minimally invasive blood samples.

**3** Patient-Derived Organoids from Uterine Aspirates of Endometrial Cancer Patients: A Preclinical Model for Testing Novel Therapies.

**Oral Communication**

**Sonia López-Landrove[1,2] , Carlota Roca[1] , Carlos Casas-Arozamena[1,2,3] , Alicia Abalo[1] , Alexia Cortegoso[1,4] , Patricia Palacio[4] , Teresa Curiel[4] , Beatriz Bernádez[5] , Efigenia Arias[6] , Victoria Sampayo[6] , Juan Cueva[4] , Ana Vilar[6] , Laura Muinelo-Romay[1,2,3]**

*1) Translational Medical Oncology Group (Oncomet), Liquid Biopsy analysis Unit, Health Research Institute of Santiago de Compostela (IDIS), University Hospital of Santiago de Compostela (SERGAS), Santiago de Compostela, Spain.*
*2) University of Santiago de Compostela (USC), Praza do Obradoiro, 0, 15705 Santiago de Compostela, Spain*
*3) Centro de Investigación Biomédica en Red de Cáncer (CIBERONC).*
*4) Department of Medical Oncology, University Clinical Hospital of Santiago de Compostela, University of Santiago de Compostela (USC), 15706 Santiago de Compostela, Spain*
*5) Department of Pharmacy, University Hospital of Santiago de Compostela (SERGAS), Santiago de Compostela, Spain.*
*6) Department of Gynecology, University Hospital of Santiago de Compostela (SERGAS), Trav. Choupana s/n, 15706 Santiago de Compostela, Spain*

**Introduction:** Endometrial cancer (EC) is the sixth most common malignancy in women with increasing incidence and mortality rates representing a growing global health concern. Although many EC cases benefit from early diagnosis and favorable prognoses, aggressive subtypes often exhibit resistance to conventional therapies such as chemotherapy. Current standard therapies are often inadequate, particularly for high-grade tumors, emphasizing the need for advanced *in vitro* models to develop and test novel therapies.

This study presents patient-derived organoids (PDOs) from uterine aspirates as a personalized preclinical model to investigate tumor biology and evaluate novel therapeutic strategies. PDOs preserve the molecular and histological characteristics of the original tumor, providing a robust platform for drug testing and biomarker validation.

**Methodology:** Uterine aspirates were collected from EC patients during surgery at the Gynecology Department of the University Hospital of Santiago de Compostela. Samples were enzymatically and mechanically dissociated into single-cell suspensions and cultured with a custom-defined medium optimized for organoid development.

PDOs were extensively characterized using immunohistochemistry (IHC), immunofluorescence (IF), digital droplet PCR (ddPCR), quantitative PCR (qPCR), methylation profiling, and DNA/RNA sequencing to analyze genomic, epigenomic, and transcriptomic landscapes and assess specific markers relevant for therapy selection.

The *in vitro* efficacy of novel therapies (compared with standard therapies), including gamma-aminobutyric acid receptor inhibitors (GABARi) and TROP2-targeting antibody-drug conjugates (TROP2-ADCs), was evaluated in PDOs using AlamarBlue-based cell viability assays.

**Results:** PDOs models were successfully established from the uterine aspirates of 28 EC patients, achieving an overall success rate of 58%, with 55% for endometrioid tumors and 40% for serous tumors (N= 48, 38 and 5 respectively). IHC and IF analyses confirmed that the PDOs expressed the same protein markers and exhibited the same genetic alterations as the tumors from which they were derived. Additionally, transcriptomic analysis revealed distinct gene expression profiles, making these PDOs highly suitable for drug assay comparisons.

The studies revealed that PDOs responded to targeted therapies based on their individual molecular characteristics. For instance, PDOs derived from high-risk tumors demonstrated heightened sensitivity to TROP2-ADCs agents and GABARi (Bicuculline) supporting their interest as alternative therapeutic strategies in the most aggressive EC. Preliminary results suggest that TROP2-ADC responses were independent of TROP2 expression. Notably, PDOs with high expression of GABA and GABRA5 exhibited a stronger response to GABARi, suggesting a potential biomarker to predict therapy efficacy.

**Conclusión:** We have established a functional preclinical platform, based on the generation of PDOs from minimally invasive samples, that allows rapid testing of novel therapies in a personalized manner. This platform has the potential to significantly enhance the early-phase drug development process by providing more accurate and patient-relevant data, ultimately leading to improved therapeutic outcomes

**4** BlaDimiRplus: A precise, multimodal, urine-based tool for non-invasive prediction of immunotherapy response in bladder cancer.

**Poster**

**Álvaro Martín de Bernardo Abellán[1,2] , Ignacio Arévalo Reina[1,2,3] , Iris Lodewijk[1,2,3] , Esther Montesinos Romero[1,2] , Marta Rodríguez Izquierdo[4] , Félix Guerrero Ramos[4] , Jesús Paramio González[1,2,3] , Marta Dueñas Porto[1,2,3] , Cristian Suárez Cabrera[1,2,3]**

*1) Molecular Oncology Unit CIEMAT, Madrid, Spain*
*2) Institute of Biomedical Research, University Hospital '12 de Octubre', Madrid, Spain*
*3) Centro de Investigación Biomédica en Red Cáncer (CIBERONC), Madrid, Spain*
*4) Urology Department, University Hospital '12 de Octubre', Madrid, Spain*

**Introduction:** Non-muscle-invasive bladder cancer (NMIBC) presents challenges due to the varied responses to treatments. The most effective treatment approach is intravesical immunotherapy with BCG (Bacillus Calmette-Guérin), which induces inflammation and an anti-tumor response in patients who respond. However, predicting which patients will benefit is difficult, with around 50% showing inflammation without clinical improvement. Liquid biopsies, especially urine-based methods, offer a promising, non-invasive alternative for personalizing NMIBC treatment. Previously, we demonstrated that miRNAs serve as effective biomarkers for bladder cancer using the BlaDimiR system. We now introduce BlaDimiR*plus*, an advanced urine-based strategy for predicting response to BCG immunotherapy, either alone or combined with anti-PD-L1 therapy.

**Objective:** To create a straightforward method using miRNAs and cytokines to predict the immunotherapy response in NMIBC patients.

**Methodology:** We identified differentially expressed (DE) miRNAs in primary tumors from responder (R) and non-responder (NR) patients using the nCounter Human v3 miRNA Nanostring panel, and analyzed cytokines in urine samples with LEGENDplex. These findings were validated in a proof-of-concept cohort using RT-qPCR for miRNAs and ELISA for cytokines. We compared samples taken before transurethral resection and before the initial BCG instillation. A multifactorial analysis helped identify the most effective biomarker combination for validation.

**Results:** Of the 26 DE miRNAs identified, those with the highest AUC and significant p-values were analyzed in urine samples. We validated individual ratios that distinguished between R and NR patients in pre-treatment samples for BCG alone (ROC AUC = 0.82-0.91) and in combination with anti-PD-L1 (ROC AUC = 0.67). In 57 urine samples, we identified four cytokines that predicted response to BCG, with CXCL10 validated in a second cohort of 74 samples (ROC AUC = 0.74). A

multifactorial analysis yielded a ROC AUC = 1 for a combination of six miRNAs and two cytokines.

**Conclusions:** BlaDimiR*plus* is an accurate urine-based tool for predicting immunotherapy response in bladder cancer patients, addressing a critical clinical need. This multimodal approach demonstrates that deregulation of miRNAs and cytokines in urine can differentiate responders from non-responders before treatment.

**5** Clinical utility of ctDNA detection in cerebrospinal fluid by NGS for diagnosis of CNS Lymphoma

**Oral Communication**

**Yanira Ruiz-Heredia[1] , Ana Jiménez-Ubieto[1] , Alejandro Martín-Muñoz[1] , Ricardo Errazquin[2] , Laura Rufián[2] , Margarita Rodríguez[2] , Esther Parra[1] , Tycho Baumann[1] , Antonia Rodríguez[1] , M Teresa Cedena[3] , Joaquín Martínez-López[1] , Rosa Ayala[1] , Inmaculada Rapado[1]**

*1) Department of Hematology, Hospital Universitario 12 de Octubre, Instituto de Investigación Sanitaria Hospital 12 de Octubre (imas12), CNIO, CIBERONC, Madrid, Spain.*
*2) R&D Department, Altum sequencing Co., Madrid, Spain.*
*3) Hematology, Instituto de Investigación Sanitaria Hospital 12 de Octubre (i+12), Madrid, Spain.*

**Introduction:** Central nervous system (CNS) involvement in lymphoma patients (CNS-L) is a finding with a poor prognosis, whether it represents a primary CNS-L or occurs in the context of a systemic aggressive lymphoma at initial diagnosis or relapse. Although infrequent in general, the risk is considerably higher in cases with certain clinical and biological features at the time of diagnosis, so the identification of this subgroup is key to guide prophylaxis strategies. However, despite efforts to stratify lymphoma patients according to the risk of CNS infiltration, predictive score has shown insufficient prognostic value to guide prophylaxis strategies. Currently, diagnostic tool involves brain biopsy in CNSL, often contraindicated, magnetic resonance imaging (MRI) and cerebrospinal fluid (CSF) analysis by cytology and flow cytometry (FMC). However, these methods often provide inconclusive results, either due to their low sensitivity or the limited number of cells present in CSF. Recently, the detection of circulating tumor DNA (ctDNA) by NGS in CSF has emerged as a valuable and promising tool to diagnose and evaluate the response to treatment of lymphoma affecting the CNS. In this study we assessed the clinical utility of ctDNA detection and monitoring by NGS in CSF in a cohort of lymphoma patients at risk of or diagnosed with CNS infiltration.

**Methodology:** CSF from 22 patients were studied: 4 primary CNS-L; 1 patient without anatomopathological diagnosis but suspicion of Primary CNS-L; 12 systemic diffuse large b cell lymphoma (DLBCL); 2 plasmocytic lymphoma; 1 mantle cell lymphoma; and 2 follicular lymphoma (FL). cfDNA was isolated for at least 2 mL of CSF. A custom NGS panel containing the 56 most relevant mutated genes in lymphoma B malignancies was applied. NGS result was compared with available gold standard methods (MRI, FC, cytology and biopsy) at diagnosis and follow-up.

**Results:** NGS panel detected lymphoma-related mutations in all confirmed CNSL patients included in the study, providing a sensitivity of 100%.. An average of 14 variants per patient with VAFs ranging from 1.1 to 95.7% were detected. The most frequently mutated genes were *KMT2D, EP300*, *NOTCH1*, *CD79B*, *MYD88, CARD11, ARID1A, BTK, CIITA, PRDM1, TP53*. MRI was able to detect CNS Lymphoma in 13/22 patients, providing an overall sensitivity of 60% while FC was only able to detect disease in CSF in 7/22patients, showing a sensitivity of 31%.

Dividing the analysis by different clinical entities, in 4 primary CNS-L with biopsy proven diagnosis, MRI and NGS were able to correctly diagnose 4/4, while FC only 2/2. There was another patient without brain biopsy available and inconclusive MRI and negative FC but positive NGS, that was treated with MATRIX scheme and achieve a partial remission. Considering 12 patients with DLBCL, all presented somatic mutation in CSF by NGS, 6/12 had MRI compatible with CNS infiltration and only 3 had CSF positive by FC. 1/2 plasmocytic lymphoma patients was diagnose by both FC and NGS positive in CSF and the other was diagnose by NGS and MRI. The patient with mantle lymphoma was diagnose of CNS infiltration by a brain biopsy and NGS, FC and MRI were inconclusive. The 2 patients with FL, in one of them CNS involvement could be confirmed by all techniques NGS, FC and MRI. In the other patient a brain biopsy was necessary to confirm the diagnosis due to negative FC and MRI results.

Finally, we also evaluated the utility of NGS-ctDNA test to monitor response to treatment in 4 patients with confirmed CNS infiltration and with available CSF obtained during follow-up monitoring. In all cases NGS result correlate with clinical outcome, while MRI and FC failed assessing real disease status in some of them. First patient with PCNS lymphoma achieved CR after MATRIX treatment. 3 months later the MRI showed signs of progression, but NGS and FC remain negative. This patient did not relapse during the 7 months of follow-up. The second patient with systemic DLBCL was positive 6 months after diagnosis by FC and NGS, but negative by MRI. This patient relapse 1 month later and died. In 2 patients with systemic DLBCL, all test NGS, FC and MRI were negative, and both remained in complete remission during the next 7 months of follow-up.

**Conclusion:** These results highlight the utility of CSF analysis by NGS, postulating as a transformative tool for improving diagnosis, monitoring and outcome of CNS-L patients. Although larger cohort are needed, our data demonstrates that NGS surpassed the capabilities of conventional methods in detecting CNS infiltration. We suggest that CSF sample should be preferably analyzed by NGS than FC in the clinical practice.

**6** Exploring the Stool Virome as a Source of Biomarkers for Colorectal Cancer Screening

**Oral Communication**

**Loretta De Chiara[1,2,3] , Nuria Estévez-Gómez[1,2] , Ryan Doughty[4] , Pilar Gallego-García[1,2] , Pilar Alvariño[1,2] , Meritxell Pujolassos[5] , M Luz Calle[5,6] , Astrid Díez Martín[2,7] , Cristina Regueiro[2,7] , Jorge Hernández Camoiras[2,7] , Davila Piñón Pedro[2,7] , Joaquin Cubiella[2,8] , David Posada[1,2,3]**

*1) CINBIO, Universidade de Vigo, Vigo, Spain.*
*2) Galicia Sur Health Research Institute (IIS Galicia Sur), SERGAS-UVIGO, Vigo, Spain.*
*3) Department of Biochemistry, Genetics and Immunology, Universidade de Vigo, Vigo, Spain.*
*4) Department of Computer Science, Rice University, Houston, Texas, USA.*
*5) Bioscience Department, Faculty of Sciences, Technology and Engineering, University of Vic - Central University of Catalunya, Spain.*
*6) Institut de Recerca i Innovació en Ciències de la Vida i de la Salut a la Catalunya Central (IRIS-CC), Spain.*
*7) Grupo de Investigación en Oncología Digestiva de Ourense (GIODO), Ourense, Spain.*
*8) Department of Gastroenterology, Complexo Hospitalario Universitario de Ourense, Red de Enfermedades Hepáticas y Digestivas (CIBERehd)Ourense, Spain.*

The standard approach for colorectal cancer (CRC) screening is the fecal immunochemical test, which, however, exhibits limited sensitivity for detecting premalignant advanced adenomas (AA) and tumors in the proximal colon. On the other hand, emerging evidence links alterations in gut microbiota composition to CRC development, highlighting the potential role of the gut microbiome in early disease stages. The virome is the most diverse component of the human microbiome but remains underexplored, and little is known about CRC-related viral communities. In this study we aim to identify virome-based non-invasive biomarkers for CRC screening.

We analyzed the virome in stool samples from 45 CRC patients, 60 AA patients and 59 individuals with no neoplasia. We performed viral shotgun metagenomics using a custom protocol, involving viral enrichment (sequential centrifugation, filtration, ultracentrifugation), DNA/RNA extraction, retrotranscription, random priming PCR, library preparation and sequencing (150 PE reads). Reads were quality-checked, preprocessed and taxonomically classified with SeqScreen (Balaji et al., 2022). Differential abundance analysis was conducted using coda4microbiome (Calle et al., 2023) and LinDA (Zhou et al., 2022) R packages.

The proportion of reads assigned to viruses largely varied (0.19–96.3%; mean 20.52%). Among the 178 viral families identified, *Microviridae* was the most abundant, followed by *Inoviridae* and *Picornaviridae*. Preliminary findings suggest

that the viral families *Betaflexiviridae* and *Baculoviridae* may serve as potential biomarkers for AA and CRC detection. The utility of these viruses as biomarkers for CRC screening should be further validated in an independent cohort.

Funding: *"Fundación Científica de la Asociación Española contra el Cáncer"* (PRYGN211425POSA).

References:

- Balaji A et al. SeqScreen: accurate and sensitive functional screening of pathogenic sequences via ensemble learning. Genome Biol. 2022; 23(1):133.
- Calle et al. coda4microbiome: compositional data analysis for microbiome cross-sectional and longitudinal studies. BMC Bioinformatics. 2023; 24(1):82.
- Zhou et al. LinDA: linear models for differential abundance analysis of microbiome compositional data. Genome Biol. 2022; 23(1):95.

**7** IDENTIFICATION OF MINIMAL RESIDUAL DISEASE USING ULTRASENSITIVE ctDNA DETECTION TO ANTICIPATE LATE RELAPSE IN EARLY BREAST CANCER

**Poster**

**Jesús Velasco-Suelto [1,2,3] , Esperanza López-López[1,2] , Maria Elena Quirós-Ortega[1,2,3] , Celia Gaona-Romero[1,2] , Alejandro Martín[4,5] , Patricia Losana [5] , Yanira Heredia[5] , Javier Pascual [1,2,3] , Emilio Alba [1,2,3,6] , Ángel Guerrero-Zotano [7] , Iñaki Comino-Méndez [1,2,3]**

*1) Hospital Universitario Virgen de la Victoria, 29010, Malaga, Spain*
*2) The Biomedical Research Institute of Málaga (IBIMA-CIMES-UMA), 29010, Malaga, Spain*
*3) Centro de Investigacion Biomedica en Red de Cancer (CIBERONC - CB16/12/00481), 28029, Madrid, Spain*
*4) Hematological Malignancies Clinical Research Unit, Centro Nacional de Investigaciones Oncológicas (CNIO), Madrid, Spain*
*5) Altum Sequencing Co., Madrid, Spain*
*6) University of Málaga, Faculty of Medicine, 29010, Malaga, Spain*
*7) Medical Oncology Department, Fundación Instituto Valenciano de Oncología, Valencia, Spain*

**Introduction:** In early-stage breast cancer (BC) reduction in mortality is directly attributable to early diagnosis. Despite five years or more of adjuvant antiestrogen therapy, there is a persistent risk of relapse. This risk is attributed to undetectable minimal residual disease (MRD). Liquid biopsies provide invaluable real-time information about the molecular architecture of cancers, aiding in early tumor detection, drug efficacy/resistance evaluation, MRD detection, and tumor heterogeneity deciphering. As early-stage cancer, circulating tumor components are highly diluted, it requires the use of extremely resource-intensive methodologies to obtain an improvement in sensitivity and specificity.

**Objectives:** This study aims to evaluate the effectiveness of the CloneSight test, a patient-specific ctDNA assay, for detecting MRD and predicting relapse in hormone receptor-positive (HR+) BC patients who have completed adjuvant endocrine therapy.

**Methodology:** Twenty HR+ BC patients were prospectively followed, having completed at least 5 years of endocrine therapy. Plasma samples were collected every 6 months over a median follow-up period of 2 years. Tumor DNA from formalin-fixed paraffin-embedded (FFPE) samples was used to select somatic variants for ctDNA analysis. ctDNA testing employed next-generation sequencing (NGS) for MRD detection.

**Results:** Among six patients who experienced relapse, MRD positivity was detected in three, with ctDNA identified up to 68 months before clinical recurrence. PT2 exhibited MRD in 50% of tested samples and later had bone metastases; PT18 recurred with brain metastasis that were detected over a year before clinical signs. Three relapsed patients showed negative ctDNA, potentially due to HR+ biology or low cfDNA levels. Persistently negative ctDNA was observed in non-recurrence cases showing high specificity for this test.

**Conclusions:** The CloneSight test demonstrated substantial potential for early MRD detection, especially in aggressive cases. The findings underscore the importance of serial ctDNA monitoring and highlight the need for a tailored approach in interpreting ctDNA results in HR+ BC. Further studies are needed to standardize monitoring protocols and explore treatment strategies informed by ctDNA results.

**8** Insights into Carboplatin Resistance in High Grade Serous Ovarian Cancer: A Study Using Ascitic Fluid-Derived Organoids

**Poster**

**Andrea Estrella Arias-Diaz[1,2] , Marta Trillo-Dominguez[2,3,4] , Miguel Hernandez-Gamarra[2,3,4] , Edurne Perez-Beliz[5] , Melek Denizli[6] , Eva Colas[6,9] , Antonio Gil-Moreno[6,9] , Ana Vilar[7] , Teresa Curiel[1] , Catarina Allegue-Toscano[3,4,8] , Eduardo Dominguez-Medina[4] , Miguel Abal[1,9]**

*1) Translational Medical Oncology Group (Oncomet), Health Research Institute of Santiago de Compostela (IDIS), University Hospital of Santiago de Compostela (SERGAS), Trav. Choupana s/n, 15706 Santiago de Compostela, Spain*
*2) Department of Medicine, University of Santiago de Compostela (USC), 15782 Santiago de Compostela, Spain*
*3) Genomic Medicine Group, Center for Research in Molecular Medicine and Chronic Diseases (CiMUS), University of Santiago de Compostela, Av. Barcelona, 15706 Santiago de Compostela, Spain.*
*4) C005, Health Research Institute of Santiago de Compostela (IDIS), Travesía da Choupana s/n, 15706 Santiago de Compostela, Spain*
*5) Department of Pathology, University Hospital of Santiago de Compostela (SERGAS), Trav. Choupana s/n, 15706 Santiago de Compostela, Spain*
*6) Biomedical Research in Gynaecology, Vall d'Hebron Institut de Recerca, 08035 Barcelona, Spain*
*7) Department of Gynecology, University Hospital of Santiago de Compostela (SERGAS), Trav. Choupana s/n, 15706 Santiago de Compostela, Spain*
*8) CB06/07/0088, Center for Biomedical Network Research on Rare Diseases (CIBERER), Instituto de Salud Carlos III, Av. Monforte de Lemos, 28029 Madrid, Spain*
*9) Center for Biomedical Network Research on Cancer (CIBERONC), Instituto de Salud Carlos III, Av. Monforte de Lemos, 28029 Madrid, Spain*

In high-grade serous ovarian carcinoma (HGSOC), first-line chemotherapy (carboplatin and taxane) has a 70–80% response rate, but resistance often develops within 12 to 36 months. Cross-resistance to non-platinum therapies limits further treatment options. While progress has been made in understanding the mechanisms of resistance, a complete understanding is still lacking, emphasizing the need for continued research to prevent or reverse this resistance. In this study, we aimed to gain insights into the mechanisms underlying carboplatin resistance using a cohort of chemo-resistant and chemo-sensitive ovarian patient-derived organoids (PDOs) from ascitic fluid, with the goal of developing therapies to prevent or reverse this resistance.

PDOs were derived from the ascitic fluids of 14 patients enrolled in the University Hospital of Santiago de Compostela and the Vall d'Hebron University Hospital in 2023 and 2024. Ascites derived-PDOs were characterized by

immunohistochemistry and immunofluorescence, while carboplatin sensitivity was assessed by exposing the models to carboplatin for 72 hours and measuring the response after a 72-hour recovery period using the alamarBlue cell viability assay. Whole transcriptome RNA sequencing was then applied for differential gene expression profiling between platinum-sensitive and platinum-resistant PDOs using Illumina platforms. After the quantitative analysis, the expression matrix of all samples was obtained and edgeR software was used to analyze the significance of expression differences, determined by pvalue<0.05 and log2FoldChange≥0. ClusterProfiler software was used for KEGG pathway enrichment analysis of differential gene sets, and validation of the selected candidate genes was carried out by TaqMan™ RTqPCR. Finally, to evaluate sensitivity of HGSOC to current anticancer drugs, a high-throughput cell-based assay was developed in 384-well plates using metabolic oxidative activity capacity of organoids as a readout. First-line cancer ovary treatment carboplatin was used as reference of sensitivity, and screening of 179 FDA-approved anticancer drugs was carried out to rank order the most active antiproliferative agents.

The cohort of advanced ovarian cancer organoids derived from ascitic fluid, present long-term proliferative capacity (>20 passages) and a predominant dense morphology. Histological characterization demonstrates resemblance to the original tumors, confirming the malignant epithelial nature and high-grade serous histology. Hematoxylin and eosin staining revealed cellular atypia, while immunohistochemistry (mutated p53 (black or null pattern), CK7, PAX8, P16, and WT1) and immunofluorescence (EpCAM, vimentin, Ki67 and mesothelin) supported the expression of markers specific for high-grade serous carcinoma. Furthermore, carboplatin sensitivity assessment in these preclinical models mirrored the clinical responses observed in patients and permitted us to classify the models into the two traditional therapy response groups (8 carboplatin sensitive and 6 carboplatin resistant PDOs) based on the carboplatin IC50. These results are of relevance, as there are currently no validated predictive markers for carboplatin-resistant disease, and these ascites-derived PDO models could be used to define the resistant setting before clinical symptoms arise in the patients. Furthermore, Next Generation RNA Sequencing and differential gene expression profiling comparing the sensitive and resistant PDO models revealed ABC transporters, MAPK, PI3K-Akt and KRAS signaling pathways, and regulation of the actin cytoskeleton, as significantly altered KEGG pathways among others. We also identified differentially expressed genes related to prostaglandin pathways (COX2 and SDCBP) and autophagy (AKR1C1), which have been previously linked to carboplatin resistance in ovarian cancer. The RTqPCR validation of the selected candidate genes differentially expressed between the resistant and sensitive PDOs further supports the involvement of these pathways in the acquisition of resistance to platinum-based therapy in advanced ovarian cancer. Finally, high-throughput screening identified a set of targeted anticancer drugs showing sensitivity in platinum-resistant setting, providing insights on the potential mechanisms related to

chemotherapy resistance, and for the design of alternative therapeutic approaches upon platinum-resistance in ovarian cancer.

In conclusion, this study describes the generation of clinically relevant organoid models in advanced ovarian cancer, highlights key molecular pathways driving carboplatin resistance using PDOs derived from ascitic fluid, and offers a foundation for developing strategies to overcome or prevent carboplatin cross-resistance.

**9** Novel patient-specific ctDNA-NGS test to predict outcome in real-word follicular lymphoma patients

**Poster**

**Alejandro Martín-Muñoz [1] , Ana Jiménez-Ubieto [1] , Yanira Ruiz-Heredia [1] , Laura Rufián [2] , Margarita Rodríguez [2] , Tycho Baumann [1] , Antonia Rodríguez [1] , María Poza [1] , Daniel Gil [1] , Pilar Sarandeses [3] , Enrique Revilla [4] , Gloria Figaredo [5] , Ricardo Errazquin [1] , Inmaculada Rapado [1] , Santiago Barrio[1] , Rosa Ayala[1] , Joaquin Martinez-Lopez[1]**

*1) Department of Hematology, Hospital Universitario 12 de Octubre, Madrid, Spain.*
*2) R&D Department, Altum Sequencing S.L., Leganés, Spain.*
*3) Nuclear Medicine, Instituto de Investigación Sanitaria Hospital 12 de Octubre, Madrid, Spain.*
*4) Pathological Anatomy Department, Instituto de Investigación Sanitaria Hospital 12 de Octubre, Madrid, Spain.*
*5) Department of Hematology, Hospital Universitario de Toledo, Toledo, Spain.*

**Introduction:** Several studies have shown the utility of cell-free DNA (cfDNA) for blood-based non-invasive genotyping of diverse human tumors, including aggressive lymphomas. However, it remains unclear whether these cfDNA advantages can also be applied to monitor minimal residual disease (MRD) in the context of follicular lymphoma (FL) real-world regimens, where deep remissions are achieved. Furthermore, current cancer detection techniques, such as PET/CT, present several limitations: PET/CT is only indicated in the middle and at the end (EOT) of treatment, and under relapse suspect; it is highly dependent on the evaluating radiologist; and it has limited sensitivity and specificity, due to nonspecific uptake. Additionally, a solid biopsy is required for confirming progression, but the extraction is impracticable in a considerable percentage of patients and there might be several masses. Thus, there is a great clinical need for less invasive monitoring methods, applicable as frequently as necessary, for the early detection of relapses. In this study, we applied CloneSight, a tumor-informed cfDNA-NGS test, in patients with FL receiving first-line and further lines of treatment to monitor MRD, and to evaluate its ability to predict clinical outcome, compared to PET/CT imaging testing.

**Methodology:** We profiled tumor samples at the time of diagnosis from a total of 93 FL patients (FFPE sample but, if not available, blood-based liquid biopsy), using a targeted NGS panel covering the 56 most frequently mutated genes in B-cell lymphomas.
MRD was serially analyzed by CloneSight (Altum Sequencing S.L.) in 363 patients' blood-based liquid biopsies extracted after treatment. Out of the identified mutations, those with higher probability of explaining the FL (based on functional

and clinical information from databases such as COSMIC, ClinVar, etc.) were selected to be sequenced with a patient-specific multiplex PCR. The median follow-up time was 15 months from start of first line therapy, and 12 months for further treatment lines. The level of circulating tumor DNA (ctDNA) measured by CloneSight was then compared to PET/CT imaging results and progression-free survival.

**Results:** Tumor genotype was identified for all 93 FL patients, enabling MRD assessment in 100% of the cohort. The median number of biomarkers per patient was 4 (1-14).
First, we analyzed the correlation between ctDNA-NGS analysis and PET/CT imaging in all paired time points included in this study (202/363) (Figure 1A). The correlation of the disease status assessed using both techniques was highly robust. However, focusing on the 43 discrepancies, CloneSight eventually converged with the imaging and clinical outcome after a short period of time in 9 time points (21%). This highlights the importance of dynamic disease monitoring, especially at the beginning of the treatment. On the other hand, CloneSight demonstrated higher sensitivity and specificity in 29 cases (67%), detecting respectively low MRD levels driving progression in 11 time points and 18 false positive PET/CT results. Next, we assessed the prognostic performance of CloneSight to predict clinical outcomes. In first line patients, ctDNA levels measured by CloneSight at both the interim and EOT cycles were significantly prognostic to risk progression (log-rank $p<0.005$ HR=12.31; log-rank $p<0.005$ HR=29.87, respectively), outperforming the predictive capabilities of PET/CT (log-rank $p=0.01$ HR=4.21; log-rank $p<0.005$ HR= 11.44, respectively) (EOT, Figure 1B). In fact, CloneSight achieve better performance than PET/CT (considering EOT, 92% versus 75% sensitivity and 89% versus 86% specificity). The predictive ability was also evaluated in subsequent lines of treatment, where greater prognostic value identifying patients at high risk of relapse was again demonstrated by CloneSight.

Finally, we studied how treatment responses are reflected in ctDNA kinetics during therapy follow-up. Refractory patients exhibited persistent disease in the blood, with higher ctDNA levels than those who progressed after initially achieving partial or complete remission. This underscores the crucial importance of serial sampling, as previously noted. In contrast, early responding patients showed the lowest ctDNA levels. A final group of patients reached delayed responses, highlighting the effectiveness of maintenance treatments, a clinical outcome that distinguishes FL from other cancers.

**Conclusion:** This study highlights the paramount potential of CloneSight as a liquid biopsy ctDNA-NGS tool for monitoring MRD and assessing treatment response in FL patients. Its non-invasive approach enables optimal patient monitoring through serial sampling. Compared to PET/CT, the current standard of care in FL, CloneSight

demonstrated higher sensitivity and specificity, as well as superior prognostic value in both response assessment and relapse detection.

**10** Testing for ESR1 Mutations to Guide Therapy in Patients with Metastatic Breast Cancer in Lleida

**Poster**

**ANA VELASCO [1], IZASKUN URDANIBIA[1], SERAFIN MORALES[2]**

*1) INSTITUT DE RECERCA BIOMEDICA DE LLEIDA (IRB LLEIDA)*
*2) HOSPITAL UNIVERSITARI ARNAU DE VILANOVA DE LLEIDA (HUAV LLEIDA)*

**Introduction:** Breast cancer is the most common and deadly cancer among women in Spain, responsible for over 6,600 deaths in 2022 (SEOM, REDECAN). Approximately 70% of breast cancer cases are estrogen receptor-positive (ER+), making endocrine therapy the standard first- and second-line treatment for these patients. However, prolonged exposure to endocrine therapies, such as tamoxifen and fulvestrant, can lead to the emergence of mutations in the ESR1 gene in 20–40% of cases. These mutations, located in the ligand-binding domain (LBD) of the estrogen receptor, result in ligand-independent activation, contributing to partial resistance to treatment, potential enhancement of metastatic processes, and the development of resistance in metastatic disease.

In recent years, the selective estrogen receptor degrader (SERD) elacestrant (Orserdu) has been approved by the FDA and EMA for treating breast tumors harboring ESR1 mutations. At the Medical Oncology Service of the Arnau de Vilanova University Hospital (HUAV) in Lleida, we have implemented routine testing for ESR1 mutations in breast cancer patients who experience recurrence after adjuvant hormonal therapy and are beginning first-line treatment with cyclin inhibitors and endocrine therapy.

**Objectives:** This study aims to:

1. Evaluate the prevalence of ESR1 mutations in breast cancer patients with recurrence following adjuvant endocrine therapy.
2. Assess the potential utility of liquid biopsy (cfDNA) as a non-invasive method for detecting ESR1 mutations and monitoring metastatic progression.
3. Contribute to the understanding of how ESR1 mutations impact treatment decisions and disease management in patients with metastatic breast cancer.

**Methodology:** A total of 76 cfDNA samples from 53 patients were analyzed to identify ESR1 mutations. Three distinct methods were employed: next-generation

sequencing (NGS), quantitative PCR (qPCR), and digital PCR (dPCR). Liquid biopsy was chosen as a non-invasive approach to obtain tumor information and monitor metastasis in relapsed patients. These samples were derived from patients who had previously undergone adjuvant endocrine therapy and were starting first-line treatment with cyclin inhibitors and endocrine therapy at HUAV.

**Results:** ESR1 mutations were detected in 26.5% of the patients analyzed. The most frequent mutations identified were Y537S and D538G, followed by Y537N, Y537C, E380Q, and D432L. These mutations were located within the ligand-binding domain (LBD) of the estrogen receptor, a region associated with resistance to traditional endocrine therapies such as tamoxifen and fulvestrant.

**Conclusions:** Liquid biopsy is a promising, non-invasive tool for obtaining tumor-related information in metastatic breast cancer patients, allowing for dynamic monitoring of disease progression and treatment efficacy. The detection of ESR1 mutations in a significant proportion of patients with recurrence highlights the challenges these mutations present in the treatment of advanced or metastatic breast cancer. Routine assessment of ESR1 mutation status is essential for optimizing therapeutic strategies and improving clinical outcomes for these patients. Given the recent approval of SERDs like elacestrant, ESR1 mutation status should be systematically evaluated in metastatic breast cancer to guide treatment decisions and better manage disease progression.

**11** Liquid biopsy as a tool for identifying actionable mutations in metastatic breast cancer: Insights from a clinical cohort

**Poster**

**Álvaro García Bauto[1] , Catalina Falo Zamora[1] , Daniel Azuara García[2] , Juan Sebastian Cruz Velez[1] , Andrea Carolina Vethencourt Casado[1] , Bartomeu Fullana Grimalt[1] , Adela Fernandez Ortega[1] , Paula Sabat Viltro[1] , Silvia Vázquez Fernández[1] , Clara Ejarque Martínez[1] , Sabela Recalde Penabad[1] , Agostina Stradella[1] , Veronica Luisa Obadia Gil[1] , Rafael Villanueva Vázquez[1] , Sonia Pernas Simon[1] , Sara González Rodríguez[2] , Conxi Lázaro García[1,2]**

*1) Institut Català d'Oncologia (ICO), L'Hospitalet de Llobregat, Barcelona, Spain.*
*2) Bellvitge Biomedical Research Institute (IDIBELL), L'Hospitalet de Llobregat, Barcelona, Spain.*

**Introduction:** Metastatic breast cancer (MBC) is a heterogeneous disease in which molecular alterations contribute significantly to disease progression and the development of resistance to treatment. Liquid biopsy (LB), particularly involving circulating tumor DNA (ctDNA), is a minimally invasive test that captures the molecular heterogeneity of MBC and allows for dynamic identification of actionable mutations (MUT), facilitating tailored therapy. This prospective study analyzes the molecular profile of MBC patients in LB and its relationship with clinical characteristics, prognosis, and actionability.

**Methodology:** This unicentric, prospective study of LB in MBC patients was conducted prior to initiating systemic treatment (mainly first or second line for metastatic disease). Samples were collected in Streck® cell-free DNA tubes and analyzed with the Plasma-Seq Sensei™ breast cancer assay from Sysmex®, a highly sensitive ctDNA-based NGS which includes the main actionable mutations in PIK3CA, ESR1, ERBB2, AKT1 and TP53, selected for their therapeutic relevance. The global percentage of MUTs was analyzed and compared based on prior treatment. Actionability of MUTs and their impact on progression-free survival after first-line metastatic disease (PFS1) were assessed. Data analysis was performed using SPSS v23.0 ($p \leq 0.05$).

**Results:** The cohort included 48 patients (97.9% women, median age 59, SD 11.8 years). Most patients had luminal tumors, with less representation of HER2+ and triple-negative (TN) subtypes. Regarding metastatic localization, 60.4% of patients presented with non-visceral metastases. In 58.3% of the cases, LB was performed after progression to previous treatment; of those, 17 cases had progressed during adjuvant hormonotherapy.

MUTs were detected in 60.4% of patients, with 48% being clinically actionable. The most frequent MUTs were TP53 (37.5%), PIK3CA (29.2%), and ESR1 (16.7%), followed by ERBB2 (6.3%) and AKT1 (4.2%). ESR1 mutations were more prevalent in patients with prior treatment (21.4% vs. 10%), confirming its role as a mechanism of resistance to hormone therapy. Although not statistically significant, several trends were observed: all patients with grade 3 tumors presented mutations, and HER2+ and TN subtypes showed higher rates of mutations compared to luminal ones (p = 0.35). TP53 mutations were associated with a higher visceral metastatic burden (64.7% vs. 58.1%, p = 0.44). Patients who progressed during adjuvant therapy and those who progressed after first-line metastatic treatment had higher frequencies of MUTs (69.6% vs. 52.0%, p = 0.17, respectively). These trends did not reach statistical significance but suggest a potential relationship between clinical characteristics and genomic alterations.

Regarding PFS1, patients without MUTs had a median of 21 months (95% CI: 16.4–26.5), significantly longer than the 11 months observed in those with actionable MUTs (95% CI: 8.3–13.7; p = 0.04). PFS1 was shorter in patients with ≥2 MUTs (12.8 months, 95% CI: 6.7–19.0) compared to those with one MUT (19.7 months, 95% CI: 12.4–44.5; p = 0.002). Although global survival analysis did not provide conclusive results due to the small cohort size and low number of events, the observed trends suggest an important potential impact of molecular alterations on disease progression.

**Conclusion:** LB is a valuable tool for characterizing molecular alterations in MBC and guiding personalized therapies. In this preliminary clinical study, 48% of the patients tested could benefit from targeted therapy. PIK3CA and TP53 were the most frequent MUTs, while ESR1 mutations were linked to resistance to aromatase inhibitors. The presence of any MUTs in LB was associated with shorter PFS1 compared to patients without MUTs. The observed trends between clinical characteristics and specific mutations highlight the need for multicenter studies to validate these findings and improve therapeutic strategies in MBC.

**12** Non-invasive epigenetic biomarkers for detection and prognosis of colorectal cancer

**Poster**

**Jaime Dubbelman-Vizcaíno[1,2*], M. Ángeles Gómez-García[1,2,3*], Nicolás Costa-Fraga[1,2,4], Aitor Rodríguez-Casanova[1,4], Vanesa Varela[5,6], Elena Brozos-Vázquez[5,6], Francisca Vázquez-Rivera5,6, Yolanda Vidal-Insua[5,6], Sonia Candamio-Folgar[4,5,6], Rafael López-López[2,4,5,6], Ángel Díaz-Lagares[1,3,4]**

*1) Unidad de Epigenómica, ONCOMET, Instituto de Investigación Sanitaria de Santiago (IDIS).*
*2) Universidad de Santiago de Compostela.*
*3) Servicio de Análisis Clínicos, Complejo Hospitalario Universitario de Santiago (CHUS).*
*4) CIBERONC, ISCIII.*
*5) Servicio de Oncología Médica, Complejo Hospitalario Universitario de Santiago (CHUS).*
*6) ONCOMET, Instituto de Investigación Sanitaria*

*de Santiago (IDIS). *Co-first authors.***Introduction:** Colorectal cancer (CRC) is one of the leading causes of mortality worldwide. Its high incidence and mortality underscore the urgent need to discover non-invasive biomarkers for detection and prognosis. Epigenetic analysis of DNA methylation in circulating free DNA (cfDNA) holds significant potential for identifying cancer, enabling diagnosis prior to the onset of clinical manifestations. Beyond detection, DNA methylation-based biomarkers in cfDNA offer a promising tool for prognosis.

**Objectives**: The objective of this study was to identify and validate novel non-invasive epigenetic biomarkers in cfDNA with potential clinical utility for detection and prognosis of CRC.

**Materials and methods**: Methylation data obtained by next-generation sequencing (NGS) of cfDNA from CRC patients and healthy subjects from a GEO cohort (GSE149438) were analyzed. A search for differentially methylated CpGs between cancer patients and healthy controls was conducted, leading to the selection of three candidate genes. To confirm their methylation status in tumors, a cohort of 604 CRC patients (tumor and non-tumor colorectal tissues) from the TCGA public database was analysed. Methylation and expression levels of these tissue samples were correlated. To validate the methylation of the 3 genes identified in cfDNA, an independent cohort of 38 individuals (19 CRC and 19 healthy controls) was recruited from the Oncology Department of the Hospital Clínico Universitario de Santiago de Compostela. Genes were pre-amplified for subsequent methylation analysis via droplet digital PCR (ddPCR) in a QX200 System (BioRad). The diagnostic performance and survival impact of methylation for the three genes were analyzed using ROC and Kaplan-Meier curves, respectively, in GraphPad Prism.

**Results**: The promoter of the three identified genes were hypermethylated in primary tumors of CRC patients compared to colorectal non-tumor tissue. This hypermethylation was observed in both early and advanced stages of the disease. A negative correlation between methylation levels and gene expression was observed. ROC curve analysis in tissue samples demonstrated a high diagnostic accuracy of the three hypermethylated genes to identify CRC patients (AUCs > 0.95). Hypermethylation of one of these three genes was significantly associated with a worse prognosis of the disease (P=0.019). Of note, in cfDNA, the promoter of the three genes showed higher methylation levels in CRC than in healthy subjects ($p<0.0001$), with a high performance to detect CRC patients (AUC for the 3 genes of 0.94, 0.89 and 0.86).

**Conclusions**: In this study we have identified the promoter hypermethylation of three genes in both cfDNA and primary tumors of CRC patients. The presence of hypermethylation in both early and advanced stages of the disease, suggests that these biomarkers could be useful for early detection of CRC. Methylation of these genes in cfDNA displayed a strong ability to detect CRC patients, indicating that its analysis in liquid biopsy could serve as a novel non-invasive clinical tool for early detection of CRC detection. In addition, methylation of one of the identified genes in cfDNA emerges as a non-invasive biomarker of worse prognosis of the disease.

**13** Impact of ctDNA test TAV16 and SMARCA4/ERCC3 co-mutations on relapse and survival dynamics in pancreatic cancer patients

**Poster**

**Belén Martínez-Castedo[1,2] , Blanca García-Micó[1,2] , Jorge Martín-Arana[1,2] , Daniel G. Camblor[1] , Marco Airoldi[3,4] , Marisol Huerta[1] , Victor Seguí[1] , Nadia Juárez-Ríos[1] , Isabel Mora[5] , Marina Garcés-Albir[5] , Dimitri Dorcaratto[5] , Elena Muñoz-Forner[5] , Luis Sabater[5] , Susana Roselló[1,2] , Desamparados Roda[1,2] , Andrés Cervantes[1,2] , Francisco Gimeno-Valiente[1,6] , Noelia Tarazona[1,2]**

*1) Department of Medical Oncology, Biomedical Research Institute INCLIVA, University of Valencia, Valencia, Spain.*
*2) CIBERONC, Instituto de Salud Carlos III, Madrid, Spain*
*3) Department of Biomedical Sciences, Humanitas University, via Rita Levi Montalcini 4, 20072 Pieve Emanuele, Milan, Italy*
*4) IRCCS Humanitas Research Hospital, Medical Oncology and Haematology Unit, via Manzoni 56, 20089 Rozzano, Milan, Italy*
*5) Department of Surgery, Liver-Biliary and Pancreatic Unit, Biomedical Research Institute INCLIVA, Hospital Clínico, University of Valencia, Spain*
*6) Cancer Evolution and Genomic Instability Laboratory, University College London Cancer Institute, London, UK*

**Introduction and objectives:** Pancreatic ductal adenocarcinoma (PDAC) is the solid tumor with the lowest 5-year survival rate. Standard biomarkers such as CA19.9 and CEA exhibit significant limitations in sensitivity and specificity, particularly for predicting relapse and monitoring treatment response. In this context, liquid biopsy emerges as a promising alternative. By analyzing plasma circulating tumor DNA (ctDNA), liquid biopsy enables minimally invasive access to the molecular landscape of PDAC, bypassing the challenges of obtaining tumor tissue biopsies.
This study explores the utility of liquid biopsy for molecular profiling and relapse prediction in localized and locally advanced PDAC patients. Specifically, we aim to assess the impact of induction chemotherapy on the genomic profile and evaluate the utility of ctDNA status both pre-surgery and in the context of minimal residual disease (MRD).

**Materials and Methods:** A total of 31 PDAC patients across clinical stages I to III from Hospital Clínico Universitario in Valencia were enrolled in the study. Among them, 20 had localized pancreatic cancer (LPC), and 11 had locally advanced pancreatic cancer (LAPC), receiving induction chemotherapy regimens
like FOLFIRINOX, gemcitabine (GEM) and gemcitabine-abraxane (GEM-ABR) prior to surgery. Serial blood samples were collected at baseline, after neoadjuvant chemotherapy in LAPC patients, and 4-8 weeks post-surgery (MRD setting). Whole exome sequencing (WES), with unique molecular identifiers, was performed on

cfDNA plasma samples, and matched white blood cells were also sequenced to filter germline mutations and error correction.

**Results:** The analysis of the global genomic profile revealed no significant changes in the top 10 oncogenic mutations detected at baseline compared to the MRD samples. Interestingly, APC and BRCA1 mutations, which were among the top 10 at baseline, were no longer prominent post-surgery, while NOTCH1 and TET2 mutations emerged in their place. Notably, only 22.6% (7/31) of patients in our cohort had KRAS mutations, as validated by ddPCR, highlighting a surprisingly low detection rate for this mutation in plasma samples.
No significant association was observed between the presence of oncogenic mutations and the administration of chemotherapy, as most oncogenic variants remained unchanged before and after induction chemotherapy. This aligns with the fact that all patients exhibited a partial to complete response, enabling them to proceed to surgery.
CEA, CA19.9 and ctDNA MRD status were evaluated. ctDNA MRD status was significantly associated with relapse-free survival (RFS) (HR:5.24; P:0.0015) and overall survival (OS) (HR:6.59; P:0.0046), independent of treatment.
Importantly, the detection of SMARCA4 and ERCC3 mutations in the MRD setting was significantly associated with shorter RFS (HR co-mutated vs none: 0.47; HR single mutated vs none: 0.12; P:<0.001). Patients harboring both mutations had the poorest outcomes, with a median RFS of 10.3 months, compared to 14.1 for those with only one mutation and 27.9 months for patients without these mutations.
In patients who relapsed, somatic mutations identified in the MRD setting were enriched for hallmarks associated with epithelial-to-mesenchymal transition (EMT) (P:0.016), further emphasizing their role in disease progression.

**Conclusion:** ctDNA MRD status serves as a robust prognostic indicator, while the presence of SMARCA4 and ERCC3 co-mutations post-surgery identifies patients at higher risk for poor outcomes. These findings highlight the potential of integrating liquid biopsy into PDAC management. Ongoing analyses, including relapse samples, aim to further validate these results and uncover the genomic basis of EMT-driven progression

**14** NSCLC Exosomes: Unlocking Biomarker Potential

**Oral Communication**

**Susana* Torres[1,2,3] , Elena* Duréndez[1,2,3] , Sandra Gallach [1,2,3] , Eva Escorihuela[1,2] , Ester Munera[1,2,3] , Paula Espinosa[1,2] , Ana Blasco[1,2,3,4] , Cristina Caballero[1,2,3,4] , Ana Giménez[5] , Miguel Ángel Molina[5] , Silvia Calabuig[1,2,3,6] , Eloisa Jantus[1,2,3,7]**

*1) Molecular Oncology Laboratory, Fundación de Investigación Hospital General Universitario de Valencia, Valencia, Spain*
*2) Unidad Mixta TRIAL CIPF-FIHGUV, Valencia, Spain*
*3) CIBERONC, Madrid, Spain*
*4) Medical Oncology, Hospital General Universitario de Valencia, Valencia, Spain*
*5) Hospital Dexeus, Oncology Department, PangeaLab, Barcelona, Spain*
*6) Department of Pathology, Hospital General Universitario de Valencia, Valencia, Spain*
*7) Department of Biotechnology, Universitat Politècnica de València, Valencia, Spain.*

**Introduction:** Exosomes are membranous vesicles (40-150 nm) that carry biological information to distant tissues, being able to regulate several tumor processes. They are a valuable tool that may represent a turning point in the clinical management of non-small cell lung cancer (NSCLC).

**Goals:** Analyze plasma NSCLC derived exosomes cargo for searching new biomarkers that could improve NSCLC clinical management.

**Materials and methods:** Plasma samples were collected from 36 patients with advanced-stage NSCLC. Exosomes were isolated from plasma by ExoGAG. Exosomal RNAs were extracted using exoRNeasy Midi Kit and were RNA integrity and concentration were assessed using the Agilent RNA Pico Chip. A transcriptomic multiplexed analysis using nCounter® Low RNA Input Amplification Kit was performed to analyze mRNA of plasma-derived exosomes. Particularly, a human custom panel consisting of 30 genes was used to analyze plasma-derived EVs, including biomarkers associated with CSCs population, immune and drug response, proliferation and cell cycle, and other genes associated with lung cancer. Non-parametric Mann–Whitney U and Kruskal–Wallis tests were used to compare continuous variables. Prognostic value was determined by Kaplan-Meier curves. A p-value $<0.05$ was considered statistically significant.

**Results:** Exosomal RNA fragments were in the range of 30-200 nt. Out of the 30 probes included in the custom panel, only 23 showed hybridization signals in more than one patient (3 probes corresponding to housekeeping genes were included). Out of the 36 plasmatic exosome samples, only 32 successfully completed the

entire amplification, hybridization, and normalization process. Transcriptomic analysis of plasma NSCLC exosomes revealed that CD24 was significantly associated with gender and histology. Moreover, markers like MICA, RIOK3, S100A2 and MMP9 were significantly associated with histology, stage, mutational status, or response to therapeutic approaches, respectively. No significant association between these genes and the patients' RFS/OS ($p>0.05$) was found.

**Conclusions:** In conclusion, our study aimed to analyze plasma-derived exosomes from NSCLC patients for identifying potential biomarkers. Exosomal RNA analysis revealed associations between certain genes and different clinical factors, though no significant correlations were found with patient survival. Further research is needed to validate these findings.

**Supported by grants CB16/12/00350 (CIBERONC), PI22/01277 and P122/01221 (AES-ISCIII) and AECC Valencia**

**15** Extracellular vesicle DNA methylation signature as a predictor of chemotherapy response in metastatic colorectal cancer

**Oral Communication**

**Nicolas Costa-Fraga[1,2,3,4] , Aitor Rodríguez-Casanova[1,2,4] , Aida Bao-Caamano[1,4] , Alicia Abalo[4] , Elena Brozos-Vázquez[4,5] , Vanesa Varela[4,5] , Francisca Vázquez-Rivera[4,5] , Yolanda Vidal-Insua[4,5] , Sonia Candamio-Folgar[4,5] , Rafael López-López[2,3,4,5] , Ángel Díaz-Lagares[1,2,4,6]**

*1) Unidad de Epigenómica, Instituto de Investigación Sanitaria de Santiago (IDIS). 2) CIBERONC, ISCIII.*
*3) Universidad de Santiago de Compostela.*
*4) ONCOMET, Instituto de Investigación Sanitaria de Santiago (IDIS).*
*5) Servicio de Oncología Médica, Complejo Hospitalario Universitario de Santiago (CHUS).*
*6) Servicio de Análisis Clínicos, Complejo Hospitalario Universitario de Santiago (CHUS).*

**Background:** Metastatic colorectal cancer (mCRC) has a variable response to standard chemotherapy, particularly FOLFOX-based chemotherapy, with only 40-50% of patients responding effectively. This variability is may be influenced by epigenetic factors, particularly DNA methylation, which is implicated in chemotherapy resistance. Recent advances in liquid biopsy technology offer a non-invasive method to analyze these epigenetic alterations.

**Objectives:** This study aimed to investigate whether extracellular vesicle DNA (evDNA) methylation patterns can serve as predictive biomarkers for FOLFOX treatment response in mCRC patients.

**Methods:** A cohort of 20 mCRC patients scheduled for FOLFOX treatment was recruited, and their responses were evaluated using RECIST 1.1 criteria. Paired tumor tissues and EVs from blood samples were obtained before therapy, and DNA from these samples was extracted. Genome-wide DNA methylation analysis was performed using Illumina's EPIC v2.0 arrays. Differential methylation analysis was conducted to identify CpGs that were significantly different between patients with progressive disease (PD) and non-progressive disease (nPD).

**Results:** The analysis identified an evDNA methylation signature of 16,304 differentially methylated CpGs between the PD and nPD groups, from which 71% were hypomethylated in nPD patients. These CpGs were predominantly located in promoter regions and were enriched in key cancer-related pathways, such as PI3K/AKT, Wnt, and Cadherin signaling. A strong correlation (R=0.95) was observed between the methylation profiles of paired tumor tissues and EVs from blood samples, suggesting that evDNA reliably reflects tumor methylation patterns.

**Conclusions:** This study provides strong evidence that evDNA methylation pattern can serve as a non-invasive biomarker for predicting FOLFOX response in mCRC patients. Although the findings are promising, further validation in larger cohorts is necessary. This approach could potentially lead to more personalized and effective treatment strategies in mCRC.

**16** CLINICAL UTILITY OF LIQUID BIOPSY FOR ALK FUSION POSITIVE NON-SMALL CELL LUNG CANCER PATIENTS

**Oral Communication**

**Lucía Robado de Lope[1] , Roberto Serna-Blasco[1] , Rosario García-Campelo[3] , Aitor Azkárate[4] , Manuel Cobo[5] , Virginia Calvo[2] , Ernest Nadal[6] , Delvys Rodríguez-Abreu[7] , Ana Callejo[8] , Manuel Dómine[9] , Oscar Juan-Vidal[10] , Ana Collazo-Lorduy[2] , Joaquín Mosquera[3] , Raquel Marsé[4] , Atocha Romero[1,2] , Mariano Provencio[2]**

*1) Instituto de Investigación Sanitaria Puerta de Hierro-Segovia de Arana*
*2) Hospital Universitario Puerta de Hierro-Majadahonda*
*3) Complexo Hospitalario Universitario A Coruña*
*4) Hospital Universitario Son Espases*
*5) Hospital Regional Universitario de Málaga, IBIMA*
*6) Institute Català d'Oncologia, L'Hospitalet*
*7) Hospital Universitario Insular de Gran Canaria*
*8) Hospital Universitari Vall d'Hebron / Vall d'Hebron Institute of Oncology*
*9) Hospital Universitario Fundación Jiménez Díaz. IIS-FJD*
*10) Hospital Universitario y Politécnico La Fe*

**Introduction:** The identification of ALK rearrangements in non-small cell lung cancer (NSCLC) is crucial to guide targeted therapies. Tumor heterogeneity and biopsies availability are important limitations of routine tissue testing. Liquid biopsies (LB) are a non-invasive source of tumor molecular information.

**Objectives:** Here, we evaluate the clinical utility of LB for the management of ALK-positive NSCLC patients using platelets and extracellular vesicles (EVs) derived RNA, and circulating tumor DNA (ctDNA).

**Methodology:** Blood and tissue samples were collected from 30 ALK-positive, stage IIIB-IV NSCLC patients prior to initiating first-line brigatinib treatment. Nucleic acids from formalin-fixed-paraffin embedded (FFPE) tissue and plasma samples were analyzed by next-generation sequencing (NGS) using the Oncomine Focus Assay or the TruSight Oncology 500 ctDNA panel, respectively. EVs and platelets were isolated from plasma samples by differential centrifugation and RNA within these compartments was evaluated by QuantStudio 3D Digital PCR System and, when possible, by nCounter.

**Results:** The overall response rate to brigatinib treatment was 93% (95%CI: 75.1, 98.4), with a median duration of response of 14.7 months, median progression-free survival (PFS) of 33.8 months (6.2 – NR months) and median overall survival (OS) not reached (NR). NGS analysis of FFPE samples enabled the detection of *ALK* fusion

variants in all available samples. LB analysis revealed fusion detection rates of 33% using EVs, 14% in platelets, and 71% by ctDNA NGS profiling, and uncovered additional variants not identified in FFPE samples in some cases. Particularly, we identified 11 patients with *EML4-ALK* variant 1 (v1), 5 with v2, 7 with v3 and the remaining with less frequent variants or a combination of more than one variant. There were no significant differences in survival outcomes according to the type of variant, however, patients with *TP53* co-mutations detected by LB NGS had worse prognostic value (HR PFS: 8.8 [1.7-47], p=0.01; HR OS: 4.8 [1-23], p=0.046). Furthermore, ctDNA levels at baseline were evaluated, observing that patients with high ctDNA (>mean) had significantly inferior PFS (HR:5.7 [1.1-29], p=0.036) and OS (HR:5.2 [1-26], p=0.047) compared to those with low ctDNA levels. Other ctDNA cut-off values (such as 1% cutoff) validated these results.

**Conclusions:** The use of LB is a complementary and promising approach for ALK fusion variants identification. Detection rate by ctDNA profiling is higher compared with RNA-based approaches and could be considered a biomarker of prognostic significance.

**17** Cross-Platform Comparison of Plasma-SeqSensei™ CRC panel with reference Technologies for Detecting RAS/BRAF Mutations in ctDNA and tissue samples.

**Poster**

**Ramón Lago Lestón[1] , Marta Toledano Fonseca[2] , Elena Brozos[1] , Sonia Candamio[1] , Yolanda Vidal[1] , Alicia Abalo[1] , Francisca Vázquez[1] , Julia Alcaide[3] , Alicia Garrido Aranda[4] , Mª del Carmen Ocaña Farfán[4] , Emilio Alba[4,5] , Martina Alvarez[4,5] , Enrique Aranda[2,5] , Antonio Rodríguez[2,5] , Laura Muinelo[1,5] , Rafael López[1,5]**

*1) Translational Medical Oncology Group (Oncomet), Liquid Biopsy analysis Unit, Health Research Institute of Santiago de Compostela (IDIS), University Hospital of Santiago de Compostela (SERGAS), Santiago de Compostela, Spain.*
*2) Maimonides Biomedical Research Institute of Córdoba (IMIBIC), Córdoba, Spain; Medical Oncology Department, Reina Sofía University Hospital, Córdoba, Spain; Department of Medicine, Faculty of Medicine, University of Córdoba, Córdoba, Spain.*
*3) Servicio de Oncología Médica, Hospital Universitario Regional de Málaga, Málaga, Spain.*
*4) Laboratorio de biología molecular del cáncer (LBMC), Centro de investigaciones médico-sanitarias (CIMES-UMA), 29010, Málaga, Spain; The Biomedical Research Institute of Málaga (IBIMA-CIMES-UMA), 29010, Málaga, Spain.*
*5) Centro de Investigación Biomédica en Red de Cáncer, CIBERONC.*

**Introduction:** Analysis of circulating tumour DNA (ctDNA) represents a powerful tool for guiding targeted therapy and monitoring tumour evolution in patients with colorectal cancer (CRC). Highly sensitive diagnostics platforms are required for the detection of clinically relevant mutation (RAS or BRAF mutations) in plasma from CRC patients. Several platforms are commercially available for this analysis and the selection of the most robust and informative option for the clinical routine is a key factor to improve the use of liquid biopsy for this tumour genotyping. This study aimed to provide insight in the accuracy of Plasma-SeqSensei™ CRC for RAS/BRAF testing by comparing with other validated liquid biopsy/tissue-based approaches.

**Methodology:** In a real-world cohort of 198 patients diagnosed with advanced CRC recruited at the Oncology Department of three Spanish Hospitals (Complexo Hospitalario Universitario de Santiago de Compostela, Hospitales Universitarios Virgen de la Victoria y Regional de Málaga y Hospital Reina Sofía de Córdoba), plasma cfDNA was extracted and analyzed with Plasma-SeqSensei™ CRC panel (PSS), which allows the detection and identification of mutations in KRAS/NRAS, BRAF, PIK3CA and EGFR genes. Data obtained were compared with results

obtained in tissue using the standard operating procedure applied in each Hospital and data obtained analysing the cfDNA by OncoBEAM CRC RAS kit.

**Results:** The cfDNA analysis with PSS and BEAMing allowed to detect pathogenic variants in RAS/BRAF genes in the 53.2% and the 51,4% of the patients, respectively. Tissue-based testing detected RAS/BRAF mutations in the 50,3% of the patients. Overall comparison for RAS status obtained with the three strategies showed a good agreement, specially between the cfDNA characterization by BEAMing and PSS with a Kappa Cohen value of 0.907. The most common mutation detected was KRAS G12D, followed by KRAS G13D and KRAS G12V. Considering the MAFs, 25% of all mutations detected with PSS were between 0.07 and 0.5%. BRAF mutations were found in 6 (11,1%) patients using PSS being the most common G469A and V600E variants. Of note, the agreement between the three approaches analyzed was poor. In addition to RAS/BRAF status PSS analyzed PIK3CA and EGFR. A total of 27 variants were detected in PIK3CA, being the most common variants E545K and E542K. The 43% of all mutations detected in PIK3CA with PSS showed a MAF between 0.07 and 0.5%. Only 2 variants were detected in EGFR with a MAF of 0.015%. The percentage of variants detected below 0.5 % of MAF reinforces the need of highly sensitive strategies for CRC cancer genotyping through cfDNA analyses.

**Conclusions:** This multicenter study demonstrated the accuracy and specificity of PSS assay to identify clinically relevant mutations in plasma samples from a real-world cohort of metastasic CRC patients to guide the therapy selection.

**18** SOLTI-2102 HOPE Prostate study to assess the feasibility and impact of liquid biopsy-based genomic profiling in metastatic prostate cancer

**Poster**

**Pablo Cresta Morgado[1,2] , Tomás Pascual[3,4] , Rubén Olivera-Salguero[3] , Iria Martínez[3] , Fernando Salvador[3] , Ángel Borque-Fernando[5] , Antonio Rosino[6] , María José Donate[7] , Antonio Gómez-Caamaño[8] , Almudena Zapatero[9] , Fernando López-Campos[10] , Elena Castro[11] , Mara Cruellas[1,2] , Arkaitz Carracedo[12,13,14,1] , Enrique Gallardo[17] , Verónica Calderero[18] , Ana Vivancos[1] , Juan Manuel Ferrero-Cafiero[3] , Joaquin Mateo[1,2] , Joan Carles[1,2]**

*1) Vall d'Hebron Institute of Oncology (VHIO), Barcelona Spain*
*2) Hospital Universitari Vall d'Hebron, Barcelona, Spain*
*3) SOLTI Cancer Research Group, Barcelona, Spain*
*4) Hospital Clínic de Barcelona, Barcelona, Spain*
*5) Hospital Universitario Miguel Servet (IIS-Aragón), Zaragoza, Spain*
*6) Hospital General Universitario Morales Meseguer, Murcia, Spain*
*7) Complejo Hospitalario Universitario de Albacete, Albacete, Spain*
*8) Hospital Clínico Universitario de Santiago de Compostela, Santiago de Compostela, Spain*
*9) Hospital Universitario de La Princesa, Instituto de Investigación Sanitaria, Madrid, Spain*
*10) Hospital Universitario Ramón y Cajal, Madrid, Spain*
*11) Hospital Universitario 12 de Octubre, Madrid, Spain*
*12) Center for Cooperative Research in Biosciences (CIC bioGUNE), Basque Research and Technology Alliance (BRTA), Bizkaia Technology Park, Derio, Spain*
*13) CIBERONC*
*14) Ikerbasque, Basque Foundation for Science, Bilbao, Spain*
*15) Traslational prostate cancer Research lab, CIC bioGUNE-Basurto, Biocruces Bizkaia Health Research Institute, Barakaldo, Spain*
*16) Biochemistry and Molecular Biology Department, University of the Basque Country (UPV/EHU), Bilbao, Spain*
*17) Parc Taulí Hospital Universitari. Institut d'Investigació i Innovació Parc Taulí (I3PT-CERCA), Universitat Autònoma de Barcelona, Sabadell, Spain*
*18) Hospital Universitario San Jorge de Huesca, Huesca, Spain*

**Introduction:** Genomic testing (GT) allows the stratification of patients for precision medicine approaches, aiming to improve patient outcomes. Nevertheless, access to GT and molecular advisory boards (MABs) for clinical interpretation of the findings is often unequal among centers/regions. A strategy to support the optimal implementation of GT for metastatic prostate cancer (mPC) is to promote the active participation of patients in the management of their disease and to empower them to have a leading role in the pursuit of GT. Thus, we designed SOLTI-2102 HOPE Prostate (HOPE-P), an academic, observational, real-world, patient-centric study to assess the feasibility and impact of GT on treatment

decision-making for the clinical management of patients with mPC. Patients have a leading role during inclusion, sample obtention, and follow-up using a digital tool (DT). The involvement of treating physicians is recommendable, but not mandatory. Here we present the trial in progress report.

**Objectives:** The primary objective of HOPE Prostate is to evaluate the feasibility and impact of a liquid biopsy-based GT strategy in the clinical management of mPC patients. Key secondary objectives are to describe the genomic landscape of mPC in a real-world population and to study the clinical impact of GT in matched therapies prescribed by treating physicians.

**Methodology:** HOPE-P will include mPC patients living in Spain, with a first interim analysis at n=240 and a final target of n=360 (Figure 1). When a patient self-registers, a dedicated team validates the eligibility criteria through a phone interview. Patients provide their clinical data through the DT and, at progressive disease, a blood sample is acquired at a designated local laboratory in Spain, where the patient also provides an archival tumor sample retrieved from their center of care. Blood samples undergo ctDNA GT using the Guardant360® assay. Tissue samples undergo capture-based targeted sequencing at VHIO. New genomic and clinical information is regularly discussed by a multidisciplinary MAB that delivers a clinical interpretation report including potential therapeutic implications. After report delivery, patients are followed with twice-a-year questionnaires for 3 years to capture 1) clinical outcome; 2) GT-matched therapies received, and 3) patient's perception of how GT impacted disease management.
Recruitment started in March 2023 and by November 2024, 191 subjects registered to participate and 157 could be successfully included. A total of 68 patients provided blood samples for liquid biopsy-based GT, and 124 patients have sent tumor tissue. In 44 cases GT could be successfully performed in both types of samples, liquid biopsy and tumor tissue. Currently, 120 cases have been discussed by the MAB with the subsequent report delivered.

**NCT05885009**

**19** Therapeutic systems based on nanoemulsions for ovarian cancer treatment

**Poster**

**Laura Fuentes Varela[1] , María Emilia Vásquez Miño[1] , Carmen Abuín[1] , Rafael López[1,2,3] , Ana Belén Dávila-Ibáñez [1,2]**

*1) Translational Medical Oncology Group, Oncomet. Health Research Institute of Santiago de Compostela (IDIS)*
*2) CIBERONC, Centro de Investigación Biomédica en Red Cáncer*
*3) Medical Oncology Department, Complejo Hospitalario Universitario de Santiago de Compostela*

**Introduction:** Ovarian cancer (OC) is a leading cause of cancer deaths among women, responsible for approximately 207,000 deaths annually worldwide. The symptomatology includes abdominal pain, diarrhoea and weight loss, among others. These unspecific disorders result in a late-stage diagnosis when widespread peritoneal dissemination occurs, leading to this high mortality rate. This underscores the urgent need for innovative therapies to improve patient outcomes.
In this sense, photodynamic therapy (PDT), a minimally invasive FDA-approved cancer treatment, uses a light source that activates a photosensitizer, generating Reactive Oxygen Species (ROS) in the malign cells, minimizing healthy tissues and, therefore, reducing treatment side effects.
Verteporfin (VP) has shown promise in cancer therapy, particularly in PDT, due to its deeper tissue penetration. However, VP faces challenges mainly related to poor water solubility. To face this, this project seeks to enhance therapeutic efficacy, improve targeting accuracy, and overcome solubility limitations by encapsulating VP into oil-in-water nanoemulsions (NEs). These biocompatible NEs enable the encapsulation of VP due its hydrophobic core and they can be functionalized to only recognize cancer cells, avoiding non-affected areas.

**Objectives:** Therefore, the objectives of this innovative approach are to enhance tumor selectivity and develop a more effective treatment for advanced OC, with the goal of improving patient quality of life and survival rates. Once optimized, this strategy could also be extended to apply PDT-based NEs to other types of tumors.

**Methodology**: NEs were synthesized spontaneously by injecting an organic phase into an aqueous phase under magnetic stirring. The organic phase consisted of phosphatidylcholine and cholesterol, both lipids commonly found in cell membranes. Two types of NEs were formulated based on the fatty acid used as the hydrophobic core: OA-NEs (Oleic Acid-based NEs) and MG-NEs (Miglyol-based

NEs). For both types, Verteporfin (VP) was encapsulated (VP-NEs) by adding it to the organic phase before injection.

Once VP-NEs were synthesized, a purification step was performed to separate free VP from encapsulated VP. This was achieved using hydrophilic syringe filters, which allowed the aqueous sample containing VP-NEs to pass through while retaining the drug on the filters. This process enabled the differentiation between the theoretical and actual VP concentrations, which were then used to calculate the encapsulation efficiency. The actual VP concentration was determined through spectroscopy measurements, taking advantage of the specific absorbance spectrum of VP. After optimizing the synthesis process, the VP-loaded NEs were characterized. They were analyzed for size, shape, charge, and polydispersity index (PDI) using electron microscopy and dynamic light scattering (DLS). Stability studies were also conducted to determine the best storage conditions for the samples, assessing the effects of temperature and room light exposure over time. Cellular uptake studies were carried out by internalizing the NEs into the OV-90 ovarian cancer cell line and monitoring them using fluorescence and confocal microscopy. Finally, viability experiments were performed by treating OV-90 and SKOV-3 ovarian cancer cell lines with free VP, VP-OA-NEs, and VP-MG-NEs. These experiments aimed to determine the safe, non-cytotoxic VP concentrations that could be used before light activation for photodynamic therapy (PDT).

**Results:** There were formulated two different NEs (VP-OA-NEs and VP-MG-NEs), which exhibited well physico-chemical properties related to their size, charge and PDI. They showed a high encapsulation efficiency for both systems, of approximately 70% for each type. However, it was observed that VP-MG-NEs, allowed the encapsulation of VP until 0.8 ppm, while VP-OA-NEs reached the maximum loaded capacity at 0.5 ppm. Stability studies showed different behaviours between both systems as VP showed higher stability when loaded into MG-NEs than in OA-NEs, in all the tested conditions. However, any system VP-OA-NEs or VP-MG-NEs manifested noticeable changes over time among these different conditions, just an increase in size in the case of VP-OA-NEs when stored at room temperature under light exposure. Viability experiments showed that VP-MG-NEs have a similar IC toxicity behaviour to free VP, while VP-OA-NEs exhibited and increment of eightfold in SKOV-3 and OV-90 OC cell lines.

**Conclusions:** We have formulated two drug delivery systems that showed differences in terms of the capability of VP that they are able to encapsulate, the stability drug encapsulation and the cell viability. The difference on the physico-chemical properties of these delivery systems resulted in an impact on their cytotoxic behaviour. We are currently analyzing the effect of VP-OA-NEs, VP-MG-NEs and free VP when activating the photosensitizer molecule (VP) by using the specific light.

**20** A novel cfDNA methylation signature for the prediction of FOLFOX-based therapy response in advanced colorectal cancer

**Poster**

**Aitor Rodríguez-Casanova[1,2,3] , Nicolás Costa-Fraga[1,2,3,4] , Aida Bao-Caamano[1,3] , Alicia Abalo[3] , Elena Brozos-Vázquez[3,5] , Vanesa Varela[3,5] , Francisca Vázquez-Rivera[3,5] , Yolanda Vidal-Insua[3,5] , Sonia Candamio-Folgar[2,3,5] , Rafael López-López[2,3,4,5] , Ángel Díaz-Lagares[1,2,3,6]**

*1) Unidad de Epigenómica, Instituto de Investigación Sanitaria de Santiago (IDIS)*
*2) CIBERONC, ISCIII*
*3) ONCOMET, Instituto de Investigación Sanitaria de Santiago (IDIS)*
*4) Universidad de Santiago de Compostela*
*5) Servicio de Oncología Médica, Complejo Hospitalario Universitario de Santiago (CHUS)*
*6) Servicio de Análisis Clínicos, Complejo Hospitalario Universitario de Santiago (CHUS)*

**Background:** The first-line treatment of mCRC is usually based on fluoropyrimidine-based chemotherapies, where the combined use of 5-FU, leucovorin and oxaliplatin (FOLFOX) is the most common treatment. However, there are patients that do not respond to this therapy, highlighting the urgent unmet clinical need to discover novel biomarkers in order to identify the patients who will benefit from this treatment. The presence of DNA methylation in biological fluids represents a promising non-invasive biomarker. Therefore, the aim of this study was to discover novel non-invasive methylation biomarkers in cfDNA to predict FOLFOX-based therapy response of mCRC patients.

**Methodology:** A retrospective cohort of 20 mCRC patients before starting first-line treatment with FOLFOX was selected. Following RECIST 1.1 criteria, therapy response was evaluated at 3-6 months of starting FOLFOX. Using the Infinium MethylationEpic (EPIC) array v2.0, we analyzed the cfDNA methylome of baseline plasma samples of 8 patients with progressive disease (PD) and 12 patients with non-progressive disease (nPD). Bioinformatic analysis of methylation data was carried out using RnBeads 2.0.

**Results:** A total of 1,174 differentially methylated CpGs (DMCpGs) were identified in cfDNA between patients with nPD and PD. These DMCpGs were widely distributed throughout all the chromosomes of the genome. Interestingly, patients with nPD showed more hypomethylated CpGs than those with PD. Of note, we were able to identify a cfDNA methylation signature (episignature) comprising 406 DMCpGs that clearly differentiated nPD and PD. Furthermore, the genes of this episignature were involved in relevant cancer pathways related to chemotherapy resistance, such as PI3K/AKT, Wnt and Cadherin signaling.

**Conclusions:** In this study, using a genome-wide non-invasive methylation approach, we discovered a novel episignature of cfDNA able to predict the response to FOLFOX therapy in mCRC patients. This cfDNA methylation signature represents a promising non-invasive tool for precision oncology of CRC. We also identified the epigenetic deregulation of relevant genes and cancer pathways involved in the response to FOLFOX therapy, opening new avenues to overcome the resistance to this treatment.

**21** Predicting BCG Failure in Bladder Cancer Patients: a microRNA-Driven Biomarker Approach

**Poster**

**Rui Ribeiro-Pereira[1,2] , Ana Teixeira-Marques[1,3] , Cláudia Martins-Lima[1] , Rui Freitas[1,4,5] , João André Carvalho[1,4,5] , Jorge Correia[4] , Marta Dueñas[4] , Sara Monteiro-Reis[1] , Rui Henrique[1,9,10] , Carmen Jerónimo[1,10]**

*1) Cancer Biology and Epigenetics Group, IPO Porto Research Center (CI IPOP); CI IPOP@RISE Porto Comprehensive Cancer Center Raquel Seruca (Porto.CCC), Portuguese Oncology Institute of Porto (IPO Porto)/R. Dr. António Bernardino de Almeida, 4200 072 Porto, Po*
*2) Master Programme in Oncology, ICBAS School Medicine and Biomedical Sciences, University of Porto, R. Jorge de Viterbo Ferreira 228, 4050 313 Porto, Portugal.*
*3) Doctoral Programme in Biomedical Sciences, ICBAS School Medicine and Biomedical Sciences, University of Porto, R. Jorge de Viterbo Ferreira 228, 4050 313 Porto, Portugal.*
*4) Urology Clinic & Department of Urology, Portuguese Oncology Institute of Porto (IPO Porto), R. Dr. António Bernardino de Almeida, 4200 072 Porto, Portugal.*
*5) Doctoral Programme in Medical Sciences, ICBAS School Medicine and Biomedical Sciences, University of Porto, R. Jorge de Viterbo Ferreira 228, 4050 313 Porto, Portugal.*
*6) Molecular Oncology Unit, CIEMAT (Centro de Investigaciones Energéticas, Medioambientales y Tecnológicas), Madrid, Spain.*
*7) Biomedical Research Institute I+12, University Hospital "12 de Octubre", Madrid, Spain.*
*8) Centro de Investigación Biomédica en Red de Cáncer (CIBERONC), Madrid, Spain*
*9) Department of Pathology, Portuguese Oncology Institute of Porto (IPO Porto), R. Dr. António Bernardino de Almeida, 4200 072 Porto, Portugal.*
*10) Department of Pathology and Molecular Immunology, ICBAS-School of Medicine and Biomedical Sciences, University of Porto, R. Jorge de Viterbo Ferreira 228, 4050 313 Porto, Portugal.*

Bladder cancer (BlCa) represents a significant global health concern. High-risk non-muscle-invasive bladder cancer (NMIBC) is typically treated with Bacillus Calmette Guérin (BCG) installations. However, the efficacy of this treatment is constrained by suboptimal response rates and significant adverse effects in certain patients. Compounding these challenges is the lack of a clinically validated biomarker to predict treatment outcomes, which poses a substantial challenge in optimising patient management. MicroRNAs (miRNAs) have gained attention as promising non-invasive liquid biopsy biomarker candidates in BlCa due to their critical roles in tumorigenesis, progression, and response to therapy. Despite their potential, the clinical application of miRNA-based biomarkers for guiding BCG therapy remains elusive.

This study aimed at validating a previously identified miRNA signature - hsa-miR-483-3p, hsa-miR-579-3p, hsa-miR-874-5p e hsa-miR-4443 - using droplet digital PCR (ddPCR) analysis of urine sediments from patients undergoing BCG therapy.

A cohort of 79 NMIBC patients treated at the Portuguese Institute of Oncology of Porto (IPO Porto) was analysed. Urine samples were collected prior to BCG treatment initiation and at the final available treatment session. Total RNA was extracted using the Trizol method, followed by cDNA synthesis with the TaqMan™ MicroRNA Reverse Transcription Kit. MicroRNA expression levels were measured using ddPCR, following pre-established optimisations for cDNA input and annealing temperature performed by our research group.

The tested miRNA signature exhibited significant discriminatory power in differentiating true BCG responders from non-responders when combined in a panel. Incorporating this signature into a nomogram model further improved predictive accuracy, by retaining their significance in the multivariate model, achieving 58% sensitivity and 76% specificity values. Moreover, the model also demonstrated that high-risk patients were significantly more likely to experience BCG treatment failure compared to their low-risk counterparts.

These findings underscore the potential of miRNA-based biomarkers to refine patient stratification for BCG therapy, thereby reducing unnecessary treatments and optimizing therapeutic outcomes. By accurately identifying patients most likely to benefit from BCG, this approach paves the way for tailored and more effective BlCa cancer patients' management.

**22** A First-Line Approach: Liquid Biopsy-Guided Osimertinib Therapy for EGFR-Mutated NSCLC

**Poster**

**Susana* Torres-Martínez[1,2,3] , Silvia* Calabuig-Fariñas[1,2,3,4] , Ester Munera Maravilla [1,2,3] , Javier Iranzo[1] , Lara Navarro Cerveró[5] , Marais Mosqueda[1,2] , Sandra Gallach[1,2,3] , Nerea Fernández[1,2] , Amaya Fernández[1,2,6] , Cristina Caballero[1,2,3,6] , Ana Blasco[1,2,3,6] , Eloisa Jantus-Lewintre[1,2,3,7,8]**

*1) Molecular Oncology Laboratory, Fundación de Investigación Hospital General Universitario de Valencia, Valencia, Spain*
*2) Unidad Mixta TRIAL CIPF-FIHGUV, Valencia, Spain*
*3) CIBERONC, Madrid, Spain*
*4) Department of Pathology, Universitat de València, Valencia, Spain*
*5) Department of Pathology, Hospital General Universitario de Valencia, Valencia, Spain*
*6) Medical Oncology, Hospital General Universitario de Valencia, Valencia, Spain*
*7) Department of Biotechnology, Universitat Politècnica de València, Valencia, Spain*
*8) Nanomedicine, Centro Investigación Príncipe Felipe-Universitat Politècnica de Valencia, 46022 Valencia, Spain*

**Introduction:** Targeted therapies, especially those utilizing epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs), have revolutionized the treatment landscape for patients diagnosed with non-small cell lung cancer (NSCLC). Among these, third-generation TKIs, such as Osimertinib, have demonstrated remarkable efficacy, not only in prolonging overall survival and delaying disease progression but also in effectively managing challenging complications like brain metastases. These advancements underline the pivotal role of precision medicine in improving clinical outcomes and enhancing the quality of life for individuals battling advanced stages of NSCLC.

**Objectives**: The primary objective of this study was to demonstrate the utility of monitoring EGFR mutational status in ctDNA for the management of NSCLC patients treated upfront with Osimertinib.

**Material and Methods:** This study included 27 advanced NSCLC patients with mutated EGFR tumors at diagnosis, all of whom received Osimertinib as first-line therapy. Monthly blood samples were collected for EGFR ctDNA monitoring using digital PCR. At the time of disease progression, Next-Generation Sequencing (NGS) was performed to identify potential resistance mechanisms. Parametric and non-parametric tests were employed for statistical analysis. For survival analysis, Cox regression and Kaplan Meier with log-rank test were performed. P-value <0.05 was considered statistically significant.

**Results:** EGFR mutations were analyzed via liquid biopsy at diagnosis, revealing the following distribution: p.L858R in 40.7% of cases and exon 19 deletions in 59.3%. A total of 234 blood samples were collected to monitor EGFR mutational status throughout disease progression. At the time of this analysis, 15 out of 27 patients had progressed, and 17 had died. The median Progression-Free Survival (PFS) was 10.7 months [range: 2.73–38.9 months], and the median Overall Survival (OS) was 13.46 months [range: 2.73–46.73 months].

Patients who became plasma ctDNA-negative within the first six months of treatment showed a higher response rate to Osimertinib (p=0.044) and experienced improved PFS and OS compared to those with detectable EGFR mutations in ctDNA (PFS: 17.40 vs. 8.53 months, p=0.026; OS: 29.43 vs. 13.47 months, p=0.037). Liquid biopsy detected molecular progression earlier than radiological progression in 66.6% of cases (10/15), providing a median molecular anticipation of 3.76 months [range: 0.73–12.97 months]. Additionally, sequencing of these patients upon progression identified resistance mechanisms such as EGFR p.L718V and p.C797S, BRAF p.K601N, PIK3CA p.E545K, and amplifications in EGFR, CCND1, MET, PIK3CA, and CD274.

**Conclusion:** These findings underscore the utility of liquid biopsy and high-sensitivity techniques like dPCR for monitoring EGFR-mutated patients during treatment, enabling early detection of molecular progression. Furthermore, NGS proves valuable in providing predictive information at the point of targeted therapy progression.

**23** Liquid Biopsy for monitoring response in KRAS-Mutated NSCLC Patients Treated with First-Line Immunotherapy

**Poster**

**Ester Munera-Maravilla[1,2,3] , Susana Torres-Martínez[1,2,3] , Pablo José Gómez-Garcia[5] , Marais Mosqueda[1,2] , Luz Bellón[1,2] , Sandra Gallach-García[1,2,3] , Lara Navarro[4] , Paula Espinosa-Olarte[5] , Ana Blasco Cordellat[1,2,3,5] , Cristina Caballero[1,2,3,5] , Eloisa Jantus-Lewintre[1,2,3,6] , Silvia Calabuig-Fariñas[1,2,3,7]**

*1) Molecular Oncology Laboratory, Fundación de Investigación Hospital General Universitario de Valencia, Valencia, Spain*
*2) Unidad Mixta TRIAL CIPF-FIHGUV, Valencia, Spain*
*3) CIBERONC, Madrid, Spain*
*4) Department of Pathology, Hospital General Universitario de Valencia, Valencia, Spain*
*5) Medical Oncology, Hospital General Universitario de Valencia, Valencia, Spain*
*6) Department of Biotechnology, Universitat Politècnica de València, Valencia, Spain*
*7) Department of Pathology, Universitat de València, Valencia, Spain*

**Introduction:** The monitoring of response through the tracking of mutations present in circulating tumor DNA (ctDNA) has proven effective not only in targeted therapies but also in response to immunotherapy. Also, it has shown to be a potential tool in capturing primary resistance to immunotherapy in *KRAS*-mutated patients. Thus, it is highly relevant to explore biomarkers to predict and monitor treatment response through a liquid biopsy-based approach, given that obtaining tissue rebiopsies during disease evolution is impractical and invasive.

**Objective:** The primary aim of this study was to demonstrate the clinical utility of monitoring KRAS mutational status in patients with non-small cell lung cancer (NSCLC) undergoing first-line immunotherapy.

**Methods:** Twenty-three advanced NSCLC patients with *KRAS* mutations who were receiving immunotherapy ± chemotherapy as first-line treatment were included in this study. Blood samples were collected, and monitoring was performed using digital PCR (dPCR, Applied Biosystems™ QuantStudio™ Absolute Q, ThermoFisher Scientific). Patients were categorized into two groups: Group A (poor prognosis) and Group B (good prognosis). All patients in Group A met one or more of the following conditions: i) VAF% > 1.97 (median VAF of shedders) at baseline, ii) VAF% became detectable during follow-up, or iii) VAF% remains detectable. Group B included patients who did not meet any of these conditions.

**Results:** 23 NSCLC patients were included in this study, and 46 blood samples were collected to monitor *KRAS* mutational status throughout disease evolution. The *KRAS* mutations identified at diagnosis were p.G12C (80%), G12A (4%), G12D

(4%) and G13D (4%). ctDNA was detectable at baseline in 18 out of 25 cases. By the time of the current analysis, 12 out of 25 patients had relapsed, from which 4 patients were exitus. The median PFS and OS were 8.27 (0.7-74.77) and 9.57 (0.7-108.93) months, respectively. It was observed that patients categorized in group A showed worse prognosis when compared to patients categorized in group B for PFS (8.97 vs 23.23 months, *p*=0.036).

**Conclusion:** These results highlight the utility of ctDNA and high sensitivity techniques such as dPCR to monitor *KRAS*-mutated patients throughout immunotherapy. Furthermore, it emphasises the implementation of liquid biopsy in the clinical practice to allow early molecular response to treatment detection.
This study was supported by GIDO 2024 and CB16/12/00350.

**24** A tumor-agnostic ctDNA whole-exome sequencing approach enhances minimal residual disease detection in localized colon cancer patients

**Oral Communication**

**Jorge Martín-Arana[1,2] , Francisco Gimeno-Valiente[3] , Tenna Vesterman Henriksen[4] , Blanca García-Micó[1,2] , Belén Martínez-Castedo[1,2] , Valentina Gambardella[1] , Marisol Huerta[1,2] , Carolina Martínez-Ciarpaglini[2,5] , Juan Antonio Carbonell-Asins[6] , Daniel G. Camblor[1] , David Moro[7] , Vicente Pla[7] , Leticia Pérez-Santiago[7] , Stephanie García-Botello[7] , Alejandro Espí[7] , Susana Roselló[1,2] , Desamparados Roda[1,2] , Claus Lindbjerg Andersen[4] , Andrés Cervantes[1,2] , Noelia Tarazona[1,2]**

*1) Department of Medical Oncology, INCLIVA Biomedical Research Institute, University of Valencia, Valencia, Spain*
*2) Instituto de Salud Carlos III, CIBERONC, Madrid, Spain*
*3) Cancer Evolution and Genome Instability Laboratory, University College London Cancer Institute, London, UK*
*4) Department of Molecular Medicine, Aarhus University Hospital, Aarhus, Denmark*
*5) Department of Pathology, INCLIVA Biomedical Research Institute, Valencia, Spain*
*6) Biostatistics Unit, INCLIVA Biomedical Research Institute, Valencia, Spain*
*7) Department of Surgery, INCLIVA Biomedical Research Institute, Valencia, Spain*

**Introduction:** Liquid biopsy is now a valuable tool for guiding adjuvant chemotherapy decisions in stage II-III localized colon cancer (LCC). Studies have shown that detecting circulating tumor DNA (ctDNA) post-surgery relates to a higher risk of recurrence and poorer disease-free survival. However, current ctDNA assays lack sensitivity in detecting minimal residual disease (MRD), especially immediately after surgery when treatment decisions are critical.

**Objectives:** To develop a specialized bioinformatics approach for the tumor-agnostic whole-exome sequencing analysis of post-surgery plasma samples, to improve the MRD detection sensitivity of current personalized assays or custom panels based on tumor-informed approaches.

**Methodology:** 40 relapsed LCC patients were recruited, with 25 patients from the Hospital Clinico de Valencia as discovery cohort, along with 21 additional relapse-free patients included as negative controls. The validation cohort consisted of 15 relapsed patients recruited from Aarhus University. WES was conducted on available primary tumor, baseline plasma, and post-surgery plasma samples, as well as paired white blood cell samples. Unique molecular identifiers (UMIs) were included for error correction. We implemented a tumor-agnostic whole-exome sequencing (WES-TA) approach on MRD. Additionally, to assess the cost-effectiveness of MRD detection using WES, the 16 mutations with the highest

variant allele frequency (VAF) in each patient's primary tumor exome were selected as monitoring candidates within a tumor-informed framework. Furthermore, we developed a new tumor-agnostic assay based on selecting the 16 mutations with the highest VAF in baseline plasma exome sequencing (TAV16).

**Results:** WES-TA somatic variant calling in postoperative plasma sequencing identified at least one somatic mutation in 86.7% (13/15) and 100% (14/14) of patients in the discovery and validation cohorts, respectively. Specificity was 95.2%, with only one relapse-free patient classified as ctDNA-positive. The tumor-informed analysis, based on primary tumor WES, revealed at least two candidate mutations in postoperative plasma in 67% (10/15) of patients in the discovery cohort and 57% (8/14) in the validation cohort. Using the TAV16 approach, sensitivity was 67% (6/9) and 86% (12/14) in the discovery and validation cohorts, respectively. However, sensitivity increased to 89% (8/9) and 100% (14/14) when a positive ctDNA status was defined by the detection of a single mutation in plasma. Notably, in the tumor-informed approach, 96% and 98% of selected variants were identified as unique to individual patients in the discovery and validation cohorts, respectively. For the TAV16 approach, 86% of the mutations were unique in the discovery cohort and 78% in the validation cohort.

**Conclusions:** In this study, we demonstrated that a whole-exome sequencing tumor-agnostic (WES-TA) approach for ctDNA analysis immediately after surgery significantly enhances the sensitivity for MRD. This approach surpasses previous studies employing personalized assays based on either tumor-informed or tumor-agnostic approaches with custom panels while maintaining high specificity. Due to the challenges of implementing this strategy in clinical practice, largely owing to the costs associated with current technologies, we have developed a more cost-effective assay: the TAV16 approach. This assay achieved sensitivity values comparable to those of the WES-TA approach when considering a single mutation for ctDNA positivity. These findings suggest that utilizing a personalized tumor-agnostic assay based on plasma WES at diagnosis, rather than primary tumor sequencing, could be pivotal in refining monitoring strategies, advancing precision medicine in this clinical context, and reinforcing the critical role of personalized assays in MRD detection.

**25** Assessing Mutational Status in NSCLC through Exosomal Liquid Biopsies

**Poster**

**Susana* Torres[1,2,3] , Elena* Duréndez[1,2,3] , Marais Mosqueda [1,2] , Ester Munera[1,2,3] , Nerea Fernández[1] , Paula Espinosa[1,2,4] , Ana Blasco [1,2,3,4] , Cristina Caballero [1,2,3,4] , Silvia Calabuig[1,2,3,5] , Eloisa Jantus-Lewintre[1,2,3,6]**

*1) Molecular Oncology Laboratory, Fundación de Investigación Hospital General Universitario de Valencia, Valencia, Spain*
*2) Unidad Mixta TRIAL CIPF-FIHGUV, Valencia, Spain*
*3) CIBERONC, Madrid, Spain*
*4) Medical Oncology, Hospital General Universitario de Valencia, Valencia, Spain*
*5) Department of Pathology, Hospital General Universitario de Valencia, Valencia, Spain*
*6) Department of Biotechnology, Universitat Politècnica de València, Valencia, Spain.*

**Introduction:** Liquid biopsy has emerged as a promising alternative for the detection and monitoring of tumor-derived genetic alterations. Among the various components analyzed in liquid biopsies, circulating free DNA (cfDNA) has been extensively studied. However, recent research suggests that exosome-derived DNA (exo-DNA) might offer a more reliable source for detecting clinically relevant mutations.

**Goals:** To evaluates exo-DNA's effectiveness compared to cfDNA in identifying mutations in NSCLC patients.

**Materials and methods:** Peripheral blood samples were collected from 6 patients with NSCLC. Exosomes were isolated from plasma and serum by ExoGAG. For cfDNA isolation and exo-DNA, a sample volume of 2 ml and 0.5-1Ml was employed, respectively, using the QIAamp DNA Micro kit. cfDNA and exo-DNA mutations were determined by different dPCR technologies, including BEAMing (beads, emulsion, amplification, and magnetics) and dPCR-3D (QuantStudio 3D Digital PCR System).

**Results:** The DNA concentration obtained from isolated exosomes is comparable to, if not more suitable, the cfDNA present in plasma for the determination of these mutations by dPCR. The comparison between cfDNA and exo-DNA revealed that the percentage of the mutant fraction (MF) obtained in exosomes was similar or higher than the detected in cfDNA for several *KRAS* and *EGFR* mutations (Table 1). In the case of patient 6890, the mutation was only detected in cfDNA using BEAMing (not detected by dPCR-3D), and it was also detected in exosomes (using dPCR-3D) after increasing the input volume. The same mutations were also detected in serum samples but MF was lower than in plasma.

**Conclusions:** This study demonstrates that exo-DNA is a promising tool for detecting clinically relevant mutations in NSCLC, using reduced sample volumes, and even in cases with low MF. Therefore, exosomes could be a valuable component of liquid biopsy strategies for monitoring advanced-stage NSCLC, though further validation in larger patient cohorts is needed.

**Supported by grants CB16/12/00350 (CIBERONC), PI22/01277 and P122/01221 (AES-ISCIII) and AECC Valencia**

**26** Genomic and Methylomic ctDNA Analysis Reveals Epigenomic Dysregulation as a Key Driver of Therapy Resistance in Metastatic Colorectal Cancer

**Poster**

**Daniel G. Camblor[1] , Francisco Gimeno-Valiente[1,2] , Jorge Martín-Arana[1,3] , Blanca García-Micó[1,3] , Belén Martínez-Castedo[1,3] , Celia Martín-Bravo[4] , Carolina Martínez-Ciarpaglini[3,5] , Brenda Palomar[5] , David Moro[6] , David Casado[6] , Vicente Pla[6] , Leticia Pérez-Santiago[6] , Stephanie García-Botello[6] , Alejandro Espí[6] , Marisol Huerta[1,3] , Susana Roselló[1,3] , Desamparados Roda[1,3] , Andrés Cervantes[1,3] , Noelia Tarazona[1,3]**

*1) Department of Medical Oncology, INCLIVA Biomedical Research Institute, Valencia, Spain 2) Cancer Evolution and Genome Instability Laboratory, University College London Cancer Institute, London, United Kingdom 3) Instituto de Salud Carlos III, CIBERONC, Madrid, Spain 4) Hospital Regional Universitario, Málaga, Spain 5) Department of Pathology, INCLIVA Biomedical Research Institute, Valencia, Spain 6) Colorectal Surgery Unit, Department of General and Digestive Surgery, INCLIVA Biomedical Research Institute, Hospital Clínico Universitario, Valencia, Spain*

**Introduction:** Therapy resistance in metastatic colorectal cancer (mCRC) remains a major clinical challenge, with current knowledge limited to well-studied genes such as *RAS* and *BRAF*. However, tumor adaptation often involves complex mechanisms that extend beyond these targets, complicating the development of effective treatments. Circulating tumor DNA (ctDNA) offers a unique opportunity to investigate the full spectrum of tumor evolution, capturing intratumor heterogeneity that is often missed by tissue biopsies and facilitating real-time monitoring of molecular changes. By integrating whole-exome sequencing (WES) and methylomic analysis of ctDNA, we aim to address gaps left by traditional approaches, offering a more comprehensive understanding of resistance mechanisms in mCRC.

**Objectives:** To elucidate the molecular mechanisms underlying therapy resistance in mCRC through integrated genomic and methylomic profiling of ctDNA.

**Methodology:** We conducted a retrospective analysis of 30 mCRC patients recruited at the Hospital Clínico Universitario de Valencia (Spain). Plasma samples were collected after diagnosis (untreated, baseline) and at first progression to chemotherapy ± anti-VEGF or anti-EGFR targeted therapy, as appropriate.

WES was carried out on samples from both timepoints (baseline and progression), along with paired white blood cells to exclude germline variation. Variant calling was performed using a custom pipeline for low-frequency variant detection,

incorporating unique molecular identifiers to correct sequencing errors. Logistic LASSO regression was applied to identify the most relevant genes in which oncogenic variants, as described by their variant allele frequency (VAF), related to progression. Further confirmation of the association between mutational status and progression was obtained with McNemar's tests, and multivariable Cox regression accounting for sex, age, tumor location and therapeutic regimen was used to assess the impact of these mutations on progression-free survival (PFS).

Methylation was assessed with Infinium MethylationEPIC v2.0 BeadChip microarrays (which target ~900k CpG sites across the genome), and preprocessed with the SeSAMe pipeline in R, masking low quality probes and filtering out cross-reactive probes as described by Peters et al. (2024). The DMRcate package was used to identify differentially methylated regions (DMRs) overlapping with gene promoters and regions 1-5 kb upstream of the transcription start site. DMRs were defined using Benjamini-Hochberg-adjusted p-values ≤ 0.05 and accounting for paired testing. Overrepresentation analysis was performed on the genes mapped to these DMRs to identify epigenomic-driven pathways related to progression.

**Results:** *ARID1A* inactivating mutations were found to be the primary acquired genetic event associated with progression across our cohort, as uncovered by logistic LASSO regression. Indeed, these mutations were more often found in progression samples compared to baseline samples (14/30 vs. 5/30; p = 0.022), and their acquisition was an independent predictor of shorter PFS (HR = 3.2, 95% CI 1.1-9.7, p = 0.036). When considering the top increases in VAF from baseline to progression at the individual patient level, we found that some of these main mutations arose in genes previously linked to therapy resistance and key epigenetic regulators: *NF1*, *MED12*, *TET2*, *ARID1B*, *KMT2C* and *KMT2D*, among others. The overall concordance of mutated genes between baseline and progression samples was high (81.2%), further supporting the involvement of resistance mechanisms beyond just genetic alterations.

Differential methylation analysis revealed 16,028 potentially functional DMRs, including regions mapping to genes that are involved in therapy resistance and cell plasticity. Specifically, hypomethylation was observed in progression for genes such as *EGFR*, *PDGFRB*, *SOX2-OT*, *HDAC7* and *RUNX1*. Conversely, hypermethylation occurred in genes such as *ABCC11*, *ELF1* and *METTL16*. Notably, *ARID1A* showed significant hypermethylation at progression, correlating with the genomic findings. This suggests a dual mechanism of *ARID1A* silencing —via both mutation and methylation— which could amplify its impact on therapy resistance. Overrepresentation analysis of DMR-associated genes revealed an enrichment in the EGFR tyrosine kinase inhibitor resistance and VEGF signaling pathways, suggesting that resistance to therapy may also be driven by methylation changes. Interestingly, we found enrichment in mTOR signaling and longevity regulation, raising the possibility that plasticity in resistant tumors may be achieved via

epigenomic regulation to counter therapeutic pressure.

**Conclusions:** Both genomic and methylomic profiling of ctDNA highlight that epigenomic dysregulation, particularly *ARID1A* inactivation, is a key factor behind therapy resistance in mCRC. Further investigation of the epigenomic landscape may reveal new avenues for therapeutic intervention.

**27** Liquid biopsy-based epigenetic biomarkers for guiding first-line treatment in metastatic pancreatic cancer

**Poster**

**Pablo Cano-Ramírez[1,2] , Marta Toledano-Fonseca[1,2] , Mª Teresa Cano-Osuna[1,3,4] , Antonio Rodríguez-Ariza[1,2,3] , Enrique Aranda[1,2,3,4] , Mª Victoria García-Ortiz[1,2]**

*1) Maimónides Biomedical Research Institute of Córdoba (IMIBIC), Córdoba, Spain.*
*2) Cancer Network Biomedical Research Center (CIBERONC), Madrid, Spain.*
*3) Medical Oncology Department, Reina Sofía University Hospital, Córdoba, Spain.*
*4) Department of Medicine, Faculty of Medicine, University of Córdoba, Córdoba, Spain.*

**Introduction:** Pancreatic cancer remains the deadliest form of cancer due to its late diagnosis, and the limited efficacy of current treatments. Optimizing first-line treatment strategies and patient follow-up could improve disease management by enabling more personalized therapeutic approaches. Circulating CA19-9 levels and computed tomography (CT) are the standard tools to assess disease progression. Recently, liquid biopsy-based methylation analysis has emerged as a promising non-invasive method for developing epigenetic biomarkers with diagnostic, response predictive and prognostic value.

**Objective:** Identification of epigenetic biomarkers based on cell-free DNA (cfDNA) methylation in blood samples from metastatic pancreatic ductal adenocarcinoma (mPDAC) patients to evaluate treatment response and monitor disease progression.

**Methods:** An Illumina Infinium EPIC 850K methylation array was used to analyze cfDNA samples, comparing methylation patterns between healthy individuals and mPDAC patients. The array findings were then validated by digital droplet PCR (ddPCR) including samples from mPDAC patients at diagnosis and sequential samples obtained during disease progression. Additional PDAC biomarkers, such as CA19-9 levels, *RAS* mutation allele fraction (MAF), cfDNA concentration and cfDNA fragmentation were also evaluated.

**Results:** Nineteen differentially methylated CpG positions were identified in cfDNA from mPDAC patients compared to healthy individuals, primarily located in gene regions, at the gene body or at their promoter region. Validation via ddPCR confirmed the presence of differentially methylated CpGs in three *genes of interest* (*GOI1*, *GOI2* and *GOI3*). Methylation analysis showed a strong prognostic value for *GOI1*, *GOI2* and *GOI3,* along with significant association with other PDAC biomarkers, including *RAS* MAF, cfDNA concentration and cfDNA fragmentation. However, no correlation was observed with CA19-9 levels. Furthermore, *GOI3*

methylation was able to stratify patients based on their response to first-line FOLFIRINOX-based treatment, and provided insights into disease progression, correlating with treatment outcomes.

**Conclusions:** Our findings demonstrate a distinct and differential methylation pattern in cfDNA from metastatic pancreatic cancer patients, highlighting the potential clinical utility of liquid biopsy-based epigenetic biomarkers for guiding first-line treatment decisions and optimizing disease management.

Funding: Junta de Andalucía ProyExcel_00734 and Beca Carmen Delgado / Miguel Pérez-Mateo 2024.

**28** Clinicopathological and Molecular Features of Recurrence Based on Circulating Tumor DNA in Resected stage II-III Colorectal Cancer Patients

**Poster**

**Judit Sanz Beltran[1] , Renata Seminerio[1,2] , María Alejandra Molina Pérez[1] , Anna C Virgili[1] , Berta Martín Cullell[1] , Francisco J. Pelegrín[1] , Pau Riera[3] , Victoria Fusté[4] , Caterina Fumagalli[4] , Justyna Szafranska[4] , Lluis Catasus[4] , Alan Gonzalez[4] , David Páez[1]**

*1) Medical Oncology Department, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain*
*2) Medical Oncology Department, Policlino Sant'Orsola, Bologna, Italy*
*3) Pharmacy Department, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain*
*4) Pathology Department, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain*

**Introduction:** Liquid biopsy, particularly through circulating tumor DNA (ctDNA), has gained significant attention as a promising biomarker in colorectal cancer (CRC) patients, especially for monitoring treatment response in metastatic cases. In resected CRC, ctDNA is a valuable tool for detecting minimal residual disease (MRD) and identifying patients at high risk of recurrence. However, the clinicopathological and molecular features of recurrence in ctDNA-positive versus ctDNA-negative patients remain poorly understood

**Objective:** To investigate the role of liquid biopsy in patients with resected CRC, analyze recurrence rates among ctDNA-negative and ctDNA-positive patients post-surgery, and characterize the clinicopathological and molecular features of patients who experience recurrence.

**Methodology:** We conducted a study (EudraCT: 2020-000451-12) including patients with surgically resected stage II-III CRC, who were candidates for adjuvant chemotherapy at our hospital. Blood samples for ctDNA assessment were collected between 4 and 8 weeks after surgery. ctDNA was analized with AVENIO Oncology Assay Surveillance Test. Two cohorts were defined: Cohort A (ctDNA-negative or non-detected) and Cohort B (ctDNA-positive). Clinical and treatment data were extracted from medical records and molecular profiles in recurrence cases were obtained using the Oncomine Precision Assay on tumor samples. Descriptive statistics were used to summarize baseline characteristics.

**Results**: From 2021 to 2024, a total of 147 patients (median age: 68 years, range: 32–85, 53% male) were included, of which 38% had stage II and 62% had stage III CRC. Cohort A included 128 patients (ctDNA-negative or non-detected), while Cohort B included 19 patients (ctDNA-positive). With a median follow-up of 20.7

months, 6 recurrences (4.7%) were observed in Cohort A and 9 recurrences (47.7%) in Cohort B. The majority of these events occurred within the first year of follow-up (66.7%), with all of them taking place within the first two years. Clinicopathological and molecular features for both cohorts are summarized in Tables 1 and 2. In Cohort A, most relapsed patients had pT4 tumors, with recurrences predominantly occurring in the peritoneum or lungs, consistent with previously reported patterns. Notably, all patients in Cohort A who relapsed exhibited alterations in NGS with a higher incidence of KRAS mutations in Cohort A (83.3%) compared to Cohort B (44.4%).

**Table 1: Cohort A (ctDNA negative)**

| Patient ID | Age | Gender | UICC stage | Stage | Location | MSS/MSI status | Adjuvant treatment | Time to recurrence | Recurrence site | Molecular profile |
|---|---|---|---|---|---|---|---|---|---|---|
| 40 | 68 | F | T4aN0 | II | Left | MSS | CAPOX (4 cycles) | 8,4 months | Peritoneal Lung | KRAS G12S PIKC3CA H1047L |
| 81 | 69 | M | T4aN0 | II | Left | MSS | CAPOX (4 cycles) | 20,7 months | Lung | KRAS G13D PIK3CA loss |
| 92 | 65 | M | T4aN2b | III | Right | MSS | FOLFOX (12 cycles) | 11,4 months | Liver Ganglionar | KRAS G13D GNAS R201C IDH1 R132C PTEN R130Q |
| 101 | 77 | F | T4aN1a | III | Right | MSS | Capecitabine (6 cycles) | 20,2 months | Peritoneal | ERBB2 amplification |
| 116 | 76 | M | T4aN0 | II | Left | MSS | CAPOX (4 cycles) | 9,3 months | Peritoneal | KRAS G12D TP53 Y236H |
| 122 | 80 | M | T1N1a | III | Right | MSS | CAPOX (4 cycles) | 10,5 months | Liver | KRAS G12D APC Q1406X CTNNB1 P16S |

**Table 2: Cohort B (ctDNA positive)**

| Patient ID | Age | Gender | UICC stage | Stage | Location | MSS/MSI status | Adjuvant treatment | Time to recurrence | Recurrence site | Molecular profile |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 70 | F | T3N1a | III | Left | MSS | CAPOX (8 cycles) | 6,9 months | Liver | NRAS Q61L |
| 2 | 69 | F | T4aN2 | III | Left | MSS | FOLFOX (12 cycles) | 14,3 months | Ganglionar | BRAF V600E |
| 3 | 75 | F | T4aN1a | III | Right | MSS | FOLFOX (11 cycles) | 5,9 months | Liver<br>Ganglionar<br>Peritoneal | KRAS Q61H<br>TP53 G245S<br>AKT1 E17K |
| 5 | 76 | F | T3N2b | III | Right | MSS | FOLFOX (11 cycles) | 6,6 months | Liver<br>Lung | KRAS G12S<br>IDH1 R132C |
| 13 | 85 | M | T4bN2b | III | Left | MSS | FOLFOX (8 cycles) | 3,2 months | Liver | Uknown |
| 14 | 73 | M | T3N1b | III | Right | MSI | FOLFOX (12 cycles) | 13,2 months | Liver | KRAS G13D |
| 17 | 63 | M | T3N2b | III | Right | MSS | FOLFOX (12 cycles) | 10,5 months | Local | WT* |
| 19 | 74 | F | T3N1b | III | Rectum | MSS | Capecitabine (8 cycles) | 7,2 months | Ganglionar<br>Lung | KRAS G12V<br>PI3KCA G545L<br>TP53 V216M |
| 20 | 65 | M | T3N2a | III | Left | MSS | FOLFOX (12 cycles) | 19 months | Ganglionar | WT* |

M: male, F: female. MSS: Microsatellite Stable. MSI: Microsatellite Instability CAPOX: capecitabine and oxalipatin. WT: wild type.

**Conclusions:** Our analysis based on ctDNA provided valuable insights. The first two years post-surgery emerged as a critical period requiring close monitoring. Most recurrences in stage II-III CRC patients with ctDNA-negative results were observed in pT4 tumors. Additionally, molecular profiling revealed distinct mutation patterns between ctDNA-negative and ctDNA-positive patients. These findings could guide the selection of adjuvant treatment selection based on risk stratification and optimizing surveillance strategies for patients with resected stage II-III CRC.

**29** KEAP1 and STK11: emerging biomarkers in the clinical management of refractory non-small cell lung cancer

**Poster**

**Josu Aurrekoetxea Oribe[1] , Begoña Calvo Martínez[1] , Mónica Saiz Camin[2] , Xabier Mielgo Rubio[1] , Raquel Casas Cornejo[1] , Berezi Ortega Gallastegi[1] , Maria Gutierrez Toribio[1] , Maria Jesus Salas Jiménez[1] , Eider Azkona Uribelarrea[1]**

*1) Department of Medical Oncology, Cruces University Hospital - Biobizkaia Health Research Institute, Barakaldo, Bizkaia, Spain*
*2) Department of Pathology, Cruces University Hospital - Biobizkaia Health Research Institute, Barakaldo, Bizkaia, Spain*

**Introduction:** Advanced-stage non-small cell lung cancer (NSCLC) accounts for more than 60% of diagnosed cases. It represents a significant challenge in oncology, particularly for patients who progress to first-line treatment due to poor prognosis and limited treatment options. In recent years, the advent of liquid biopsy techniques has revolutionized the way we approach cancer diagnosis and monitoring, particularly through the analysis of circulating tumor DNA (ctDNA). Next-Generation Sequencing (NGS) analysis of a targeted gene set implicated in oncological processes provides a comprehensive evaluation of tumor-specific genetic alterations, offering insights into tumor heterogeneity and resistance mechanisms, ultimately facilitating personalized therapy strategies for advanced NSCLC.

**Objectives:** This study aimed to investigate the molecular characterization of ctDNA in patients with advanced NSCLC who progressed on first-line therapy to identify potential biomarkers to improve treatment selection and patient outcomes.

**Methodology:** Patients diagnosed with advanced NSCLC who had progressed or failed to respond to first-line therapy were recruited for the study between October 2022 and December 2023. Peripheral blood samples were collected at the time of relapse. Comprehensive molecular profiling was performed using the blood-based test *FoundationOne® Liquid CDx* (Foundation Medicine, Inc.) designed to detect genetic variations in 324 genes. Univariate and multivariate Cox regression models were applied to evaluate progression-free survival (PFS), defined as the time from disease progression to death. Kaplan-Meier was employed to calculate median PFS months between variables.

**Results:** A total of 30 patients, predominantly male (90%), with a mean age of 66.24 ± 7.94 years were included in this study. Following relapse, treatment strategies

included chemotherapy (63%), immunotherapy (30%), or no further treatment. Molecular analysis of ctDNA identified mutations in 52 different genes, with up to 27% of patients harboring mutations in either the STK11 or KEAP1. The median PFS at 2 years from baseline was 8.2 months (95% CI: 5.4-11.0). Immunotherapy treatment was associated with improved PFS (HR 0.34, 95% CI: 0.13-0.84, p = 0.020), with a median survival increase of 5.7 and 12.3 months compared to chemotherapy and no further treatment, respectively. Conversely, the presence of mutations in either KEAP1 (HR 2.76, 95% CI: 0.99-7.63, p = 0.050) or STK11 (HR 2.99, 95% CI: 1.13-7.96, p = 0.027) was associated with a worse prognosis, regardless of treatment modality (carrying a pathogenic alteration in either the KEAP1 or STK11 genes resulted in a decrease in median PFS of 6.3 months). Multivariate analysis confirmed these findings, demonstrating that treatment modality (HR 0.41, 95% CI: 0.17-0.99, p = 0.048) and the presence of STK11 or KEAP1 mutations (HR 2.88, 95% CI: 1.12-7.35, p = 0.027) were significantly associated with PFS. These two variables were independent of each other (interaction analysis not significant, p = 0.73).

**Conclusions:** These findings highlight the clinical significance of STK11 and KEAP1 mutations as negative prognostic markers in advanced NSCLC, underscoring the need for novel therapeutic strategies for these patients and the importance of incorporating genomic profiling into the clinical management of the disease.

**30** NON-CODING RNAs AS BIOMARKERS IN THE EARLY BREAST CANCER SETTING

**Poster**

**Celia Gaona-Romero[1,2] , Esperanza López-López[1,2] , María Elena Quirós-Ortega[1,3] , Ana Godoy-Ortiz[1,2,3] , Jesús Velasco-Suelto[1,3] , Rocío Lavado-Valenzuela[1,2,3] , Martina Álvarez[1,2,3] , Javier Pascual[1,2,3] , Emilio Alba[1,2,3,4] , Iñaki Comino-Méndez[1,2,3]**

*1) Hospital Universitario Virgen de la Victoria, 29010, Malaga, Spain.*
*2) The Biomedical Research Institute of Málaga (IBIMA-CIMES-UMA), 29010, Malaga, Spain.*
*3) Centro de Investigacion Biomedica en Red de Cancer (CIBERONC - CB16/12/00481), 28029, Madrid, Spain.*
*4) University of Málaga, Faculty of Medicine, 29010, Malaga, Spain.*

**Introduction:** Breast cancer (BC) remains the most prevalent neoplasm in women worldwide and early detection is crucial for enhancing survival rates in these patients. While the response to neoadjuvant chemotherapy (NAC) is well-established as a prognostic factor, current approaches leave room for significant advancement. In this context, the identification and validation of novel, highly sensitive biomarkers are essential to facilitate earlier disease detection and more accurate prediction of NAC response, ultimately enhancing treatment outcomes. For this purpose, non-coding RNA-based liquid biopsy has emerged as a promising approach. This includes the analysis of various cell-free components, such as microRNAs (miRNAs), piwi-interacting RNAs (piRNAs), small nucleolar RNAs (snoRNAs) among others. In this regard, ncRNAs are promising biomarkers because of their stability in body fluids, their abundance, and their specificity. Unlike traditional biomarkers, circulating ncRNAs provide dynamic insights as they can be non-invasively sampled, offering real-time information on disease status. Here, we present a novel strategy integrating the detection of ncRNAs into early-stage BC screening and the prediction of NAC response utilizing blood samples.

**Objectives:** This study aims to assess expression patterns of multiple ncRNAs in the plasma of NAC-treated early BC patients. Using small plasma volumes collected before and after NAC, we evaluate the ncRNAs potential as non-invasive blood-based biomarkers for early BC detection and NAC response prediction. We integrate less-explored RNA types, such as piRNAs and snoRNAs, in addition to miRNAs.

**Methodology:** Discovery phase: ncRNA sequencing was performed on 42 samples from 21 NAC-treated early BC patients and 10 healthy controls. An optimized QIAGEN protocol was employed utilizing spike-ins for extraction and hemolysis

monitoring. Libraries for RNA species ranging from 15-40 nucleotides were constructed with Unique Molecular Identifiers (UMIs). A robust bioinformatic pipeline, employing DESeq2 and EdgeR tools, ensured ncRNAs expression profiling through differential expression analysis (DEA). Differentially expressed (DE) ncRNAs were considered at an adjusted p-value < 0.05 and |logFC| ≥ 1.5, with an intersection analysis to reduce false positives.

Validation phase: We evaluated 121 plasma samples (65 pre-NAC, 29 post-NAC, and 27 controls from healthy individuals). Cell-free total RNA purification was performed employing the miRNEasy Serum/Plasma Advanced Kit (Qiagen) and first-strand cDNA was synthesized using the miRCURY LNA RT Kit. Spike-ins for extraction and RT were included for monitoring. Quantitative Real-Time PCR (qPCR) was performed on a LightCycler using individual miRCURY LNA miRNA PCR Assays for 14 different miRNAs, 4 piRNAs, 2 snoRNAs, 2 hemolysis, 4 spike-ins, and 3 endogenous genes. Differential expression of ncRNAs was assessed and predictive models were generated for individual miRNAs, piRNAs, and snoRNAs. Subsequently, we selected the ncRNAs that, in combination, yielded the most effective predictive model.

**Results:** Discovery phase: miRNAs, piRNAs, snoRNAs, and lncRNAs were explored, with miRNAs being the most abundant (≈ 49.3% of reads). Noteworthy dysregulations were observed in pre-treatment samples versus controls. DE ncRNAs between BC samples and healthy controls were selected for validation by qPCR.

Validation phase: DE of 11/14 miRNAs, 4/4 piRNAs and 2/2 snoRNAs was observed by qPCR in BC samples compared to healthy controls. Logistic regression showed that the combination of 3 miRNAs and 2 piRNAs generate an efficient predictive model with an AUC of 0.979 demonstrating a high accuracy for the diagnosis of BC.

**Conclusions:** While further validation in independent cohorts is ongoing, our study underscores the potential of circulating ncRNAs as biomarkers for early BC using minimal blood volumes. Our findings suggest that combining diverse ncRNAs into panels significantly enhances the accuracy of BC diagnosis.

**31** miRNAs in Blood and Urine as Companion Diagnostic Biomarkers in Genitourinary Tumors.

**Poster**

**Marta Dueñas[1], Álvaro Martín de Bernardo[1] , Carolina Rubio[1] , Esther Montesinos[1] , Ignacio Arévalo[1] , Marta Rodríguez-Izquierdo[2] , Félix Guerrero[2] , Pablo Álvarez[2] , Guillermo de Velasco[2] , Jesús M. Paramio[1] , Cristian Suárez[1]**

*1) CIEMAT-Fundación 12 de Octubre*
*2) Hospital 12 de Octubre*

MicroRNAs (miRNAs) are small non-coding RNAs that play a critical role in gene regulation and have emerged as promising biomarkers for several diseases, including genitourinary malignancies. Liquid biopsy, a non-invasive approach to detect biomarkers in body fluids such as blood, urine or serum, has gained significant attention for its potential in cancer diagnosis, monitoring and prognosis. MiRNAs that are stable in these fluids can be selectively enriched and quantified, making them ideal candidates for use in liquid biopsy. In the context of genitourinary cancers, specific miRNA signatures have been identified that correlate with tumor presence, stage and response to therapy.

In our group, we have developed miRNA-specific signatures for bladder cancer diagnosis and monitoring as well as companion diagnostics for immunotherapies such as BCG and anti-PDL1 in urine samples. We have performed a blinded validation for diagnosis and monitoring called BlaDimiR® with a diagnostic accuracy of over 95%. Following a similar experimental approach, we have also identified a miRNA signature in urine samples that is able to predict response to BCG treatment in those patients at high risk of relapse, this second urine-based test we have named BlaDimiRplus®. With the BlaDimiRplus® predictive tool, we were able to validate BCG response prediction with an accuracy between 80 and 100% in a proof-of-concept validation cohort. We are also evaluating miRNAs in plasma samples, either free or from plasma EVs, using prediction to Cabozantinib in a renal cell carcinoma clinical trial. We will show the results comparing miRNA from EVs and free in plasma in terms of quantity, quality and differential expression between responder and non-responder patients.

In conclusion, we demonstrate that cell-free miRNAs as liquid biopsy biomarkers have great potential as companion biomarkers for early detection, risk stratification and treatment monitoring, enabling personalised therapeutic strategies. Furthermore, the combination of miRNAs with other molecular markers could improve the sensitivity and specificity of liquid biopsy assays, providing more reliable tools for clinical practice in genitourinary cancers.

**32** Liquid Biopsy in Bladder Cancer: Uncovering the clinical value of DNA methylation-Based biomarkers to predict Bacillus Calmette-Guérin response

**Poster**

**Marcel Figueras[1,2,3] , Fiorella L. Roldán[1,2] , Mercedes Ingelmo-Torres[1,2] , Claudia Mercader[1,2] , Bernat Padullés[1,2] , Ariadna Bernal[1] , Josep L. Carrasco[4] , Agustín Franco[1] , Laura Izquierdo[1,2] , Antonio Alcaraz[1,2] , Lourdes Mengual[1,2,5]**

*1) Laboratori i Servei d'Urologia, Hospital Clinic de Barcelona, Barcelona, Spain.*
*2) Genètica i Tumors Urològics, Fundació de Recerca Clinic Barcelona-Institut d'Investigacions Biomèdiques August Pi i Sunyer (FRCB-IDIBAPS), Barcelona, Spain.*
*3) Departament de Biomedicina, Facultat de Medicina i Ciències de la Salut, Universitat de Barcelona (UB), Barcelona, Spain.*
*4) Unitat de Bioestadística. Departament de Fonaments Clínics, Facultat de Medicina i Ciències de la Salut, Universitat de Barcelona (UB), Barcelona, Spain.*
*5) Unitat de Genètica. Departament de Biomedicina, Facultat de Medicina i Ciències de la Salut, Universitat de Barcelona (UB), Barcelona, Spain.*

**Introduction:** Predicting bacillus Calmette-Guérin (BCG) response in high-risk non-muscle-invasive bladder cancer (NMIBC) patients remains a clinical challenge.

**Objective:** To evaluate the performance of Bladder Epicheck (BE) in predicting tumor recurrence and BCG failure during the first year after induction treatment.

**Methods:** Prospective study including 65 NMIBC patients treated with BCG between 2018–2021. Urine samples analyzed with BE were collected before and after BCG induction. Logistic binary regression was used to assess the association between clinical-pathological variables and BE results with tumor recurrence and BCG failure during the first year after induction treatment.

**Results:** During follow-up, 16 (24.6%) patients experienced a bladder cancer (BC) event, 11 (68.8%) of which were BCG failure (high-grade recurrence) and five (31.2%) were low-grade recurrences. The median time to overall recurrence was 7.3 (3.8–17.4) months. A significant association was found between the risk of tumor recurrence/BCG failure and post-BCG cystoscopy (OR 10.0; $p<0.001$ and OR 13.1; $p<0.001$, respectively), post-BCG BE result (OR 16.9; $p<0.001$ and OR 33.1; $p<0.001$, respectively) and pre/post-BCG EpiScore value variation (OR 14.4; $p=0.001$ and OR 7.1; $p=0.018$, respectively). A nomogram including these three variables outperformed the CUETO risk tables to predict any BC event after BCG induction (AUC 95.1% vs. 67.1%). Result validation in a larger and independent series is needed.

**Conclusions:** BE post-BCG status and variations in EpiScore values can help us identify patients at higher risk of any BC event and BCG failure promptly. These data can have an impact on disease management.

**33** Genomic diversity and BCL9L status in CTC-pools predict overall survival in metastatic colorectal cancer

**Poster**

**Joao M Alves[1,2] , Nuria Estévez-Gómez[1,2] , Roberto Piñeiro[3,4] , Laura Muinelo-Romay[4,5] , Patricia Mondelo-Macía[5] , Mercedes Salgado[6] , Agueda Iglesias-Gómez[7] , Laura Codesido-Prada[7] , Joaquin Cubiella[7] , David Posada[1,2,8]**

*1) CINBIO, Universidade de Vigo, 36310 Vigo, Spain*
*2) Galicia Sur Health Research Institute (IIS Galicia Sur), SERGAS-UVIGO.*
*3) Roche-Chus Joint Unit, Translational Medical Oncology Group, Oncomet, Health Research Institute of Santiago de Compostela (IDIS), Santiago de Compostela, Spain*
*4) Centro de Investigación Biomédica en Red de Cáncer (CIBERONC), Madrid, Spain.*
*5) Liquid Biopsy Analysis Unit, Translational Medical Oncology Group, Health Research Institute of Santiago de Santiago de Compostela (IDIS), Santiago de Compostela, Spain*
*6) Department of Oncology, Hospital Universitario de Ourense, Research Group in Gastrointestinal Oncology-Ourense, Ourense, Spain 7) Department of Gastroenterology Hospital Universitario de Ourense, Research Group in Gastrointestinal Oncology-Ourense, Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Ourense, Spain 8) Department of Biochemistry, Genetics, and Immunology, Universidade de Vigo, 36310 Vigo, Spain*

Given that cancer dissemination primarily occurs via the bloodstream—where tumor cells detach from the primary tumor, enter circulation, and migrate to distant tissues—there is growing interest in circulating tumor cells (CTCs) due to their purported role in metastasis and potential as a liquid biopsy tool for cancer diagnosis and management.

Although the clinical significance of CTCs has already been established, - with elevated CTC counts correlating with aggressive disease in several cancer types, including colorectal cancer (CRC) - early sequencing studies have revealed that CTCs can exhibit considerable genomic and phenotypic heterogeneity within patients. Importantly, increasing evidence suggests that intratumoral genetic heterogeneity is a critical factor influencing clinical outcomes in CRC. We therefore hypothesize that higher genetic variation within the CTC population may contribute to increased disease aggressivness and reduced overall survival.

As such, to investigate the biomarker potential of CTC genomics in predicting survival outcomes, we collected and performed whole-exome sequencing on CTC pools from 30 metastatic CRC patients. Our analysis revealed substantial differences in mutational burden and copy number alterations across the cohort, with a high number of somatic mutations detected in key CRC driver genes such as APC, KRAS, and PIK3CA. Notably, higher CTC genomic heterogeneity, as indicated

by elevated MATH scores, and the mutation status of BCL9L gene were significantly associated with poorer prognosis and overall survival.

These findings underscore the relevance of CTC-derived genomic information as a robust prognostic biomarker in metastatic CRC and highlight the potential of liquid biopsies in personalized cancer management.

**34** Liquid Biopsy in Localized Prostate Cancer Patients Undergoing Radiotherapy: Urinary Exosomal miRNAs as Potential Predictive Biomarkers

**Poster**

**María del Mar Martínez Olivo[1] , Carlos Tarín Cerezo[1] , Nadina Erill Sagalés[1] , Rocio Cantalapiedra[2] , Carmen María Ponte García[3] , José Reyes Rodríguez Garrido[3] , Patricia Diezhandino[4] , María Jesús Vega Chicote[5] , Marco Panichi[6] , Luis Antonio Corchete Sánchez[7,8] , Judit González García[1] , Laura Lledó Bryant[1] , Víctor González Rumayor[1] , Juan Luis García Hernández[7,8] , Juan Jesús Cruz Hernández[7,8,9]**

*1) Atrys Health, Barcelona & Madrid*
*2) Servicio de Onocología Radioterápica, Hospital Recoletas Campo Grande Valladolid*
*3) Servicio de Oncología Radioterápica, Hospital Clínico de León*
*4) Servicio de Oncología Radioterápica, Hospital Clínico Universitario de Valladolid*
*5) Servicio de Oncología Radioterápica, Complejo Asistencial de Zamora*
*6) Instituto de Oncología Avanzada (IOA) Atrys-Sanitas Barcelona*
*7) Instituto Universitario de Biología Molecular y Celular del Cáncer (IBMCC), Salamanca*
*8) Instituto de Investigación Biomédica de Salamanca (IBSAL)*
*9) Departmento de Oncología Médica, Hospital Universitario de Salamanca*

Liquid biopsy has emerged as a new diagnostic and prognostic tool to guide cancer diagnosis, providing comprehensive information and follow-up on the progression of the tumour. It enables the development of different treatment strategies as well as the monitoring of therapy response. This innovative approach is particularly relevant for prostate cancer, where non-invasive methods can provide critical insights into tumour behaviour, aiding in early detection, risk stratification, and treatment monitoring. Prostate cancer is a major health issue and the most common cancer in men, with over 417,000 new cases reported annually in the European Union. While most prostate cancer cases are initially diagnosed as localized, approximately 30% of patients progress to advanced stages and eventually, all will develop progressive disease, a condition called castration-resistant prostate carcinoma (CRPC).

We present a multicentre study involving six recruiting centres located in León, Valladolid, Zamora, Salamanca, and Barcelona. The main objective is to develop a liquid biopsy molecular signature based on the analysis of urine exosomal miRNAs for treatment response and early relapse prediction in patients with prostate cancer undergoing external radiotherapy and androgen deprivation therapy. The encapsulated nature of exosomal miRNAs protects them from degradation and preserves their integrity, making them reliable for downstream analysis.

The analysis cohort consists of 115 patients with localized prostate cancer, classified according to the Prostate Cancer NCCN Clinical Practice Guidelines in Oncology Version 1.2023.

Urine samples were collected at the urology unit in accordance with the patients' information sheet approved by the Ethics Committee of the Biomedical Research Institute of Salamanca before the start of radiotherapy treatment. The collected clinicopathological data include information such as age, weight, PSA, Gleason score, and other relevant parameters. Additionally, patients are being followed up to study potential recurrences.

Exosomes were isolated using ultracentrifugation, and RNA was extracted using Qiagen's RNeasy Mini Kit. For the miRNAs analysis, a customized OpenArray (Thermo Fisher) including a selection of 54 miRNA probes based on existing literature and their possible relation to prostate cancer has been used in conjunction with the QuantStudio 12K Flex Real-Time System.

The $2^{-\Delta\Delta Ct}$ values obtained from the Ct (cycle threshold) values of the different miRNA amplifications for each sample were statistically analyzed using the GraphPad Prism program version 10.2.3.
Based on the clinicopathological risk of recurrence, the distribution of patients is: 38 very high risk (VHR), 40 high risk (HR), and 37 intermediate risk (IR). Statistical analyses were performed based on patient distributions by risk of recurrence and total Gleason score (group 6+7: 61 patients and group 8+9+10: 54 patients).

Multiple comparisons on miRNA analysis between risk groups showed statistical differences with miR-181a-5p between VHR and IR (adjusted p-value 0.0452) and miR-423-5p between VHR and IR (adjusted p-value 0.0196).

When comparing the two groups based on the total Gleason score, significant differences were observed in miR-26a-5p (p-value 0.0418) and miR-27a-3p (p-value 0.0496).

This study highlights the potential of urinary exosomal miRNAs as predictive biomarkers for treatment response and relapse in localized prostate cancer patients undergoing radiotherapy and androgen deprivation therapy. The findings demonstrate that specific miRNAs exhibit significant expression differences across patient subgroups, providing valuable insights into tumour biology and patient outcomes. Previous studies have reported differential expression of the described miRNAs in other types of cancer, as well as their involvement in the regulation of epithelial-mesenchymal transition via TGF-beta pathway.

**35** Non-invasive immunogram to characterize and monitor immune status in Non-Small Cell Lung Cancer patients treated with immunotherapy

**Poster**

**Andrea Moreno-Manuel[1] , Susana Torres-Martínez[2,3,4] , Sandra Gallach[2,3,4] , Marais Mosqueda[2] , Maria Luz Bellón[2] , Eva Escorihuela[2] , Ana Blasco[5] , Silvia Calabuig-Fariñas[2,3,4,6] , Carlos Camps[2,3,4,5,7] , Eloísa Jantus-Lewintre[2,3,4,8]**

*1) Cancer Heterogeneity and Immunomics, Aragon Health Research Institute, Zaragoza, Spain*
*2) Molecular Oncology Laboratory, Fundación Investigación Hospital General Universitario Valencia, Valencia, Spain*
*3) Unidad Mixta TRIAL CIPF-FIHGUV, Valencia, Spain*
*4) CIBERONC, Madrid, Spain*
*5) Department of Medical Oncology, Hospital General Universitario Valencia, Valencia, Spain*
*6) Department of Pathology, Universitat de València, Valencia, Spain*
*7) Department of Medicine, Universitat de València, Valencia, Spain*
*8) Department of Biotechnology,Universitat Politècnica de València, Valencia, Spain*

**Introduction:** Non-Small Cell Lung Cancer (NSCLC) represents 80% of lung cancer cases, being one of the most frequent and death causing cancers. Recently developed treatments with immunotherapy have improved patient prognosis. However, a significant number of patients do not respond to treatment, thus there is an urge for biomarkers.

**Objectives:** The main objective of this study was to obtain a combination of non-invasive biomarkers capable of predicting which NSCLC patients will benefit from immunotherapy.

**Methodology:** This study included 52 advanced-stage NSCLC patients treated with Anti-PD1 or Anti-PD1 in combination with chemotherapy (Anti-PD1+CT) in the first-line setting. Non-invasive biomarkers were analysed using peripheral blood samples, which were obtained before first cycle and at first response assessment. The potential biomarkers analysed in this study were i) haematological and immunological parameters, ii) immune related gene expression analysed on Peripheral Blood Mononuclear Cells (PBMCs), iii) T Cell Receptor (TCR-β) repertoire, and iv) HLA genotype. We then used RStudio to apply machine-learning techniques to prioritize the analysed variables with greater influence on patient outcomes, creating a multivariate model, which was called non-invasive immunogram.

**Results:** The integration of the analysed variables has resulted in a proposal of a multivariate model capable of predicting patients with improved outcomes to treatment with anti-PD1 therapy. Our immunogram combined PD-L1 tumour tissue expression with other biomarkers such as Neutrophil-to-Lymphocyte Ratio (NLR) and certain HLA alleles to predict prognosis, in the subgroup of patients treated with anti-PD1 monotherapy.

Furthermore, we developed two independent non-invasive immunograms with the ratios of analytical variables to identify patients with DCB, which could be of significant utility to monitor patients throughout treatment. Interestingly, patients with improved prognosis had an increase in the number of TCR-β clones, but also an increase in clonality and higher number of shared clonotypes between pre- and on-treatment samples. Despite immunotherapy-based treatment boosts anti-tumour immune responses, pre-existing anti-tumour immunity might be required to trigger and maintain effective responses.

**Conclusions:** Altogether, this study highlights the role of composite non-invasive biomarkers to characterize and monitor immune status in NSCLC patients treated with immunotherapy or chemoimmunotherapy.

Study supported by AECC Valencia Scientific Foundation, CB16/12/00350 and PI18/00266 (ISCIII) and AICO/2021/333 (GVA).

**36** ESR1 Mutation Testing in Breast Cancer: Clinical Relevance and Therapeutic Applications

**Poster**

**SERAFIN MORALES MURILLO[1] , ANA VELASCO SANCHEZ[2] , IZASKUN URDANIBIA[2] , ARIADNA GASOL CUDOS[1] , NOIEMI TUSET DER-ABRAIN[1] , GARA RODRIGUEZ GONZALEZ[1]**

*1) HOSPITAL ARNAU DE VILANOVA DE LLEIDA*
*2) INSTITUT DE RECERCA BIOMEDICA DE LLEIDA*

Estrogen receptor (ESR1) mutations represent an acquired mechanism of hormonal resistance that leads to treatment failure in metastatic luminal phenotype breast cancer. However, recent innovations have introduced new therapies as ER degraders (SERDs) that have demonstrated efficacy in this setting, offering a novel therapeutic opportunity for patients harboring this mutation.
We studied ESR1 mutations through liquid biopsy in a cohort of patients with metastatic luminal breast cancer under various clinical conditions to identify the optimal setting for performing this determination.
We analyzed ESR1 mutation status in 49 patients undergoing hormonal therapy for metastatic breast cancer. Of these, 32 patients (65%) were receiving treatment with aromatase inhibitors, either alone or in combination with CDK inhibitors, while the remaining patients were on fulvestrant. The clinical setting prior to ESR1 mutation testing included 17 patients (37%) in the adjuvant phase, 27 (55%) undergoing first-line treatment, and the rest receiving second-line or later therapies. At the time of testing, half of the patients were still responding to treatment, while the other half had progressed and were initiating a new line of therapy.
We identified the ESR1 mutation in 15 cases (30%), with no correlation observed with histopathological variables (hormonal receptor status, Ki-67) or clinical parameters. Additionally, no association was found with the duration of prior hormonal therapy exposure (median: 44 months). While the limited sample size precluded analysis of the mutation's relationship with the timing of metastasis onset, a higher prevalence was noted in cases of early relapse (43%; 7/16), followed by de novo metastasis (30%; 3/10) and late relapse (21%; 5/24). Among 17 patients evaluated at progression during adjuvant therapy, only 1 case (6%) was positive for the mutation, compared to 14 out of 30 cases (46%) where the mutation was detected after at least one line of therapy for metastatic disease.
The detection of ESR1 mutations is not associated with any routinely used clinical or histopathological variable, nor with the duration of prior exposure to hormonal therapy. However, a higher proportion of positive cases is observed in patients with early relapse (43%) and after at least one prior line of treatment for metastatic disease (46%). Therefore, its analysis should be considered in these scenarios to optimize its clinical utility.

**37** ANALYZING IMMUNOTHERAPY RESPONSES IN UROTHELIAL CANCER USING LEGENDPLEX TECHNOLOGY

**Poster**

**Esther Montesinos[1,2] , Julia del Prado Montero[3] , Ignacio A. Reina[1,2,4] , José Carlos Casalvilla-Dueñas[3] , Álvaro Martín de Bernardo[1,2] , Iris Lodewijk[1,2,4] , Marta Rodríguez Izquierdo[5] , Félix Guerrero Ramos[5] , Eduardo López Collazo[3] , Jesús María Paramio[1,2,4] , Marta Dueñas[1,2,4] , Carolina Rubio[1,2,4]**

*1) Molecular Oncology Unit. Center for Energy, Enviromental and Technological Research (CIEMAT), Madrid, Spain*
*2) Cellular and Molecular Oncology Genitourinary Tumor Group. Institute of Biomedical Research, Hospital Universitario 12 de Octubre, Madrid, Spain*
*3) Innate Immunity and Tumorimmunology Group of the Research Institute of the Hospital de la Paz (IdiPaz), Madrid, Spain*
*4) Center for Biomedical Research in Network (CIBER), Madrid, Spain*
*5) Urology, Hospital Universitario 12 de Octubre, Madrid, Spain*

**Introduction:** Urothelial cancer (UC), a malignancy of the urinary system, is among the most common cancers worldwide, affecting a significant number of individuals each year. This type of cancer is known for its tendency to recur, with a considerable percentage of patients that experience recurrence of the disease and over half of all cases eventually progress to an invasive form. Immunotherapy, particularly through the use of immune checkpoint inhibitors, has emerged as a innovative approach, offering new hope for patients with advanced stages of the disease. Liquid biopsy has gained grip as a minimally invasive technique to monitor disease progression and treatment response. LEGENDPlex™ technology, a multiplex immunoassay, allows measuring cytokine levels and other immune biomarkers like immune checkpoints, providing a detailed landscape of the tumor microenvironment. The interaction between cytokines and immune checkpoints provides valuable insights into the mechanisms of immune response in bladder cancer. This work aims to explore the integration of these emerging tools and technologies in enhancing the understanding and management of bladder cancer.

**Objectives:** The main objective of this study is to investigate the role of cytokines and immune checkpoints in patients diagnosed with bladder cancer. This study aims to not only to establish a relationship between the patients that show a response to the treatment and those who do not, but also to explore for a possible interplay between the cytokines and the immune checkpoints in these patients across different stages of the disease. The samples were collected at various stages of bladder cancer to capture a comprehensive view of the immune landscape as the disease progresses. Additionally this study seeks to evaluate the

benefit of liquid biopsy as a non-invasive method for tracking disease progression and treatment responsiveness.

**Methodology:** Liquid biopsy samples from peripheral blood plasma were obtained from a cohort of patients diagnosed with bladder cancer across different stages of the disease. The samples were subjected to LEGENDplex™ human cytokines and immune checkpoint panels, allowing us to simultaneously quantify and measure a diverse range of cytokines and immune checkpoints associated with inflammation and immune response. After that, the data was acquired through flow cytometry and then statistical analysis was conducted using Graphpad software to interpret the cytokine and immune checkpoint quantification, providing insights into the systemic immune response associated with immunotherapy treatment in urothelial cancer.

**Results:** Our preliminary results show different cytokine and immune checkpoint profiles for both responder and non-responder patients. Elevated levels of proinflammatory cytokines, including IL-6, IFN-γ and TNF-α, were consistently observed in treatment-responsive patients along with an increase in the immune checkpoint sCD27. In contrast, patients who are non-responsive to the treatment show a more immunosuppressive immune system with an increased TGF-β1 immune checkpoint. Even though these results are preliminary, they highlight the need to investigate how systemic immunotherapy the disease in UC patients.

**Conclusions:** LEGENDplex™ technology has demonstrated its effectiveness as a useful tool for cytokine and immune checkpoint profiling. The patterns observed in both cytokines and immune checkpoint signatures prove to be potential biomarkers for the diagnosis and prognosis of the disease. Additionally, it proves to be helpful for the guidance of the development of targeted immunotherapies. The variations shown in the expressions of cytokines and immune checkpoint among different disease stages and responsiveness to treatment emphasize the potential for employing these molecules as markers that reflect the landscape of urothelial cancer. By utilizing liquid biopsy in combination with LEGENDplex™ technology as a non-invasive biomarker presents the opportunity to develop minimally invasive diagnostic methodologies and treatment monitoring based on plasma cytokine and immune checkpoint profiles, paving the way for more personalized therapeutic treatments.

**38** Early cancer detection from liquid biopsy using cell-free RNA

**Poster**

**Phil Sanders[1] , Lucila Gonzalez[1] , Laura Sanchez[1] , Beatriz Neto[1] , Julien Lagarde[1] , Marc Weber[1] , Pablo Monteagudo[1] , Cristina Tuñi[1] , Giovanni Asole[1] , Elena Rusu[1] , João Curado[1] , Sol Garcia[2] , Pilar Simón-Extermera[2] , Sonia Panadero[3] , Maria Concepcion Romero[3] , Purificación Catalina[3]**

*1) Flomics Biotech*
*2) Leitat Technological Center*
*3) Andalusian Public Health System Biobank*

Liquid biopsies have become increasingly relevant diagnostic tools for early cancer detection due to their high sensitivity and minimal invasiveness. Within liquid biopsy samples, cell-free RNA (cfRNA) is a promising biomarker source for early cancer detection. This is because cancer-associated cfRNA molecules are actively secreted into the blood by both healthy and tumour cells, which makes the blood cfRNA profile a powerful tool in obtaining direct insight into the status of the patient. Furthermore, cancer-associated changes of the blood cfRNA profile are detectable much earlier than mutations in cell-free DNA, which makes cfRNA better suited to the detection of small and early stage cancers where the number of tumour cells is low.

However, as an emerging field rife with technical challenges, cfRNA profiling for diagnostic purposes lacks gold standards for both laboratory and bioinformatic protocols. At Flomics Biotech, we have overcome these challenges by developing our proprietary cfRNA-Seq platform that profiles human plasma cfRNA in a robust and reproducible manner.

We are currently applying our cfRNA-Seq platform to the ongoing LiquiDx pre-clinical study, with the objective of developing a cfRNA-based multicancer early detection test (MCED).

In this study, we apply our cfRNA-Seq platform to plasma samples from a cohort of 1,300 donors. The cohort consists of healthy individuals, and patients with colorectal, lung, breast, pancreatic or prostate cancer, or non-cancer diseases of the same organs. For each cancer type, patients with early or late stage disease were recruited.

We analyze the cfRNA-Seq data using a combination of gold-standard bioinformatics tools and advanced machine learning methods to identify cancer type-specific biomarker signatures and develop predictive machine learning models for early cancer detection.

The study is ongoing, but at this stage over 800 samples have been processed and analysed to generate a high quality data set where more than 6000 genes are detected in 80% of samples. This includes the robust detection of a broad range of gene biotypes including protein coding genes, long non-coding RNAs, and a diverse range of small RNAs. Amongst these genes are many oncogenes, tumour suppressor genes, and other known cancer-associated genes.

Although data analysis is still ongoing, we have already identified promising biomarker signatures both for the separation of cancer and non-cancer donors, and for the identification of specific cancer types. Analyses of the genes contributing to these signatures indicate perturbations in cellular processes implicated in cancer.

Using these biomarker signatures we have begun to develop machine learning-based models for cancer prediction, with some of these models already demonstrating promising sensitivity and specificity for the accurate prediction of cancer type.

Based on the results of the study so far, we have observed that the cfRNA-Seq platform has potential beyond early cancer detection in areas such as infectious disease detection. This is due to further analysis of the data set identifying a small subset of donors with viral infections at the time of blood collection, with these infections subsequently being confirmed via the clinical data provided with the samples.

We demonstrate that the Flomics cfRNA-Seq platform generates high quality data from liquid biopsies, and has great potential for the development of biomarker signatures and predictive models for early cancer detection, which will accelerate treatment and result in more favorable patient outcomes.

Beyond early cancer detection, our technology can be applied to other biofluids and diseases, including infectious and neurodegenerative diseases. This technology can also be applied to areas such as Minimal Residual Disease (MRD) testing, guiding treatment selection, and tracking response to therapies.

With many potential applications across a diverse range of diseases and research areas, the Flomics cfRNA-Seq platform can have a significant impact on global health.

**39** Utility of CTC Gene Expression in the Molecular Characterization of Metastatic Recurrence in Breast Cancer

**Poster**

**Maribel Aybar[1] , Carmela Rodríguez[2] , Miriam González-Conde[1] , Carmen Abuín[1] , Laura Muinelo-Romay[1,3] , Patricia Palacios[2] , Juan Cueva[2] , Teresa Curiel[2] , Alexandra Cortegoso[2] , Natalia Fernández-Diaz[2] , Rafael López-López [1,2,3] , Clotilde Costa[1,2,3]**

*1) Translational Medical Oncology Group, Oncomet. Health Research Institute of Santiago de Compostela (FIDIS). Travesia da Choupana s/n 15706 Santiago de Compostela, Spain.*
*2) Department of Oncology, Complexo Hospitalario Universitario de Santiago de Compostela (SERGAS), Santiago de Compostela, Spain.*
*3) CIBERONC, Centro de investigación Biomédica en Red Cáncer, Madrid, Spain.*

**Introduction:** Breast cancer is the most common type of cancer among women worldwide and is classified into distinct subtypes based on the expression of hormone receptors (HR), human epidermal growth factor receptor 2 (HER2), and the proliferation rate (determined by the KI67 biomarker). Moreover, therapeutic strategies (including endocrine therapy, HER2-targeted therapies, chemotherapy, or immunotherapy, among others) vary according to the subtype. Therefore, proper molecular subtype identification, primarily done via immunohistochemistry of tumour biopsies, is essential for personalised treatment.

After treatment for localised breast cancer, about 20% of patients experience disease recurrence with metastasis. A major challenge in clinical practice is the lack of early-detection tests for metastatic breast cancer. Current diagnostic tests like CT scans, MRIs, and biopsies are performed when symptoms manifest and are often limited by inaccessible tissue or imaging uncertainties. Besides, due to tumour evolution and therapy resistance, subtypes may change over time, requiring updated molecular characterisation in the advanced state. Liquid biopsy offers a minimally invasive alternative for real-time tumour monitoring and treatment adjustment, particularly for advanced cases with inaccessible metastatic tissue.

**Aim:** In this work, it was explored if the expression profile of CTCs from the peripheral blood of metastatic breast cancer patients that were being studied for a suspected recurrence of breast carcinoma could be supportive in the clinical decision-making of different clinical cases.

**Methodology:** 40 mL of peripheral blood was collected from 6 advanced breast cancer patients in Cellsave (1 tube) and 3 BD Vacutainer® EDTA tubes. The enumeration of circulating tumour cells (CTCs) of epithelial origin (CD45-, EpCAM+, and cytokeratins 8, 18+ and/or 19+) in the blood of patients was performed using

the CELLSEARCH® Circulating Tumour Cell Kit. From EDTA tubes, the CTC fraction was isolated using a negative enrichment approach (RosetteSep™ CTC Enrichment Cocktail Containing Anti-CD56) and PBMCs were isolated by density gradient centrifugation using Lymphoprep (StemCell tech). Then, RNA was extracted using AllPrep DNA/RNA mini kit (Qiagen) from both fractions. Subsequently, cDNA was synthesised and preamplified from RNA using the SuperScript™ IV First-Strand Synthesis System. Next, gene expression was performed by RT-qPCR (LightCycler 480, Roche) for a selected set of genes including hormone receptors (CDH1, ERS1, PGR, AR, BCL11A, ERBB2, and MKi67) using Taqman probes. B2M was used as a reference gene and CTC gene expression was normalised by PBMCs expression. Clinical data was collected from the clinical history of these patients.

**Results:** In this series of cases involving patients with breast carcinoma treated with radical interventions, circulating tumour cell (CTC) enumeration and gene expression analysis were investigated as supportive tools in the diagnostic evaluation of potential metastatic disease during clinical follow-up. CTC presence associated with metastasis confirmation. Besides, CTC gene expression of hormone receptors, such as ESR1 and PGR, and TNBC-associated markers like BCL11A were observed. Notably, the liquid biopsy findings showed strong concordance with pathological results from metastatic tissue samples, supporting the reliability of molecular profiles derived from CTCs.

**Conclusions:** Although CTC analysis is not routinely recommended for the diagnosis of metastatic breast cancer, in our case series, it supported the diagnostic process and associated with subsequent tests performed. Preliminary data suggest that CTC analysis holds value as a real-time, non-invasive tool that can provide insights into tumour characterisation to assist physicians in developing personalised treatment strategies. Despite the limited number of patients studied, further research is needed to draw definitive conclusions

**40** Identification of treatment response biomarkers in tumor-educated platelets from patients with luminal breast cancer

**Poster**

**Izaskun Urdanibia Ascanio[1], Ana Velasco Sánchez[1,2], Ariadna Gasol Cudos[2], Serafin Morales Murillo[1,2]**

*1) Instituto de Investigación Biomédica de Lleida Fundación Dr. Pifarré (IRBLleida)*
*2) Hospital Universitario Arnau de Vilanova de Lleida-HUAV*

Liquid biopsy provides a direct source of biomolecules derived from tumor cells. It is a fast, non-invasive, and reproducible sampling method that can dynamically reflect changes in the tumor's gene expression profile and provide
a solid foundation for individualized therapy and early cancer diagnosis.

One of the components of liquid biopsy is tumor-educated platelets (TEPs). It has long been known that platelets play important roles in hemostasis, thrombosis, and wound healing, and they are now also recognized as mediators in malignant diseases, influencing various aspects of cancer progression, most notably tumor cell metastasis. The interaction between tumor cells and platelets induces platelet activation, which can alter their RNA profile. Additionally, platelets can directly uptake and store tumor-derived factors such as messenger RNA (mRNA), circular RNA, long non-coding RNA, mitochondrial RNA, and other non-coding RNAs (ncRNAs). This gives TEPs a highly dynamic mRNA repertoire with potential applicability for cancer diagnosis. It has been demonstrated that platelets isolated from cancer patients often show distinct RNA and protein profiles. Using platelet RNA sequencing, it was possible to identify patients with six types of localized or metastatic tumors and healthy individuals with 96% accuracy, as well as the primary tumor location with 71% accuracy.

The objective of this study was to characterize gene expression in platelet RNA from liquid biopsies in patients with luminal breast cancer. Twelve patients diagnosed with luminal breast cancer at the Arnau de Vilanova Hospital in Lleida were recruited. Multiple blood samples were taken at different stages (at diagnosis, after neoadjuvant treatment, and after surgery). Platelets were purified using two low-speed centrifugations, and platelet RNA was purified using a commercial kit. A custom panel of 55 clinically relevant cancer genes was evaluated using AnyGenes technology through real-time PCR.

Differentially expressed genes were identified, including a decrease in SELP

and CASP3 and an increase in KRAS, TERT, TK1, MET, NOTCH4, ESR1, ESR2, MTOR, EGFR, IL1, CTLA4, HTR1A, CACNA2D1, and CDK2. The results suggest a potential association between the differential expression of these genes and treatment response, as well as disease prognosis. The decrease in SELP and CASP3 expression in breast cancer may contribute to greater tumor aggressiveness and treatment resistance, while the overexpression of oncogenes such as KRAS and EGFR could be associated with therapeutic resistance and poorer prognosis. This underscores the importance of these markers in prognostic evaluation and the design of therapeutic strategies.

These preliminary findings highlight the importance of platelet gene expression and its potential as a biomarker for predicting treatment response. The study is currently ongoing with the recruitment of additional individuals.

**41** 3D Modelling of CTC Clusters: focusing on immune-cancer cells interaction

**Poster**

**Adriana Carneiro[1,2] , Paulina Pairo[1] , Tamagno Pesqueira[1] , Rodirgo Mariño[1] , Luís Lima[2] , Lorena Diéguez[1]**

*1) International Iberian Nanotechnology Laboratory (INL), Braga, Portugal*
*2) Centro de Investigação do Instituto Português de Oncologia do Porto (CI-IPOP), Porto, Portugal*

Cancer metastasis is a leading cause of cancer-related mortality, driven by the dissemination of cancer cells from the primary tumour to distant organs. Circulating tumour cells (CTCs), shed from active tumours into the bloodstream, are critical mediators of this process, facilitating the formation of metastatic lesions in distant tissues. During circulation, CTCs can travel as single cells or as clusters, and their presence has been extensively reported in a wide variety of cancers and correlated with increased drug resistance and poorer prognosis [2-4]. Importantly, CTC clusters can be homotypic or heterotypic, often escorted with immune cells, and exhibit significantly higher metastatic potential compared to single CTCs, highlighting their role in disease progression and poor patient prognosis [1]. CTC clusters confer protection against immune recognition and conventional therapies, representing an effective tumour escape mechanism [5]. Despite these findings, the analysis of CTC clusters remains underexplored, particularly in the context of their interaction with the immune system. Hence, this study aims to understand the interactions between CTCs and immune cells, particularly in the formation of CTC clusters, and their involvement in metastatic disease. Understanding the nature of these interactions is crucial for developing targeted therapeutic strategies.

To address this gap, we developed an in vitro model to study CTC-immune cell clusters. Firstly, we tested different microwell designs (spherical, conical, and square) to optimize cluster formation with low initial cell seeding densities. As the spherical design performed best for cluster formation, growth and imaging, we proceeded with testing different cell densities until achieving an initial cell seeding of 10 cells using different colorectal cancer cells lines (HT-29 and SW480 cells). Cells were kept in culture and monitored for 7 days to promote cell adhesion. By performing viability assays with Calcein-AM (viable cells) and Bobo-3 (death cells) we observed that most cells seemed viable up to seven days. Hence, we proceeded with cluster-model characterization assays to assess both its morphology and phenotypical profile through SEM microscopy and immunocytochemistry, respectively. Focusing on the heterotypical cluster model, after a five-day growth

period to promote cell adhesion, the resulting cell clusters were co-cultured with monocytes isolated from healthy whole blood samples. To facilitate the co-culture monitorization, monocytes were stained with cell tracker green upon its isolation and, imaged 5, 24 and 48 hours after seeding to evaluate the interaction between both cell types.

This study provides a novel 3D culturing framework for understanding the mechanisms of CTC cluster formation and their impact on metastasis. As such, next steps include the extraction of the genetic material for RNA whole genome sequencing, aiming to elucidate the genetic profiles associated with CTC-immune cell clusters, ultimately offering valuable insights for the development of innovative therapeutic approaches.

**42** Clinical Translational Study in Gastrointestinal Cancer (GI): EMT Status of Circulating Tumor Cells (CTCs) and Clinical Utility

**Poster**

**Paulina Piairo[1,2] , Adriana Carneiro[1,3,4] , Daniela A. R. Santos[3,4] , Mariana Eiras[3,4] , Lúcio Lara Santos[3,5] , Luís Lima[3] , Lorena Diéguez[1,2]**

*1) Medical Devices Group, International Iberian Nanotechnology Laboratory (INL), Braga, Portugal 2) RUBYnanomed Lda., Braga, Portugal 3) Experimental Pathology and Therapeutic Group, Research Center of IPO Porto (CI-IPOP), RISE@CI-IPOP (Health Research Network), Portuguese Oncology Institute of Porto (IPO-Porto)/Porto Comprehensive Cancer Center Raquel Seruca (Porto.CCC), Porto, Portugal; 4) Instituto de Ciências Biomédicas Abel Salazar (ICBAS) da Universidade do Porto, Porto, Portugal 5) Department of Surgical Oncology, Portuguese Institute of Oncology (IPO-Porto), 4200-072, Porto, Portugal*

Gastrointestinal (GI) cancers account for 1 in 4 cancer cases and 1 in 3 cancer deaths globally. GI cancer includes oesophageal, gastric, liver, pancreatic, and colorectal cancers. Late-stage detection, aggressive tumour behaviour, and a lack of effective treatments for advanced patients, most often contribute to low survival rates in GI.

In this context, translational research has its efforts critically directed into overcoming difficulties presented by conventional diagnostic approaches and improving the general prognosis. Liquid biopsies offer a unique way to assess cancers at all stages of treatment and capture prognostic cues. Unlike traditional biomarkers, CTCs are putative multimodal biomarkers allowing for the investigation of enumeration, phenotyping and genetic profiling of the tumour cells, thus enabling an encompassing view into a patient's unique tumour.

In this study, 83 GI cancer patients (33 gastric, 37 colorectal, 5 hepatocellular, 4 pancreatic and 4 oesophageal) were enrolled at Portuguese Oncology Institute of Porto (IPO Porto) and whole blood samples were collected for CTCs isolation using the RUBYchip™, a microfluidic device designed for efficient tumour cell capture. After which, the entrapped cells were identified through cytokeratin (for epithelial cells) and vimentin (for mesenchymal cells), white blood cells are excluded by a combination of CD45 and CD15 markers.

This assay allows for a sensitive detection of CTCs in clinical samples, independently of the GI cancer type, this cohort presents varying phenotypic profiles within the epithelial-mesenchymal transition (EMT). CTCs were identified in over 50% of stage I-II patients, CTC mesenchymal phenotype is predominant in over

60% of cases. However, further clinical correlation studies are under investigation to demonstrate CTC clinical relevance in relation to GI clinical outcomes.

Herein clinical applicability of label-free CTC isolation, highlights CTCs and particularly EMT phenotyping as promising biomarkers for monitoring and prediction of GI cancer progression, harbouring potential in supporting personalized treatment decisions.

**43** Establishment and Characterization of a CTC-Derived Cell Line from Mouse Blood Reveals Prognostic Gene Signatures in Breast Cancer

**Poster**

**Cristóbal Fernández-Santiago[1,2] , Inés Martínez-Pena[1] , Pablo Hurtado[1] , Marta Paramés[1] , Mario Rodriguez[1] , Carmen Abuín[1] , Clotilde Costa[1,2] , Ana Belén Dávila-Ibáñez[1,2] , Rafael López-López[1,2,3] , Roberto Piñeiro[1,2]**

*1) Translational Medical Oncology Group, Oncomet. University Hospital of Santiago de Compostela (CHUS)*
*2) CIBERONC, Centro de Investigación Biomédica en Red Cáncer, 28029 Madrid, Spain*
*3) Medical Oncology Department, Complejo Hospitalario de Santiago de Compostela*

**Background:** Circulating tumor cells (CTCs) and CTC-clusters are key in breast cancer metastasis. Developing cellular models that replicate CTCs' in vivo traits is vital for understanding metastasis mechanisms and identifying biomarkers. This study aimed to establish a CTC-derived cell model (mCTC) from a breast cancer mouse xenograft to explore metastatic behavior and molecular profiles.

**Methods:** CTCs and CTC-clusters were isolated from mice injected with metastatic MDA-MB-231 cells and cultured to develop the mCTC model. Comparative analyses included cell cycle evaluation, colony formation, invasion, adhesion, and metastasis in zebrafish models. Transcriptomic profiling identified differentially expressed genes (DEGs), validated in molecular assays. Public gene expression datasets from GEO and TCGA were analyzed to identify a prognostic gene signature, which was validated in an independent cohort.

**Results:** mCTC cells exhibited enhanced colony formation, invasion, adhesion, and metastatic behavior compared to parental MDA-MB-231 cells. Transcriptomic analysis identified 1,696 DEGs, with SPARC significantly upregulated. Functional assays showed SPARC overexpression may be related to increased invasiveness and migration. Analysis of public data confirmed SPARC's high expression in breast cancer CTCs and its association with bone metastasis. A 4-gene signature—involving SPARC—was identified, showing strong prognostic value for overall survival (OS) and distant metastasis-free survival (DMFS) in breast cancer patients. This signature effectively distinguished high-risk patients with poorer outcomes in validation cohorts, with elevated expression in CTCs linked to increased mortality risk.

**Conclusion:** The mCTC model reveals distinct metastatic traits, highlighting SPARC's role in CTC biology and its potential as a prognostic marker in breast cancer metastasis. The 4-gene signature offers a valuable tool for assessing patient

risk and guiding patient monitoring strategies, potentially uncovering new targets for managing breast cancer progression.

**44**

# Exploring the Mutational Landscape in Locally Advanced HNSCC Using a Targeted Next-Generation Sequencing

**Poster**

**Ana-María Rodríguez-Ces [1,2,3] , Óscar Rapado-González [1,2,3,4,5] , Santiago Agüín[6] , Inés Formoso-García [7] , Roberto Rodríguez-Álvarez[8] , Alicia Abalo [2] , Arantza Alzueta [9] , Rafael López-López [6] , María-Mercedes Suárez-Cunqueiro [1,3,4,6]**

*1) Surgery and Medical Surgical Specialties, Medicine and Dentistry School, Universidade de Santiago de Compostela (USC), Santiago de Compostela, Spain.*
*2) Liquid Biopsy Analysis Unit, Translational Medical Oncology ( ONCOMET ), Health Research Institute of Santiago.*
*3) Galician Precision Oncology Research Group (ONCOGAL), Medicine and Dentistry School, Universidade de Santiago de Compostela (USC), 15782 Santiago de Compostela.*
*4) Centro de Investigación Biomédica en Red en Cáncer (CIBERONC), Instituto de Salud Carlos III, 28029 Madrid, Spain.*
*5) Cancer Biology & Epigenetics Group, Research Center of IPO Porto (CI-IPOP)/CI-IPOP@RISE (Health Research Network), Portuguese Oncology Institute of Porto (IPO Porto)/Porto Comprehensive Cancer Center Raquel Seruca (Porto.CCC), Rua Dr. António Bernardino de 6) Translational Medical Oncology Group (ONCOMET), Health Research Institute of Santiago (IDIS), Complexo Hospitalario Universitario de Santiago de Compostela (CHUS, SERGAS), 15706 Santiago de Compostela, Spain. 7) Service de Oncología Radioterápica, Hospital Universitario Lucus Augusti, Lugo, Spain. 8) Department of Pathology, Complexo Hospitalario Universitario de A Coruña (CHUAC, SERGAS), A Coruña, Spain. 9) Department of Oral and Maxillofacial Surgery, Complexo Hospitalario Universitario de A Coruña (SERGAS), 15006 La Coruña, Spain.*

**Introduction:** A high percentage of head and neck squamous cell carcinomas (HNSCC) develop disease recurrence after treatment, however, there are no predictive biomarkers. Taking in mind the heterogenous molecular landscape of HNSCCs, liquid biopsies represent an attractive strategy to provide a more comprehensive view of molecular tumor heterogeneity in advanced stages diseases. This study aims to investigate the mutational profile of locally advanced-HNSCC (LA-HNSCC) patients in tumor tissue and liquid biopsies (blood and saliva) using a targeted-NGS panel.

**Materials and Methods:** Thirty-one HPV-negative LA-HNSCC patients (26 males, 5 females; median age: 67 years) with tumors in the oral cavity (n=12), oropharynx (n=12), hypopharynx (n=5), and larynx (n=2) were included, with staging as follows: III (n=4), IVA (n=22), and IVB (n=4). Tumor, blood, and saliva samples were collected at baseline. Saliva samples were analyzed only for cases with oral or oropharyngeal tumors (n=22). DNA from tumor, plasma, saliva and leukocytes was sequenced using the QIAseq Human Comprehensive Cancer Panel (275 genes) on NovaSeq™ 6000 (Illumina). Quality controls included a pilot MiSeq run to ensure sequencing

homogeneity and a DNA fragment analysis step before sequencing to ensure data reliability, variants with low call quality or significant strand bias were excluded. Variants were identified with Mutect2 and annotated using VEP and ANNOVAR, with concordance assessed across sample types.

**Results:** A total of 1289 variants were detected in plasma, 1215 in saliva, and 679 in tumor samples. The median number of variants per sample was 46 (range 17–88) in plasma, 64 (range 5–125) in saliva, and 19 (range 1–69) in tumor. The most frequently mutated genes in plasma were *CDK12*, *SETD2 CD79B*, and *PIK3CA*, in saliva were *KDR*, *CDK12*, *FLT4*, and *NOTCH1* and in tumor samples were *STAT3*, *KDR*, *TSC2*, and *PIM1*. Single nucleotide variants (SNVs) were the most common mutation in tumor (99.26%), plasma (98.68%) and saliva (99.59%). Concordance analysis revealed 79 shared variants between tumor and saliva, 125 between plasma and saliva, 37 between tumor and plasma, and 37 variants common across all three sample types.

**Conclusion:** This study highlights the potential of liquid biopsies for detecting pathogenic tumor somatic mutations with clinical significance in LA-HNSC, representing a minimally invasive strategy for exploring the molecular mechanism underlying the disease relapse.

**Key words:** somatic mutation, cancer, head and neck cancer, next-generation sequencing, liquid biopsy.

**45** Development of an error corrected deep whole-exome ctDNA sequencing pipeline for evolutionary dynamics analysis in metastatic gastro-oesophageal adenocarcinoma

**Poster**

**Neil McCafferty[1] , Louise Barber[1] , Lucy Flanders[1] , Marta Buzzetti[1] , Thaneswari Karpanasamy[1] , Yuliia Kravchuck[1] , Rosemary Lane[1] , Lucas Baumard[1] , Dimitrios Kleftogiannis[1] , Andrew Woolston[1] , Marco Gerlinger[1]**

*1) Barts Cancer Institute, QMUL*

**Introduction**: The evolution of metastatic cancers over time can be assessed in circulating tumor DNA (ctDNA) but sequencing and bioinformatics tools for ultra-deep ctDNA whole-exome sequencing (WES) analysis are lacking. Development and optimisation of ultra-deep WES paired with novel duplex DNA strand error correction detection provides opportunity to extract clinically important genomic features in serial blood samples from metastatic gastro-oesophageal adenocarcinoma (GOA).

**Objectives**: Apply ultra-deep ctDNA WES and assess duplex in mixing samples to determine the detection limit of duplex error correction. Apply ultra-deep ctDNA WES to GOA patients (pts). Assess the impact of duplex and error correction filtering on the number of mutations called.

**Methodology**: We developed ultra-deep ctDNA WES that only requires 15ng DNA to achieve sequencing depths of ~1000x. Error correction with molecular barcodes and duplex DNA detection allowed calling of mutations ≥0.5% variant frequency (VF); demonstrated by SNP variants in a 1% mixing sample experiment. A cohort of 22 healthy donors (HD) applied deep WES on plasma, to further error correction applications of mutation calling and copy number. HD mutation error correction considered mutation VF across all HD; an error limit based on median VF plus 3 standard deviations was set to account for clonal expansion. Pooled HD copy references were generated and applied to ctDNA copy ratios using an adjusted CNVkit pipeline.

**Results**: ROC assessment of 6476 SNPs in three mixing samples showed median threshold detection for duplex reporting as 0.49% (76% sensitivity, 73.5% specificity, 80.29 AUC). The median age of 20 pilot cohort pts was 71y, 95% had distant metastases and 5% locally advanced GOAs. A median sequencing depth of 1254x after de-duplication at baseline ctDNA. To date, WES has been extended to 44 pts: 27 baseline biopsy, 91 ctDNA timepoints and 44 matched gDNA. Further cases and timepoints have been collected and are awaiting sequencing. Duplex

and HD error correction has been applied to current timepoints; improvements from these mutation filters vs normal mutation calling will be presented. HD copy number references were applied to improve the quality of raw copy profiles, improving examination of low purity ctDNA profiles and subsequent clonal mutation burden. The (sub)clonal structures demonstrate changes over time which will be shown in multi-timepoint comparisons.

**Conclusions**: ctDNA WES can assess the genetics of entire metastatic cancer cell populations over time and deconvolute their evolutionary trajectories. Application of novel mutation error-correction allows for accurate mutation calling to targeted ≥0.5% VF, facilitating investigations of evolving subclonal structures.

**46** Development of a Long-term Circulating Tumor Cells Organoid Model Derived from Metastatic Triple Negative Breast Cancer

**Poster**

**Miguel Abal[1,2] , Carmen Abuin[1,2] , Clotilde Costa[1,2] , Andrea Estrella Arias-Diaz[1,2] , Teresa Curiel[1,2] , Juan Cueva[1,2] , Patricia Palacios[1,2] , Carmela Rodriguez[1,2] , Alexia Cortegoso[1,2] , Jorge Barbazan[1,2] , Laura Muinelo-Romay[1,2] , Juan Ruiz[1,2] , Eduardo Dominguez[3] , Rafael Lopez-Lopez[1,2]**

*1) Translational Medical Oncology Group (Oncomet), Health Research Institute of Santiago de Compostela (IDIS), University Hospital of Santiago de Compostela (SERGAS), Trav. Choupana s/n, 15706 Santiago de Compostela, Spain*
*2) Centro de Investigacion Biomedica en Red de Cancer (CIBERONC), Monforte de Lemos 3-5, 28029 Madrid, Spain 3) C005-Genetics, Health Research Institute of Santiago de Compostela (IDIS), Travesía da Choupana, 15706 A Coruña, Spain*

Modeling metastasis and circulating tumor cell (CTC) dissemination remains a challenge, hindering appropriate preclinical studies targeting the cellular and molecular events associated with the vast majority of cancer-related deaths. In particular, the development of CTC-derived models has been largely restricted to short-term cultures with occasional relevant long-term cases derived from CTC-xenograft models. In this work we report the generation of a Triple Negative Breast Cancer (TNBC) long-term CTC-derived organoid model by combining viable and functional CTC enrichment with the fundaments of organoids' culture. CTCs were purified from peripheral blood of a metastatic TNBC patient by a negative enrichment approach, with CTC enumeration accounting for with > 1000 CTCs/7,5mL of blood as by CellSearch system. CTCs enriched fraction was embedded in a basement membrane-derived matrix and cultured in the presence of growth-factor enriched organoid medium to generate long-term (>10 passages) proliferative organoids with dense morphology. Immunohistochemistry analysis confirmed the triple negative ER/PR/HER2 histology, and immunofluorescence characterization described an acinar organoid structure, as expected for a breast cancer model. Using dose-dependent cell viability assays and videomicroscopy, we demonstrated that CTC-TNBC organoids are highly resistant to the standard platinum-based therapy, mimicking patient's therapy response and further confirming the reliability of this model. Finally, we conducted a high-throughput screening with a set of 179 FDA-approved anticancer drugs, and identified potential compounds with activity in a metastatic TNBC setting. In addition to explore for efficient therapies targeting these metastatic CTC organoids, this unique model represents an excellent preclinical tool to explore the biology of CTCs in TNBC, including fundamental dissemination and colonization mechanisms, biomechanics and circadian rhythmicity, organotropism, resistance to therapy, clonal evolution or the study of immunomodulation and advanced cell therapy strategies.

**47** Comparative Evaluation of Circulating Tumor Cells Enrichment Technologies in Colorectal Cancer

**Poster**

**Olalekan Bamidele[1] , Ceres Fernández Rozadilla[1]**

*1) instituto de investigacion sanitaria*

- comparing several CTCs enrichment technologies, to evaluate their capture efficiency across different cell lines
- Testing these technologies with diverse cell lines ensures that their performance is both reliable and consistent

**48** Comparison of CTC isolation technologies in patients with advanced urothelial carcinoma

**Poster**

**Ignacio Arévalo [1,2,3] , Esther Montesinos[1,3] , Álvaro Martín de Bernardo[1,3] , Iris Lodewijk[1,3] , Daniel Castellano[4] , Jesús María Paramio[1,2,3] , Marta Dueñas[1,2,3] , Carolina Rubio[1,3]**

*1) Molecular Oncology Unit, Biomedical Innovation Department, Centro de Investigaciones Energéticas, Medioambientales y Tecnológicas (CIEMAT), 28040 Madrid, Spain*
*2) Centre for Biomedical Research Network of Oncological Diseases (CIBERONC), 29029 Madrid, Spain.*
*3) Institute of Biomedical Research, University Hospital '12 de Octubre', Madrid, Spain.*
*4) Medical Oncology Department, University Hospital '12 de Octubre', Madrid, Spain.*

Urothelial cancer (UC) is the ninth most prevalent cancer worldwide, with an incidence rate that results in over 200,000 deaths each year. Treating UC remains a significant clinical challenge due to several factors.

Despite efforts in recent years to improve the survival of patients with advanced disease, the life expectancy of patients is still very short. In addition, there is no effective method of monitoring progress in anticipation of the worst clinical symptoms.

This underscores the ongoing challenges in effectively managing advanced conditions. Furthermore, there is currently no effective method available for monitoring disease progression in a way that could anticipate the most severe clinical symptoms. This lack of predictive tools emphasize the urgent need for advancements in both treatment and monitoring technologies.

Identifying reliable biomarkers that can help detect UC and monitor its development is essential to overcome these inconveniences. Recent research has shown that **Circulating Tumor Cells (CTCs)** and **Tumor Hybrid Cells (THCs)** hold promise as tools for prognosis and disease monitoring. These cells, which shed from the tumor and circulate in the bloodstream, could provide valuable information about the status of the cancer, including its likelihood of spreading.

However, there are challenges in using CTCs and THCs in clinical practice. A major problem is that they are rarely found in the bloodstream, with only a small number of these cells usually found circulating. This small amount makes it difficult to isolate, study, and characterize them in a way that could inform personalized treatment strategies.

With this in mind, new technologies based on different methodologies have been developed to isolate these types of cells. These methodologies take advantage of the larger size and lower plasticity of CTCs and THCs compared to other cells in the bloodstream. Multiple technologies have been developed with this isolation idea such Genesis (BioRad), Parsortix (Angle) and RubyChip (RUBYnanomed) systems and we decide to compare them.

To do this comparison have isolated the circulating tumor cells (CTCs) and tumor hybrid cells (THCs) from patients with advanced bladder cancer using three different technologies based on cell size and plasticity. A comparison have been made to evaluate how many CTCs and THCs each method can enrich. The goal is to select the best technology for isolating CTCs in bladder cancer, which will be useful for future research and applications.

To achieve this, we collected blood samples from patients with advanced bladder cancer to isolate, stain, and analyze CTCs and THCs using different technologies. For each patient, three tubes of blood were drawn, one for each technology. We compared the number of CTCs obtained under the same conditions in the same patient. The cells were stained with Vimentin, EpCAM, Pan-Cytokeratin, and DAPI to identify and characterize the CTCs effectively.

Within the comparison for four patients, we observed remarkable differences among the three technologies. Based on our preliminary results, the best isolation and visualization method would be the genesis system, because despite the fact that is the technology which enables to run the least quantity of blood we have achieved the mayor quantity of CTCs and its visualization under the microscope is the best. RubyChip had a very good results too although its visualization is not so clear and we have isolate less CTCs. Last, Parsortix had two samples very difficult to analyze due to the blood contaminations that remains in the chip and which difficults a lot to visualize the results using cassette immunostaining. Despite these results in Parsortix, it is probably that using other methods to visualize the cells like recovering them and doing an immunostaining out of the cassette will provide better results.

This analysis will be expanded to a larger cohort of at least 20 patients to determine, in a statistically significant manner, the most effective method for isolating CTCs in UC patients in the advance disease stage. This approach will allow us to select a single technology for future work, maximizing the quantity of isolated CTCs and enabling further analysis to characterize these cells and potentially identify new biomarkers.

**49** Identifying biomarkers of senescence after chemotherapy to guide cancer treatment

**Poster**

**Sabela Da Silva-Álvarez[1,2] , Alex Miralles-Dominguez[1] , Picallos-Rabina Pilar[1] , Valentín Estévez-Souto[1] , Patricia Lado-Fernández[1] , Víctor Núñez-Quintela[1] , Migue Angel Prados[1,3] , Alejandro Failde-Fiestras[1] , Raquel Paredes-Paredes[1] , Miguel González-Barcia[4] , Manuel Collado[1,5]**

*1) Laboratory of Cell Senescence, Cancer and Aging, Health Research Institute of Santiago de Compostela (IDIS), E15706 Santiago de Compostela, Spain.*
*2) Center for Research in Biological Chemistry and Molecular Materials (CiQUS), Department of Biochemistry and Molecular Biology, University of Santiago de Compostela, E15705 Santiago de Compostela, Spain.*
*3) Departamento de Fisioloxía and Cell Cycle and Oncology Group, Centro de Investigación en Medicina Molecular (CiMUS), Universidade de Santiago de Compostela, Health Research Institute of Santiago de Compostela (IDIS), E15782 Santiago de Compostela, Spain*
*4) Servicio de Farmacia, Xerencia de Xestión Integrada de Santiago (XXIS/SERGAS), E15706 Santiago de Compostela, Spain*
*5) Department of Inmunology and Oncology (DIO), Centro Nacional de Biotecnología (CNB-CSIC), Madrid, Spain*

Despite the opposing roles associated with senescent cells in the context of cancer, the therapeutic potential of modulating senescence is well defined and characterized. In fact, some anticancer therapies are based, in part, on their ability to re-engage the senescence program. However, the remnants of senescent cells decrease the efficiency of treatments leading to a poor response of the patients and relapses. For this reason, a 'one-two punch' strategy, combining senescence-inducing agents with senolytic agents, has been proposed. To implement such therapeutic strategy, we need to be capable of identifying senescent cells after cancer treatments to link the senescence response with the prognosis of patients. Besides, biomarkers of this senescence response would allow the evaluation of current therapies to find more robust, stable, and less toxic cancer treatments.

Target-specific anticancer drugs, such as CDK4/6i for breast cancer, block tumour growth by inducing cellular senescence. We set up experimental systems that allow us the identification of biomarkers in breast cancer cell lines and mouse models treated with CDK4/6i. The presence of these biomarkers in the blood as soluble factors or associated with extracellular vesicles could be used as a molecular signature to identify senescence induction after chemotherapy treatment. These biomarkers could serve as the basis for new diagnostic systems that would

represent powerful tools with considerable clinical implications for guiding cancer therapy.

**50** Characterization of CTCs and CTC-clusters in breast cancer xenografts

**Poster**

**Nair Varela-Rouco[1,2] , Nuria Estévez-Gómez[1,2] , Cristóbal Fernández-Santiago[3] , Laura Tomás[1,2] , Joao Miguel Alves[1,2] , Roberto Piñeiro[3] , David Posada[1,2,4]**

*1) CINBIO, Universidade de Vigo, 36310 Vigo, Spain.*
*2) Galicia Sur Health Research Institute (IIS Galicia Sur), SERGAS-UVIGO.*
*3) Roche-Chus Joint Unit, Translational Medical Oncology Group, Oncomet, Health Research Institute of Santiago de Compostela (IDIS), Santiago de Compostela, Spain.*
*4) Department of Biochemistry, Genetics, and Immunology, Universidade de Vigo, 36310 Vigo, Spain*

Preclinical and clinical studies suggest that circulating tumor cells (CTCs) are pivotal in the metastatic cascade. CTC-clusters are aggregates of cells that break away from primary tumors and are typically linked to poor clinical outcomes. Although it is hypothesized that cells within these clusters cooperate during dispersal, dissemination, and colonization processes, the degree of genomic heterogeneity within these CTC-clusters and their connection to primary tumor and metastatic clones remains largely undefined.

To address these issues, we developed mouse xenografts using the triple-negative breast cancer cell line MDA-MB-231. After tumor growth and dissemination occurred, we collected tissue samples, from primary tumors and metastases, together with blood samples to isolate single CTCs and CTC-clusters. We performed whole-exome sequencing on these samples and identified single-nucleotide and copy-number variants using a custom bioinformatics pipeline. This study presents the genomic landscape of CTCs and CTC-clusters compared to primary tumors and metastases.

# X SIMPOSIO · SYMPOSIUM | 2025
# BIOPSIA LÍQUIDA · LIQUID BIOPSY

## EL CAMINO A LA ONCOLOGÍA DE PRECISIÓN · THE WAY TO PRECISION MEDICINE

**23, 24 Y 25 DE ENERO 2025** · JANUARY 23rd, 24th and 25th 2025

**Sede · Venue:**
*San Francisco Hotel Monumento*
*Campillo de San Francisco, 3, Santiago de Compostela*

www.simposiobiopsialiquida.com


**Director científico:**
*Scientific director:*

**Dr. Rafael López López**
Medical Oncology Service
Complexo Hospitalario Universitario de Santiago de Compostela


**Con el soporte de:**
*With the support of:*

USC Universidade de Santiago de Compostela

**Organizado por:**
*Organized by:*

UFV Universidad Francisco de Vitoria