

III SIMPOSIO

Biopsia líquida

EL CAMINO A LA ONCOLOGÍA DE PRECISIÓN



25 - 27 DE ENERO 2018 · JANUARY 25TH - 27TH · SANTIAGO DE COMPOSTELA



#SimposioBiopsiaLiquida

www.simposiobiopsialiquida.com

Coordinación científica

Scientific coordination:

Dr. Rafael López López

Complejo Hospitalario Universitario
de Santiago

Sede

Venue:

San Francisco Hotel Monumento

Campillo de San Francisco, 3
Santiago de Compostela

Organizado por

Organized by:



Universidad
Francisco de Vitoria
UFV Madrid

III SIMPOSIO *Biopsia líquida*

EL CAMINO A LA ONCOLOGÍA DE PRECISIÓN

Bienvenidos al **III Simposio Biopsia Líquida: El camino a la oncología de precisión.**

Tras el éxito de las dos primeras ediciones, donde superamos gratamente las expectativas, os traemos el **tercer simposio**, con las mismas ganas de aportar y compartir el conocimiento más reciente sobre las diferentes posibilidades de la biopsia líquida. Como hemos hecho anteriormente, abordaremos la temática desde perspectivas tan atractivas como la utilidad clínica, los progresos en el conocimiento biológico y los continuos avances tecnológicos, todo ello en un escenario de formación, discusión y planteamiento de retos futuros.

Como organizador del simposio, quiero presentaros el **nuevo programa científico**, en el que contamos con un grupo de destacados expertos para liderar las diferentes sesiones y ponencias, en las que trataremos los diferentes aspectos de la biopsia líquida y las diferentes patologías oncológicas.

El San Francisco Hotel Monumento, situado en el corazón de Santiago, nos acogerá para poder celebrar el simposio en un entorno sereno y agradable.

Por todo esto, esperamos que disfrutes del simposio y sea una fuente de **enriquecimiento profesional** en el camino de la **oncología de precisión.**

¡Bienvenidos a Santiago!

*Welcome to the **III Simposio Biopsia Líquida: El camino a la oncología de precisión.***

*After the success of the first editions, which pleasantly surpassed our expectations, we bring you this **third Symposium**, with the same desire to offer and share the most recent knowledge about the different possibilities of liquid biopsy. As done before, we will approach the topic from interesting perspectives such as clinical utility, progress made in biological knowledge and the continuous technological advances, all of it in a scenario of training, discussion and addressing future challenges.*

*As organiser of the Symposium, I would like to introduce you to the **scientific programme**, bringing together a group of prominent experts who will lead a range of sessions and talks, during which we will address specific aspects of liquid biopsy and the different oncological pathologies.*

The San Francisco Hotel Monument, located in the centre of Santiago, will welcome us with a relaxing and pleasant environment to hold the Symposium.

*Therefore, we hope you will enjoy the event and will regard it as a source of **professional enrichment** on the road to **precision oncology.***

Welcome to Santiago!



Dr. Rafael López López
Servicio de Oncología Médica,
Complejo Hospitalario Universitario de Santiago



COMITÉ CIENTÍFICO *SCIENTIFIC COMMITTEE*

Dr. Rafael López López

Jefe de Servicio de Oncología Médica
Oncomet
Complejo Hospitalario Universitario
de Santiago

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Unidad de Análisis de Biopsia Líquida
Oncomet
Complejo Hospitalario Universitario
de Santiago

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Oncomet
Complejo Hospitalario Universitario de
Santiago

Dr. Miguel Abal

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Oncomet
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de Santiago

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Unidad mixta ROCHE CHUS
Oncomet
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Santiago

Dra. Teresa Curiel

Servicio de Oncología Médica
Oncomet
Complejo Hospitalario Universitario de
Santiago

SIMPOSIO SATÉLITE. TECNOLOGÍA APLICADA • SATELLITE SYMPOSIUM. APPLIED TECHNOLOGY

16:00 - 16:30 **INSCRIPCIÓN • REGISTRATION**

16:30 - 16:35 **BIENVENIDA • WELCOME**

Dr. Rafael López López, Complejo Hospitalario Universitario de Santiago

Dra. Laura Muínelo, Complejo Hospitalario Universitario de Santiago

16:35 - 18:10 **AVANZANDO EN TECNOLOGÍA Y APLICACIONES: SESIÓN I**

ADVANCING IN TECHNOLOGY AND APPLICATIONS: SESSION I

Moderadores • Chairpersons

Dr. Miguel Abal, Complejo Hospitalario Universitario de Santiago

Dra. Beatriz Sobrino, Fundación Pública Galega de Medicina Xenómica, Complejo Hospitalario Universitario de Santiago

16:35 - 16:50 **Nucleosomas como nueva prueba basada en sangre para caracterizar tumores**

Nucleosomes as new blood based test to characterize tumours

Mr. Mark Eccleston, Business Development Director at Volition. Inses, Belgium

Espensoriada por: / Sponsored by: **Volition**

16:50 - 17:05 **Perfiles genómicos utilizados en biopsia líquida**

Genomic profiles employed in liquid biopsy

Dr. Emiliano Calvo, START Madrid CIOCC, Centro Integral Oncológico Clara Campal. Madrid

Espensoriada: / Sponsored by: **Roche**

17:05 - 17:20 **Sistemas de microfluidica para aislamiento de CTCs**

Microfluidic technologies for CTCs isolation

Dra. Lorena Diéguez, International Iberian Nanotechnology Laboratory - INL. Braga, Portugal

17:20 - 17:35 **ERASE-SEQ: Detección precisa de variantes somáticas de baja frecuencia en cfDNA**

ERASE-Seq: accurate detection of low frequency somatic variants in cfDNA

Mr. Cristian Ionescu, Chief Technology Officer at Fluxion Biosciences. Alameda, California, USA

Espensoriada por: / Sponsored by: **IZASA Scientific**

17:35 - 17:50 **Métodos ultrasensibles para detección de ctDNA**

Ultrasensitive methods for ctDNA detection

Dr. Alain R. Thierry, Institut National de la Santé Et de la Recherche Médicale - INSERM. Montpellier, France

17:50 - 18:10 **Discusión • Discussion**



18:10 - 18:20 **Compra pública innovadora aplicada a oncología: Plan de innovación código 100**
Public procurement of innovation applied to oncology: Código 100 innovation plan

Dr. Luis León, Agencia de Coñecemento en Saúde-ACIS. Santiago de Compostela

18:20 - 18:40 **PAUSA CAFÉ • COFFEE BREAK** 

18:40 - 20:00 **AVANZANDO EN TECNOLOGÍA Y APLICACIONES: SESIÓN II**
ADVANCING IN TECHNOLOGY AND APPLICATIONS: SESSION II

Moderadores • Chairpersons

Dr. Roberto Piñeiro, Unidad mixta ROCHE CHUS. Complejo Hospitalario
niversitario de Santiago

Dra. Silvia Calabuig-Fariñas, Hospital General Universitario de Valencia

18:40 - 18:55 **Aplicaciones de Droplet-Based digital PCR**

Applications of Droplet-Based digital PCR

Dra. Valérie Taly, Université Paris Descartes. Paris, France

18:55 - 19:10 **La experiencia de la biopsia líquida de OncoBEAM en la práctica clínica del mundo real**

The OncoBEAM liquid biopsy experience in real-world clinical practice

Mr. Frederick S. Jones, Director Medical Affairs Liaison at Sysmex-Inostics.
Mundelein, Illinois, USA

Esponsorizada por: / *Sponsored by:* **Sysmex**

19:10 - 19:25 **Enriquecimiento CTC con PARSORTIX™: clinical applications**

CTC enrichment with PARSORTIX™: clinical applications

Dra. Eva Obermayr, Medical University Vienna. Austria

Esponsorizada por: / *Sponsored by:* **Parsortix**

19:25 - 19:40 **Detección de mutaciones ctRAS-BRAF con la plataforma Idylla™ en tan solo horas . Las ventajas de Idylla™ en la práctica clínica**

RAS-BRAF mutation detection on the Idylla™ System in just 2 hours. How Idylla™ can bring real value into clinical practice

Mrs. Marijke Van der Auwera, Product Manager at Biocartis. Mechelen, Belgium

Esponsorizada por: / *Sponsored by:* **Biocartis**

19:40 - 20:00 **Discusión • Discussion**

09:30 - 10:35 **BIOPSIA LÍQUIDA: DÓNDE ESTAMOS Y HACIA DÓNDE VAMOS**

LIQUID BIOPSY: WHERE WE STAND AND WHERE WE GO

Moderadores • *Chairpersons*

Dr. Eduardo Díaz-Rubio, Hospital Clínico San Carlos. Madrid

Dra. Teresa Curiel, Complejo Hospitalario Universitario de Santiago

09:30 - 09:45 **¿Qué hemos aprendido hasta hoy?**

What have we learned so far?

Dr. Luis Marques da Costa, Hospital de Santa Maria. Lisboa, Portugal

09:45 - 10:00 **Situación actual en biopsia líquida**

Current situation of liquid biopsy

Dr. Carlos Camps, Hospital General Universitario de Valencia

10:00 - 10:15 **Generación de modelos predictivos**

Generation of predictive models

Dr. David Tamborero, Institute for Research and Biomedicine - IRB. Barcelona

10:15 - 10:35 **Discusión** • *Discussion*

10:35 - 11:00 **INAUGURACIÓN OFICIAL** • *OFFICIAL OPENING*

11:00 - 11:45 **ENTENDIENDO LA DISEMINACIÓN**

UNDERSTANDING DISSEMINATION

Moderadores • *Chairpersons*

Dr. Jorge Barbazán, Institute Curie. Paris, France

Dr. Urbano Anido, Complejo Hospitalario Universitario de Santiago

11:00 - 11:15 **Diseminación temprana en cáncer de próstata**

Early dissemination in prostate cancer

Dr. Miodrag Guzvic, University of Regensburg. Germany

11:15 - 11:30 **Vesículas extracelulares en biopsia líquida y cáncer**

Extracellular vesicles in liquid biopsy and cancer

Dr. Guido Jenster, Erasmus University Medical Center Rotterdam. Netherlands

11:30 - 11:45 **Discusión** • *Discussion*



11:45 - 12:10 PAUSA CAFÉ • COFFEE BREAK 

12:10 - 12:55 MÁS ALLÁ DE LA SANGRE
BEYOND THE BLOOD

Moderadores • Chairpersons

Dr. Jesús García Mata, Complejo Hospitalario Universitario de Ourense
Dr. Juan Jesús Cruz, Hospital Clínico Universitario de Salamanca

12:10 - 12:25 **Detección de marcadores de cáncer de vejiga en orina**
Detection of bladder cancer markers in urine

Dr. Jesús Paramio, Hospital Universitario 12 de Octubre. Madrid

12:25 - 12:40 **En busca de nuevos marcadores tumorales en saliva**
Looking for new tumor markers in saliva

Dra. Blanca Majem, Vall d'Hebron Institut de Recerca - VHIR. Barcelona

12:40 - 12:55 **Discusión • Discussion**

12:55 - 13:40 TUMORES DE BAJA PREVALENCIA
LOW PREVALENCE TUMORS

Moderadores • Chairpersons

Dr. Antonio Antón, Hospital Universitario Miguel Servet. Zaragoza
Dra. Pilar García Alfonso, Hospital General Universitario Gregorio Marañón. Madrid

12:55 - 13:10 **Retos para el manejo clínico del melanoma a través de biopsia líquida**
Current challenges in the clinical management of melanoma by liquid biopsy

Dr. Josep Maria Piulats, Institut Català d'Oncologia-Hospital Duran i Reynals.
L'Hospitalet de Llobregat

13:10 - 13:25 **Oportunidades y retos de la aplicación del ctDNA en neuroblastoma**
Challenges and opportunities for the application of ctDNA in neuroblastoma

Dra. Rosa Noguera, Fundación para la Investigación del Hospital Clínico de la
Comunidad Valenciana- Fundación INCLIVA. Valencia

13:25 - 13:40 **Discusión • Discussion**

13:40 - 15:00 ALMUERZO Y VISITA PÓSTERES • LUNCH & POSTER VIEWING 

15:00 - 15:45 INMUNO BIOPSIA LÍQUIDA

IMMUNE LIQUID BIOPSY

Moderadores • Chairpersons

Dr. Alfredo Carrato, Hospital Universitario Ramón y Cajal. Madrid

Dr. Ignacio Chacón, Hospital Virgen de la Salud. Toledo

15:00 - 15:15 **Nuevas técnicas de seguimiento de respuesta a inmuno terapia por biopsia líquida**

New techniques for monitoring the response to immunotherapy by liquid biopsy

Dr. Eduardo Sotomayor, George Washington University Hospital. Washington DC, USA

15:15 - 15:30 **Contribución de la biopsia líquida al avance de la inmunoterapia**

Contribution of the liquid biopsy to the immunotherapy advance

Dr. Jesús García-Foncillas, Hospital Universitario Fundación Jiménez Díaz. Madrid

15:30 - 15:45 **Discusión • Discussion**

15:45 - 16:50 CÁNCER COLORRECTAL

COLORECTAL CANCER

Moderadores • Chairpersons

Dr. Juan de la Cámara, Complejo Hospitalario Universitario de Ferrol

Dra. Sonia Candamio, Complejo Hospitalario Universitario de Santiago

15:45 - 16:00 **Potencial y limitaciones de la técnica de secuenciación de biopsia líquida en pacientes con carcinoma de colon**

Potential and limitations of sequencing techniques of liquid biopsy in colon carcinoma patients

Dr. Nicola Normanno, Istituto Nazionale dei Tumori. Napoli, Italia



16:00 - 16:15 **Del laboratorio a la clínica: análisis preliminar del estudio VISNU**
From the lab to the clinic: preliminary analysis of the VISNU study

Dr. Javier Sastre, Hospital Clínico San Carlos. Madrid

16:15 - 16:30 **Potencial clínico de ARNs no codificantes como biomarcadores de biopsia líquida en cánceres gastrointestinales**

Clinical potential of noncoding RNAs as liquid biopsy biomarkers in gastrointestinal cancers

Dr. Ajay Goel, Baylor Scott & White Research Institute and Charles A. Sammons Cancer Center. Dallas, Texas, USA

16:30 - 16:50 **Discusión** • *Discussion*

16:50 - 17:20 PAUSA CAFÉ • COFFEE BREAK 

17:20 -18:40 CÁNCER DE MAMA
BREAST CANCER

Moderadores • *Chairpersons*

Dr. Vicente Guillem, Insituto Valenciano de Oncología - IVO. Valencia

Dr. Juan Cueva, Complejo Hospitalario Universitario de Santiago

17:20 - 17:35 **Desafío para la aplicación clínica de biopsia líquida en cáncer de mama**
Challenges to overcome the applicability of liquid biopsy in breast cancer

Dr. Isaac García-Murillas, Breast Cancer Now Research Center. The Institute of Cancer Research. London, United Kingdom

17:35 - 17:50 **CTCs vs ctDNA en el manejo del cáncer de mama metastásico**
CTCs vs ctDNA in the clinical management of the metastatic breast cancer

Dra. Evi Lianidou, Analysis of Circulating Tumore Cells - ACTC Lab, University of Athens. Grece

17:50 -18:05 **Modelo de grupos cooperativos para la aplicación de biopsia líquida**
Model of cooperative groups for the liquid biopsy application

Dr. Miguel Martín, Hospital General Universitario Gregorio Marañón. Madrid

18:05 - 18:20 **Generación de CDX a partir de una paciente con cáncer de mama metastásico triple negativo**
Establishment of a CDX from a triple-negative metastatic breast cancer patient

Dra. Clotilde Costa, Unidad mixta ROCHE CHUS. Complejo Hospitalario Universitario de Santiago

18:20 - 18:40 **Discusión** · *Discussion*

18:40 - 19:40 **COMUNICACIONES ORALES**

ORAL COMMUNICATIONS

Moderadores · *Chairpersons*

Dra. Ana Belén Dávila, Unidad Mixta ROCHE CHUS, Complejo Hospitalario Universitario de Santiago

Dr. Juan Ruiz, Complejo Hospitalario Universitario de Santiago

18:40 - 18:55 **La caracterización molecular de CTCs de pacientes con NSCLC avanzada es una estrategia valiosa para predecir la respuesta de quimioterapia de primera línea**

Molecular characterization of CTCs from patients with advanced NSCLC is a valuable strategy to predict first line chemotherapy response

Dra. Silvia Calabuig-Fariñas, Hospital General Universitario de Valencia

18:55 - 19:10 **Análisis de células tumorales circulantes (CTCs) a nivel de célula única para inferir cambios genéticos y de fenotipo en respuesta a presiones terapéuticas en cáncer de vías biliares**

Analysis of single Circulating Tumor Cells (CTCs) to infer phenotype and genome changes in response to therapeutic pressures in biliary tract cancer

Dra. Carolina Reduzzi, Fondazione IRCCS Istituto Nazionale dei Tumori. Milano, Italy

19:10 - 19:25 **Niveles de ctDNA por ddPCR en cáncer de pulmón de células no pequeñas: implementación en la práctica clínica**

ctDNA levels by ddPCR in non-small-cell lung cancer: implementation in clinical practice

Dr. Aitor Azkárate, Hospital Universitari Son Espases. Mallorca



19:25 - 19:40 **El ctDNA refleja la heterogeneidad intrapaciente en pacientes con cáncer colorrectal metastásico (mCRC) que progresan a FOLFIRI+ Panitumumab**
Circulating tumor (ct)DNA captures inpatient heterogeneity in metastatic colorectal (mCRC) patients progressing to FOLFIRI+panitumumab
Dra. Joana Vidal, Hospital del Mar-Parc de Salut Mar. Barcelona

19:40 - 20.40 CÓCTEL Y VISITA PÓSTERES - COCKTAIL & POSTER VIEWING

09.00 - 10:05 **CÁNCER DE PULMÓN**

LUNG CANCER

Moderadores • Chairpersons

Dr. Sergio Vázquez, Hospital Universitario Lucus Augusti. Lugo

Dr. Jorge García González, Complejo Hospitalario Universitario de Santiago

09:00 - 09:15 **Búsqueda de marcadores tempranos en sangre**

Searching for early markers in blood

Dra. Eloisa Jantus, Hospital General Universitario de Valencia

09:15 - 09:30 **Uso de técnicas de secuenciación de nueva generación en ctDNA para caracterizar tumores de pulmón**

Application of next generation sequencing on ctDNA for lung cancer characterization

Dr. José Luis Costa, Institute of Molecular Pathology and Immunology of the University of Porto - IPATIMUP. Porto, Portugal

Esponsorizada por: / Sponsored by: **ThermoFisher**

09:30 - 09:45 **¿Podemos predecir la recurrencia del cáncer de pulmón monitorizando el ctDNA?**

Can we predict recurrence in lung cancer by ctDNA monitoring?

Dr. Luis Paz-Ares, Hospital Universitario 12 de Octubre. Madrid

09:45 - 10:05 **Discusión • Discussion**

10:05 - 11:10 **CÁNCER DE PRÓSTATA**

PROSTATE CANCER

Moderadores • Chairpersons

Dr. David Olmos, Centro Nacional de Investigaciones Oncológicas-CNIO. Madrid

Dr. Juan Antonio Virzuela, Hospital Universitario Virgen de la Macarena. Sevilla

10:05 - 10:20 **AR-V7. Significado clínico en pacientes con cáncer de próstata metastásico**

AR-V7. Clinical significance in metastatic prostate cancer patients

Dr. Emmanuel Antonarakis, Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins. Baltimore, Maryland, USA



10:20 - 10:35 **ctDNA como guía para el tratamiento de cáncer de próstata con inhibidores de PARP**

ctDNA as a guide for the treatment of prostate cancer with PARP inhibitors

Dr. Joaquín Mateo, Vall d'Hebron Institut d'Oncologia - VHIO. Barcelona

10:35 - 10:50 **Ensayos clínicos activos para validar el uso clínico de biopsias líquidas en cáncer de próstata**

Ongoing clinical trials for the validation of liquid biopsy in prostate cancer

Dr. José Ángel Arranz, Hospital General Universitario Gregorio Marañón. Madrid

10:50 - 11:10 **Discusión** • *Discussion*

11:10 - 11:40 PAUSA CAFÉ • COFFEE BREAK 

11:40 - 12:45 BIOPSIA LÍQUIDA Y HETEROGENEIDAD TUMORAL

LIQUID BIOPSY AND TUMOR HETEROGENEITY

Moderadores • *Chairpersons*

Dr. Ángel Díaz-Lagares, Complejo Hospitalario Universitario de Santiago

Dra. Elena Brozos, Complejo Hospitalario Universitario de Santiago

11:40 - 11:55 **Análisis evolutivo de genomas de células individuales**

Evolutionary analysis of single-cell genomes

Dr. David Posada, Universidad de Vigo

11:55 - 12:10 **Factores clave para la supervivencia celular durante la diseminación sanguínea**

Key factors in cell survival during blood-borne dissemination

Dr. Nicola Aceto, University of Basel. Switzerland

12:10 - 12:25 **Manejo de la heterogeneidad tumoral en clínica**

Clinical management of tumor heterogeneity

Dr. Emilio Alba, Hospital Universitario Virgen de la Victoria. Málaga

12:25 - 12:45 **Discusión** • *Discussion*

12:45 - 13:45 MESA DE DEBATE: RIVALES O COMPLEMENTARIOS EN BUSCA DE UNA MEDICINA DE PRECISIÓN?

ROUND TABLE: RIVAL OR COMPLEMENTARY IN SEARCH OF A PRECISION MEDICINE?

Moderadores • *Chairpersons*

Dra. Laura Muínelo, Complejo Hospitalario Universitario de Santiago

Dr. Luis León, Axencia de Coñecemento en Saúde-ACIS. Santiago de Compostela

12:45 - 13:05 Ronda 1: Biopsia de tejido versus biopsia líquida

Round 1: Tissue biopsy versus liquid biopsy

Dra. Mar Varela, Institut d'Investigació Biomèdica de Bellvitge-IDIBELL, Barcelona

Dr. César A. Rodríguez, Hospital Clínico Universitario, Salamanca

13:05 - 13:25 Ronda 2: ctDNA vs CTCs

Round 2: ctDNA vs CTCs

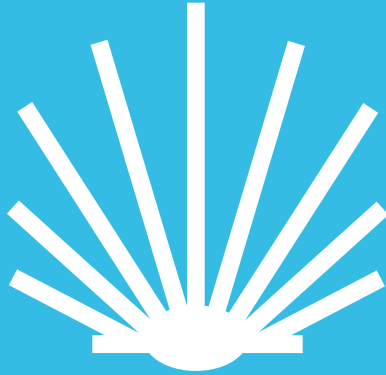
Dra. Joana Vidal, Hospital del Mar-Parc de Salut Mar. Barcelona

Dr. Roberto Piñeiro, Unidad mixta ROCHE CHUS. Complejo Hospitalario Universitario de Santiago

13:25 - 13:45 Discusión • *Discussion*

13:45 - 14:00 CLAUSURA Y ENTREGA DE PREMIOS DEL CONCURSO DE PÓSTERES

CLOSING AND POSTER COMPETITION AWARDS CEREMONY



III SIMPOSIO
Biopsia líquida
EL CAMINO A LA ONCOLOGÍA DE PRECISIÓN
ABSTRACTS

1- EVALUATING THE METASTATIC POTENTIAL OF BREAST CANCER CTC-CLUSTERS BY IN VITRO AND IN VIVO ASSAYS.

Authors: Inés Martínez Pena1*, Pablo Hurtado Blanco1*, Carmen Abuín Redondo1, Laura Sánchez Piñón2, Rafael López López3, Roberto Piñeiro Cid1‡.

1Roche-CHUS Joint Unit, University Hospital of Santiago (CHUS). 2Genetics Department, Veterinary Faculty, University of Santiago de Compostela (USC). 3Translational Medical Oncology Group, Health Research Institute of Santiago (IDIS), University Hospital of Santiago (CHUS).

* These authors has contributed equally. ‡ Tutor.

Introduction

Breast cancer (BC) is among the major tumour types diagnosed annually. In fact, BC is the most frequent tumour in female population. The majority of deaths associated with cancer are caused by the complex process of metastasis, in which new tumour foci are established in regions far from the primary tumour. Circulating tumour cells (CTCs), which are those cells released into the blood stream from the primary tumour, have the potential to seed metastatic lesions. CTCs can travel either as single cells or in groups called CTC-clusters. CTC-clusters are believed to have a greater metastatic capacity than single CTCs and their presence in the blood of BC patients is associated with a worse prognosis, indicated by a decrease in metastasis-free survival and the formation of new metastatic foci. Therefore, these CTCs-clusters could be useful to study the prognosis and progression in BC. However, little knowledge is yet available about what characteristics make them more metastatic than single CTCs, and overall, what is their contribution to metastasis.

Objective

The objective of our research is to better understand the biology of CTC-clusters. For this reason, we seek to functionally characterise CTC-clusters in order to identify differential properties compared to single CTCs, and to understand their contribution to metastasis.

Materials and methods

We use an in vitro model of CTCs and clusters generated from human breast cancer cell lines. Cells are spiked into blood from healthy donors, purified and allowed to aggregate in culture. Once aggregates are formed, they are mechanically dissociated to generate either a single or cluster cell suspension. The performance of both populations is compared in different in vitro assays of metastasis. Moreover, we assess their in vivo metastatic potential by using a xenograft model in zebrafish embryos (*Danio rerio*) in which fluorescently labelled cells are injected into the circulation of the fish (Duct of Cuvier) allowing tracking tumour spread and metastasis.

Results

Initial in vitro results are showing that our model of clusters have different properties typically associated with malignance, such as higher migration, invasion and tumourigenic capacity, when compared to individual cells. On the other hand, zebrafish xenograft experiments are showing that CTCs and CTC-clusters can survive in the circulation of the fish and efficiently disseminate and seed metastases. However, when analysing tumour volumes, no significant differences have been observed between both populations.

Conclusions

Preliminary differences between single CTCs and CTC-clusters have been observed in our in vitro functional assays. These differential properties could partially be the underlying causes that could explain the differential contribution of the clusters to the metastasis. Furthermore, we are using a novel in vivo approach to study the biology of CTCs, zebrafish xenografts. So far, our data show the ability of circulating tumour cells to disseminate and form metastases, although under these experimental conditions we have not detected significant differences between single cells and CTC-cluster populations. Therefore, our CTC-cluster model is a promising model to study the biology of these groups of cells and their contribution to metastasis.

2- ISOLATION AND MOLECULAR CHARACTERIZATION OF A NEGATIVE ENRICHED CTCs POPULATION IN METASTATIC BREAST AND PROSTATE CANCER PATIENTS BEFORE AND AFTER TREATMENT

Thais Pereira-Veiga^{1*}, Mónica Martínez-Fernández^{2,3,4}, Carmen Abuín¹, Rafael López-López^{1,4,5}, Laura Muínelo-Romay^{4,5} and Clotilde Costa^{1,4}

1. Roche-Chus Joint Unit. University Hospital of Santiago. Travesía da Choupana s/n 15706 Santiago de Compostela, Spain.
2. Molecular Oncology Unit. CIEMAT. Avda Complutense 40 28040 Madrid, Spain.
3. Molecular and Celular Oncology Unit. University Hospital 12 Octubre. Avda Córdoba s/n 28041 Madrid, Spain.
4. CIBERONC, Centro de Investigación Biomédica en Red Cáncer, Madrid, Spain.
5. Liquid Biopsy Analysis Unit, Oncomet, Health Research Institute of Santiago (IDIS), Complejo Hospitalario Universitario de Santiago de Compostela (SERGAS); Trav. Choupana s/n, Santiago de Compostela 15706, Spain.

Introduction:

Breast and prostate cancer are the two most prevalent invasive cancers in women and men, respectively. Being hormone-dependent cancers, the emergence of hormonal resistance is common. Tumour recurrence, resistance to therapy and metastasis formation are the leading causes of death in these patients, being CTCs principal actors for tumour dissemination in both cancers.

Detection of CTCs with CellSearch is based on the expression of EpCAM and CK8, 18 and 19. Therefore, the detection of CTCs that undergo a downregulation expression of epithelial markers, like more mesenchymal populations due to the EMT process, is limited using this strategy.

So far, few studies have investigated CTCs expression profile changes with the treatment but, to our knowledge, none of them have studied a negative enrichment approach in order to cover all the tumoral heterogeneity.

Objective:

The aim of the present study was to characterize a non-labelled CTCs population and to provide new insights into the dynamic evolution that occur during treatment and progression.

Methods:

CTCs from 11 metastatic breast (mBC) and 10 prostate (mPC) cancer patients were enriched before and after one cycle of treatment, and at recurrence, using CellSearch (Menarini-Silicon Biosystems) and RosetteSep (STEMCELL Technologies). Gene expression analysis was performed with a customized panel of 36 genes. Peripheral blood from 12 healthy donors was used to normalize the obtained data. In a number of samples cytospin and immunostaining was performed to assess the absence of CD45 expression in the isolated population.

Results:

Eleven out of 21 patients (52%) were positive for CTCs by CellSearch at baseline. Epithelial markers were associated with CTCs positivity by CellSearch in all of the mBC and mPC samples. Overexpression of both epithelial (EpCAM, CDH1 and KRT19) and mesenchymal markers (SNAI1) were found when comparing patient samples with healthy donors at baseline, and an increase of mesenchymal markers (SNAI1) was identified after treatment.

Conclusion:

Negative enrichment allows the isolation of a heterogeneous CTCs population, overcoming the absence of a universal marker. This approach may help better monitoring the evolution of the disease.

3- ASSESSMENT OF QMSP SENSITIVITY FOR THE DETECTION OF IGFBP-3 PROMOTER METHYLATION IN PLASMA FROM PATIENTS WITH ADVANCED STAGE NON-SMALL-CELL LUNG CANCER.

Olga Pernía^{1,2*}, Rocío Rosas^{1,2}, Julia Jiménez^{1,2}, Isabel Esteban³, Ana M. Rodríguez García³, Patricia Cruz-Castellanos⁴, Darío Sánchez-Cabrero⁴, Javier de Castro^{2,4} and Inmaculada Ibáñez^{1,2}

1. Cancer Epigenetics Laboratory, INGEMM, La Paz University Hospital, Madrid, Spain;
2. Biomarkers and Experimental Therapeutics in Cancer, IdiPAZ, Madrid, Spain;
3. Department of Pathology, La Paz University Hospital, Madrid, Spain;
4. Department of Oncology, La Paz University Hospital, Madrid, Spain.

* Olga Pernía Arias. Cancer Epigenetics Laboratory, INGEMM, Biomarkers and Experimental Therapeutics in Cancer, IdiPAZ, Paseo de la Castellana 261, Madrid, 28046.

Introduction:

Cancer is the main cause of morbidity and mortality, with 14 million new cases and 8 million deaths related 2012. Non-small cell lung cancer (NSCLC) represents the 80-85% of the lung cancers. Approximately 80% of the patients are diagnosed in advanced stages with a very poor prognosis. Cisplatin-based chemotherapy is the first line therapy for NSCLC; however, it also induces de novo DNA-hypermethylation, a process that may be involved in the development of drug-resistant phenotypes by inactivating genes required for drug-cytotoxicity. We have previously reported that IGFBP-3 promoter hypermethylation results in a reduction of tumor cell sensitivity to cisplatin in advance-stages NSCLC. Liquid biopsy is a useful tool for the analysis of tumors with limited-sample size as advanced stages of NSCLC. DNA methylation detection has been shown affordable in plasma sources, showing concordance between ctDNA and corresponding tumor tissue DNA. Knowing the IGFBP-3 methylation status in ctDNA may provide robust information associated with prognosis and treatment response for the clinical management of NSCLC.

Objective:

Assessment the sensitivity of qMSP to detect the methylation status of the IGFBP-3 gene promoter in ctDNA from NSCLC patients with advanced stages and correlate with the methylation levels in paired tumor tissue.

Methodology:

53 FFPE/plasma paired samples from stages III-IV NSCLC patients have been collected in the tumor bank at Hospital La Paz, following the ethical and confidentiality issues. Blood samples were processed within the first 60 min from the extraction and plasma stored at -80C. Tumor samples from FFPE or cytologies were reviewed by an expert pathologist to confirm the diagnosis and guarantee at least 90% tumor content. DNA and ctDNA was isolated and bisulfite modified following routine methodology. Methylation status of the IGFBP3-promoter was measured by qMSP methodology which presents a 1:10000 sensitivity, obtaining quantitative methylation levels ($C_{meth} = 100 / [1 + 2(CTCG - CTTG)]$).

Results:

We analyzed IGFBP-3 methylation in 52 matched tumor/ctDNA samples. Four samples were undetermined. 65% (31 out of 48 determined) were unmethylated in the tumor sample, and 35% (17 out of 48) were methylated. Those results are in accordance with previous cohorts analyzed. When comparing DNA methylation from tumor and ctDNA, 69% (33 out of 48) of the patients presented the same methylation pattern. We found 90% correlation between T/ctDNA in unmethylated tumor samples, but the power to find methylated ctDNA when the tumor DNA was methylated decrease to 29%. It was consider methylated when the % of methylation was >30%

Conclusion:

Firstly, we have found the expected methylation ratios when analyzing DNA from tumor tissue. Secondly, DNA methylation status was determined in 48 out of 52 ctDNA samples (92% sensitivity). Thirdly, the presence of IGFBP-3 methylation in ctDNA from plasma by using qMSP was found in 29% of advanced-stages NSCLC patients harbouring a methylated tumor. Although it is necessary to improve the detection ratio at ctDNA, these results indicate that the epigenetic marker IGFBP-3, able to predict the response to therapy or monitor the progression in NSCLC patients, could be potentially used in liquid biopsy.

4- NANOSYSTEMS FOR CIRCULATING TUMOUR CELLS CULTURE

Carmona-Ulle, Nuria Nano-Oncology Laboratory of the Roche-Chus Joint Unit. Hospital Gil Casares. Travesía da Choupana, 0, 15706 Santiago de Compostela, La Coruña
Abuín-Redondo, C.; de la Fuente, M.; López-López, R. and Dávila-Ibañez, A.B.

Introduction:

The study of Circulating Tumour Cells (CTCs) have gained attention during the last years due to their applicability in Liquid Biopsy related with the detection and progression of cancer metastasis. Some of the potential clinical applications for CTC analysis include monitoring, prognosis and the selection of the most appropriate therapy. However, it is still a challenge to translate these studies to the clinic because of the low efficiency of the isolation methods and the difficulty of CTC culture. Nanotechnology is a tool that offer great advantages in nanomedicine, particularly nanoemulsions (NEs) which can interact with the cellular machinery by entering the cells through different cellular pathways, as opposed to many common small drugs or free substances that simply diffuse according to their solubility.

Objectives:

The main objective is to formulate NEs from phospholipids involved in cell migration, invasion, angiogenesis and cancer cell proliferation to prove their activity as pro-proliferative nanosystems in metastatic breast cancer cell lines. Then, experimental conditions will be modified (NE concentrations, cell lines, incubation times, cell seeding concentration, etc) to mimic the CTCs culture conditions, since the final goal will be to use these NE as proliferative nanosystems to stimulate the proliferation of CTCs. The achievement of CTCs expansion will allow to analyze their molecular pattern to get more information about these cells and achieve more precise diagnostic tools by testing personalized therapies in vitro.

Methods:

The NEs were prepared by the previously reported low-energy emulsification oil in water method and were physically characterized. Mean average size, polydispersion index (PI) and Zeta potential (ZP) were determined by ZetaSizer Nano. The activity of the NEs was evaluated in vitro by cell viability assays (AlamarBlue®). Moreover, uptake of the NEs by breast cancer cell lines was proved using confocal laser scanning microscopy.

Results:

NEs using several phospholipids and fatty acids were successfully formulated in a wide range of sizes. Their chemico-physical characterization was done showing adequate colloidal stability in different media and with time. The cell viability assays to test the activity of the nanoemulsions related to their pro-proliferative activity showed a concentration and cell line dependence. Cellular uptake of the nanoemulsions was confirmed by confocal microscopy images.

Conclusions:

1. We have formulated colloidally stable NEs with different biocompatible components. Additionally, we were able to modify their sizes opening their applicability for different areas of nanomedicine.
2. It was proved that NEs increased the cell viability of breast cancer lines.
3. Preliminary experiments with CTCs isolated from metastatic patients showed that NEs provide advantages in the maintenance of CTCs culture over time.

5- ONCODYNAMICS LIQUID BIOBANKING: A HOLISTIC PERSPECTIVE TOWARDS PREDICTIVE MEDICINE IN METASTATIC CANCER

Authors: Patrícia Corredeira¹, Patrícia Martins², Pedro Andrade², André Mansinho³, Arlindo Ferreira ^{1,3}, Sandra Casimiro¹, Julie Ribot¹, Bruno Silva-Santos¹, Lorena Dieguez⁴, Luis Costa^{1,3}

Affiliation:

1: Instituto de Medicina Molecular – João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa, Av Prof. Egas Moniz, 1649-028 Lisbon

2: Centro de Investigação Clínica, Hospital Santa Maria, Lisbon

3: Oncology Division, Hospital de Santa Maria, Centro Hospitalar Lisboa Norte, Lisbon

4: International Iberian Nanotechnology Laboratory, Braga

Introduction:

Clinical outcomes in oncology result from complex interactions between the tumour, host microenvironment and immune response. Therefore, to fully understand such complexity, it is essential to integrate the dynamics of clonal evolution with each one of these players during disease progression.

Major technological advances in the past years have led to important discoveries about tumor genetics that translate into new targetable therapies. A major breakthrough has been the recent success of immunotherapy. But although is consensual that clonal evolution of the tumor is a major driver of therapy resistance, the interplay between clonal evolution and host immune response was never addressed.

The Oncodynamics Biobank is a prospective collection of liquid biopsies, designed to characterize both the host immune response and tumor clonal evolution during disease progression under therapy, offering new insights of Precision Medicine in cancer.

Objectives:

Oncodynamics Biobank focuses on de novo stage IV breast, prostate, colorectal, melanoma, gastric and glioblastoma cancer patients. It was planned to generate genetic and biological information along with clinical data, and provide a repository of samples obtained from liquid biopsies, including: quantification and characterization of circulating tumor cells (CTCs); DNA and RNA from CTCs; circulating tumor DNA (ctDNA); exosomes; immune profile.

Methodology:

Oncodynamics Biobank includes patients diagnosed at Oncology Department of Hospital de Santa Maria, Lisbon, with de novo stage IV breast, prostate, colorectal, melanoma, gastric and glioblastoma cancer, that referred at the Multidisciplinary meetings. Liquid biopsies (whole blood and urine) are collected at baseline (before starting systemic treatment) and at each tumour objective evaluation (every 12-16 weeks), until two consecutive progression episodes. Electronic Case report forms include demographic and real time clinical data.

CTCs are isolated in a microfluidic device by cell size and morphology (PCT/EP2016/078406, patent pending), and stained in situ with antibodies against pan-cytokeratin (pan-CK to stain Epithelial CTCs), vimentin (to stain mesenchymal CTCs), CD45 (leukocyte common antigen) and DAPI (for nucleus). Each cell is imaged in a fluorescence microscope for further analysis. Extraction of DNA and RNA from CTCs is being optimized.

Immune profile is assessed by Flow Cytometry analysis of different populations, namely: monocytes, myeloid dendritic cells, plasmacytoid dendritic cells, basophils, memory B cells, naïve B cells, monocytic myeloid-derived suppressor cells, polymorphonuclear myeloid-derived suppressor cells, natural killer T cells, effector T cells, regulatory T cells, $\alpha\beta$ T cells and $\gamma\delta$ T cells ($\gamma\delta 1$ and $\gamma\delta 2$).

Serum and plasma is collected and stored for posterior ctDNA and exosome analysis. Urine aliquots and buffy coat are stored at -80°C . Genomic DNA is extracted with NZY Blood gDNA Isolation kit (Nzytech).

Results:

Since April 2017 the Oncodynamics Biobank has included 28 patients from three different pathologies (breast cancer n=15; colorectal cancer n=9; prostate cancer n=4), with baseline and respective follow-up collections. The majority of patients presented with bone metastases (100% of prostate cancer patients and 39% of breast cancer patients), liver metastases (83% of colorectal cancer patients and 8% of breast cancer patients) and lung metastases (66% of colorectal cancer patients and 15% of breast cancer patients). The prospective collection of biological samples includes ~700 aliquots (plasma, serum, buffy coat and urine).

Preliminary analysis suggest that decrease of CTCs during follow-up is accompanied by a significant clinical improvement. Evolution in the immune profile in association with therapy and clinical outcome will be reported.

Conclusion:

The Oncodynamics Biobank will allow us to generate data to characterize both the host immune response and tumour clonal evolution during disease progression under therapy. The enrolment rate and preliminary data show that such a comprehensive collection of data, together with a valuable biological collection, is a major resource for Oncobiology-driven research.

6- EXPLORING LIQUID BIOPSY AND GLYCOSYLATION FOR EARLY DETECTION OF BLADDER CANCER

Authors

Sandra Carvalho¹, Manuel Neves^{1,2}, Catarina Abreu¹, Dylan Ferreira², Luis Lima², Avelino Fraga³, José Alexandre Ferreira², Lorena Diéguez¹, Marta I Oliveira¹

1 INL- International Iberian Nanotechnology Laboratory, Braga, Portugal

2 Portuguese Institute of Oncology, Porto, Portugal

3 Centro Hospitalar do Porto – Hospital de Santo António

Bladder cancer (BC) is the most common malignancy of the urinary tract, being associated with substantial morbidity and mortality. Despite the great advances in diagnosis, prognosis and therapy monitoring, BC has one of the highest recurrence rates and progression from non-muscle to muscle invasive BC commonly leads to metastasis [1]. Liquid biopsy has recently been exploited as a promising non-invasive tool towards the molecular profiling of circulating tumour cells (CTC) and/or circulating molecules in various biological fluids, envisaging early detection and monitoring of cancer [2]. In BC, identification of circulating molecular biomarkers will greatly improve and guide the clinical management of patients.

One emerging classes of tumour biomarkers are cancer-associated glycans, particularly sialyl Tn (STn), expressed at the membrane of glycoproteins or secreted into the bloodstream. In fact, STn expression has been directly associated with cancer progression and metastatic potential of neoplastic cells [3]. Recent studies in BC have shown overexpression of STn antigens in primary tumours, lymph nodes and distant metastasis, which correlated with decreased overall survival [4]. However, the sialylation signature of tumour cells in biological fluids and the precise role of STn glycans in BC dissemination and metastasis remains to be determined.

In this study, size-based microfluidic devices were developed to efficiently (70%) capture and characterize tumour cells from body fluids of BC patients, regardless of the cell phenotype. Unprocessed whole blood from metastatic BC patients was run in the chips, and trapped cells immunostained in situ with fluorescently labelled antibodies to identify specific profiles (Fig. 1). Of note, more than 90% of the isolated CTCs expressed STn glycans (Figure 1), mimicking the primary tumour and the metastasis, in contrast to healthy controls. Concomitantly, bladder wash and urine samples from BC patients and healthy donors were processed in distinct size-based microfluidic devices, which also showed good isolation efficiency (50%). Of note, captured cells revealed different morphologies and a phenotypic diversity. More importantly, expression of STn glycans was also observed in bladder wash/urine samples from BC patients (Figure 2).

Our preliminary findings demonstrate that besides the primary bladder tumour and metastatic lesions, tumour-associated cells in circulation also express STn, confirming its malignant nature, and highlighting its potential as a novel BC biomarker. Further studies are ongoing to elucidate the molecular nature and the clinical relevance of STn positive cells in BC patients.

Additional tables and figures:

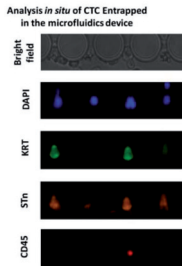


Figure 1. Photomicrograph of cancer cells trapped inside the device from whole blood of metastatic BC patients and stained for DAPI (blue), cytokeratin (green), and STn (orange).

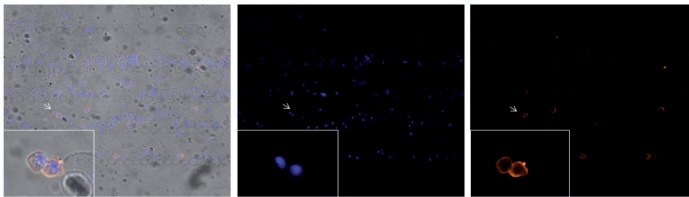


Figure 2. Photomicrograph of cells trapped inside the device from BC patients and stained for DAPI (blue) and STn (orange).

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7- COMPARATIVE GENOMIC HYBRIDIZATION IN PLASMA CIRCULATING TUMOR DNA FOR THE MONITORING OF MELANOMA PATIENTS

Main author:

Yolanda Ruano Domínguez, Instituto de Investigación Hospital 12 de Octubre.

Other authors:

Leyla Blanco, Instituto de Investigación Hospital 12 de Octubre

José Antonio López Martín, Servicio de Oncología, Hospital 12 de Octubre

Rocío Cubo, Instituto de Investigación Hospital 12 de Octubre

Erica Riveiro-Falkenbach, Instituto de Investigación Hospital 12 de Octubre

José Luis Rodríguez Peralto, Servicio de Anatomía Patológica, Hospital 12 de Octubre.

Introduction:

Melanoma is a cutaneous cancer with an increasing worldwide prevalence and high mortality due to unresectable or metastatic stages. The analysis of cell-free circulating tumor DNA (ctDNA) from blood (liquid biopsies) appears to be a promising noninvasive, repeatable, and systemic sampling tool for detecting and monitoring melanoma. Mutations in BRAF are present in approximately 50% of melanoma cases and they are proposed as a useful blood-based biomarker for the clinical monitoring of melanoma patients. However, an equivalent biomarker for wild type BRAF melanoma has not yet been fully developed.

Objectives:

Melanoma shows a recurrent pattern of chromosomal aberrations that are distinct from benign melanocytic lesions and are independent of mutational status profile. Hence, we propose the analysis of DNA copy number changes in ctDNA for monitoring patients with metastatic melanoma using comparative genomic hybridization (CGH).

Methods:

We performed CGH analysis by using the OncoScan kit (ThermoFisher Scientific) in ctDNA from liquid biopsies and paraffin-embedded tissues from solid biopsies at diagnosis of metastatic melanoma patients. The BRAF status was determined by quantitative PCR analysis by Idylla (Biocartis) in plasma and Cobas (Roche) in tissue.

Results:

The CGH analysis in the plasma from a BRAF V600E metastatic melanoma patient revealed chromosome gains at 5p,7q and 21, and chromosome losses at 4q, 6q, 7p, 10, 12p and 14. The chromosome gains at 5p and 7q, as well as chromosome loss at 4q were present in the tissue sample of the patient at diagnosis. The CGH analysis in the plasma from a BRAF wild-type melanoma patient with metastasis in several organs revealed chromosome gains at 3q and 8q and a chromosome lost at 8p. The 8q gain and 8p loss were detected in the tissue sample at diagnosis in addition to another chromosome alterations such as 1q gain, 9p, 10, 11q, 14, 16 and 20 losses and MET amplification at 7q.

Conclusions:

The CGH analysis by using OncoScan kit allowed us to detect chromosome alterations in the plasma from metastatic melanoma patients independently of BRAF status. In addition, most of the genetic aberrations detected in plasma were present in their respective tumor tissues at diagnosis. Therefore, CGH analysis would be a suitable technique for the analysis of ctDNA and monitoring metastatic melanoma patients.

8- DEVELOPMENT OF NEW, FAST AND ECONOMIC PROTOCOLS FOR THE ELIMINATION OF HIGH ABUNDANCE PROTEINS IN PLASMA SAMPLES

María del Pilar Chantada-Vázquez 1*, Ceres Fernández-Rozadilla2, MV Álvarez-Sánchez3, Ángel Carracedo-Álvarez4, Susana B. Bravo5, María García-Vence5, Cristina Núñez1, Sergio Vázquez Estévez6, Marta Covela Rúa6, Natalia Fernández Núñez6

1Laboratory of Nanoproteomic, Research Unit, Hospital Universitario Lucus Augusti (HULA), Servizo Galego de Saúde (SERGAS), 27002 Lugo (Spain)

2 Cancer Genomics and Transcriptomics Group. Fundación Pública Galega de Medicina Xenómica. Instituto de Investigación de Santiago (IDIS). Choupana s/n, 15706 Santiago de Compostela (Spain)

3 Digestive System Service. Hospital University Complex of Pontevedra. EOXI Pontevedra e o Salnés. Institute of Health Research Galicia Sur (IISGS)

4Fundación Pública Galega de Medicina Xenómica. Instituto de Investigación de Santiago (IDIS). Choupana s/n, 15706 Santiago de Compostela (Spain)

5 Proteomic Service. Instituto de Investigación Sanitaria de Santiago (IDIS). Choupana s/n, 15706 Santiago de Compostela (Spain)

6Oncology Division, Hospital Universitario Lucus Augusti (HULA), Servizo Galego de Saúde (SERGAS), 27002 Lugo (Spain)

Introduction:

The analysis of human body fluids is one of the most important approaches to the diagnosis of disease and following therapeutic interventions. Human body fluids carry information about the status of the organism that may help in the recognition of physiological imbalances when overt pathological symptoms are not yet present.

Human plasma proteins originate from a variety of tissue and blood cells as a result of secretion or leakage. Numerous biomedical studies have demonstrated that plasma protein levels reflect human physiological or pathological states and can be used for disease diagnosis and prognosis. Sample preparation and handling is a critical for plasma proteome analysis. Other critical issue is the complexity of the proteome. Plasma contains a huge number of proteins differing by the extraordinary dynamic range of at least 9-10 orders of magnitude. How to globally quantify the proteins in free, bound, or modified forms remains a critical challenge.

Some of the plasma highly abundant proteins including: albumins, immunoglobulins (IgG), alpha-1-antitrypsin (A1AT), fibrinogen, and haptoglobin (HG). Depletion of these highly abundant proteins is often desired prior to proteome analysis. Immunodepletion methods

remove a good/variable portion, but not all of the highly abundant proteins; however, more problematic is that these methods may also remove other proteins by "nonspecific" binding. A possible approach to address this problem is to disrupt the union between a low molecular weight (LMW) proteins and carrier proteins as albumin/IgG.

For instance, denaturing conditions by adding acetonitrile (ACN) may disrupt this union, result in proteins detected increased when compared to native conditions.

In addition, rather than depletion of highly abundant proteins, it would be promising to develop methods such as functionalized nanoparticles to enrich the low abundant proteins or reduce the protein concentration range.

Objectives:

The objectives of this work are to develop a rapid, cheap and effective protocol for the elimination of the majority proteins of plasma samples. In our case we development a centrifugal strategy using Amicon® with different NMWL to enrichment the serum samples in low abundant proteins. Thus, this methodology can be implemented in proteomic platforms which the number of plasma samples is high in recent years.

ABSTRACTS

Methodology:

Two different protocols for the removal of high abundance proteins have been tested. One of them is to pass the plasma sample through an affinity column using the manufacture protocol.

The other protocol is to mix the sample with a special denaturing buffer (200mM NaCl / 10% AcN in 200 nM AmBic) and pass the sample through Amicon®(Millipore) with decreased NMWL. In both protocols the proteins are identified with a Triple-TOF 6600 (AbSciex).

Results:

With both protocols the number of proteins identified with 1% of error is very high. It has even been possible to compare the different fractions obtained in the case of the size separation method with Amicons®. Both are quickly methods, have a relative low cost and are easy to implement in proteomic platforms in which the entry of complex biological samples such as serum increases every day.

Findings

Two valid protocols have been obtained, which provide a good pretreatment of plasma samples in order to make a proteomic analysis and give good results.

9- CTDNA LEVELS BY DDPCR IN NON-SMALL-CELL LUNG CANCER: IMPLEMENTATION IN CLINICAL PRACTICE.

Main author:

Aitor Azkárate Martínez.

Oncología Médica. Hospital Universitari Son Espases.

Other authors:

Mónica Enver. Advanced Therapies and Biomarkers in Clinical Oncology Group. IdISBa.

Margarida Mut. Oncología Médica. Hospital Universitari Son Espases.

Esther Martínez-Font. Advanced Therapies and Biomarkers in Clinical Oncology Group. IdISBa.

Magda Cordeiro. Oncología Médica. Hospital Universitari Son Espases.

Tutor:

Antonia Obrador-Hevia. Advanced Therapies and Biomarkers in Clinical Oncology Group. IdIS-Ba.

Josefa Terrasa Pons. Oncología Médica. Hospital Universitari Son Espases.

Introduction:

Lung cancer is the second most frequent tumor and the leading cause of death by cancer. About 15-20% of non-small-cell lung cancer (NSCLC) presents with driver mutation in the EGFR domain. At progressive disease, about 60% of these patients will harbour T790M mutation. In the last years, liquid biopsy has emerged as a promising new tool to complement tumor biopsies data. Nowadays digital PCR may overcome real-time PCR which is the gold standard in this setting. Liquid biopsy has shown to be useful in early diagnosis, monitoring responses, early detection of progressive disease and as prognostic and predictive tool.

Objectives:

In this first analysis of our data we aim to calculate how many days in advance progressive disease can be detected by droplet digital PCR (ddPCR) compared to conventional follow-up by CT scan. We also wanted to prove whether or not tumor burden is related to the amount of circulating tumor DNA (ctDNA) detected by ddPCR; and how a greater amount of T790M cop-ies/mL may have influence on progression free survival (PFS) during the first line treatment.

ABSTRACTS

Methods:

Blood samples were collected from 18 patients diagnosed with advanced or metastatic NSCLC patients who carried an activating EGFR mutation treated with first or second generation TKI and T790M mutation detection was carried out as described below. Blood samples were ob-tained every 3 months.

Plasma cfDNA was extracted with the Qiagen Circulating Nucleic Acids kit and quantified by QuantiFluor® dsDNA System. Finally, the mutational study of EGFR was carried out by digital droplet PCR (ddPCR) with the QX200 Droplet Digital PCR System with specific probes and pri-mers. Statistical analysis was performed with IBM SPSS Statistics 22 software.

CT-scan was performed according to the criteria of the attending physician. The clinical re-sponse was evaluated according to RECIST criteria v1.1.

Results:

Data of serial blood samples from 3 out of 18 patients was fully available at the time of this study and ctDNA was assessed to evaluate the advance in diagnosis of progressive disease by ddPCR ddPCR in comparison to imaging tests. Using ddPCR, we could detect progressive disease from 71 to 200 days before imaging (image 1). We detected a trend that correlates tumour burden with ctDNA levels by ddPCR with a R2 of 0.565 (p=0.015) (image 2). Finally, patients harbouring T790M mutation had a trend to have worse PFS comparing with those who didn't express this mutation (image 3), although these findings weren't statistically significant.

Conclusions:

In this first analysis of our data, we could detect progressive disease by ddPCR in advance of imaging tests in the same way other works have done recently (Provencio et al) and correlated with tumor burden. Those findings set the bases to question whether T790M positive patients by dPCR should start second line treatment or should wait until progressive disease by CT-scan is detected. Clinical trials should be designed to answer this question.

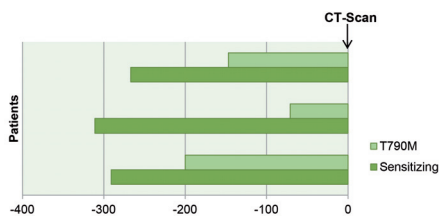


Image 1. Number of days since the earliest identification of sensitizing and T790M mutations in blood and assessment of disease progression by CT-scan.

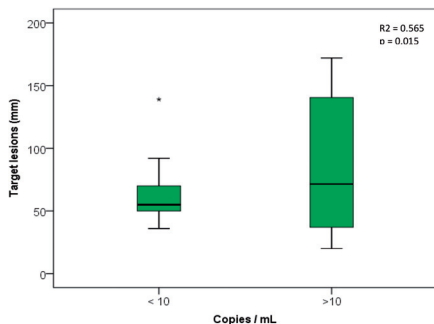


Image 2. Correlation between tumor burden and copies/mL of ctDNA by ddPCR.

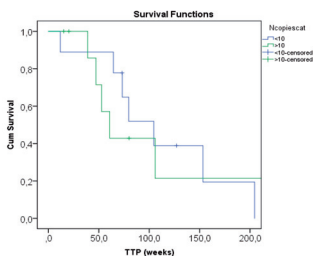


Image 3. PFS by number of copies/mL.

10- CIRCULATING TUMOR (CT)DNA CAPTURES INPATIENT HETEROGENEITY IN METASTATIC COLORECTAL (MCRC) PATIENTS PROGRESSING TO FOLFIRI+PANITUMUMAB

J. Vidal1, J.M. Viéitez2, D. Paez3, C. Santos4, D. Azuara5, A. Dalmases6, B. Bellosillo6, R. Salazar7, E. Aranda Aguilar8, C. Montagut1 en representación del Grupo de Tratamiento de los Tumores Digestivos (TTD)

1. Oncología Médica, Hospital del Mar, Barcelona
2. Oncología Médica, Hospital Universitario Central de Asturias, Oviedo
3. Oncología Médica, Hospital de la Santa Creu i Sant Pau, Barcelona
4. Oncología Médica, Institut Català d'Oncologia Hospital Duran i Reynals, Barcelona
5. Laboratorio Investigación Traslacional, Institut Català d'Oncologia Hospital Duran i Reynals-IDIBELL, Barcelona
6. Molecular Biology Laboratory H. del Mar, Barcelona. Spain
7. Oncología Médica, Institut Català d'Oncologia Hospital Duran i Reynals-IDIBELL., CIBERONC, Barcelona
8. Oncología Médica, University Hospital Reina Sofia. CIBERONC Instituto de Salud Carlos III, Cordoba

Background:

CRC cells evade EGFR blockade by several mechanisms of acquired resistance, mainly mutations in RAS, EGFR ECD, HER2 and MET. ctDNA is shed into the bloodstream by tumor cells and can be effectively used to track tumor heterogeneity and to evaluate the acquisition of molecular alterations at tumor progression.

Objective:

to analyze the acquisition of gene mutations in plasma ctDNA from patients with mCRC progressing to antiEGFR treatment and to correlate with laterality of primary tumor.

Methods:

We included mCRC patients treated within a phase II study: FOLFIRI + panitumumab in irinotecan-refractory mCRC. Plasma samples were collected at baseline and at the end of treatment and ctDNA was analyzed with the OncoPrint colon ctDNA Assay. The resulting library was sequenced in the Ion PGM NGS System and analysed with the Torrent Suite Software. The detectable cutoff mutant allele fraction (MAF) was 0.1%. Subclonal mutations were defined as mutations with MAF \leq 20% of the greatest somatic MAF in the sample.

Results:

Clinical characteristics of 16 patients were 69% male; median age 61.5 years; 75% left vs 25% right colon. ctDNA from all 16 patients was analyzed at progression. At least one mutation was detected in 94% of patients (15/16); median mutations per sample was 2.5 (range 1-13). The frequency of detected mutations was: 13 TP53, 3 APC, 1 CTNNB1, 15 KRAS, 8 NRAS, 7 EGFR, 4 BRAF, 4 PIK3CA, 4 MAP2K1, 1 GNAS, 1 SMAD4. While TP53, APC, PIK3CA and BRAF were most likely to be clonal, EGFR, RAS (KRAS+NRAS) were generally subclonal and all MAP2K1 and SMAD4 mutations were subclonal. All EGFR ECD mutations emerged in the left colon and co-existed with RAS mutations plus at least one additional acquired mutation (median 6, range 3-11). In the right colon, acquired mutations were detected at a low frequency (median 2.5; range 1-6). RAS/BRAF mutations emerged in 100% and 66% of right and left colon respectively being always clonal in right colon whereas only in 50% of cases were clonal in left colon. In 10 out of 16 patients plasma baseline was available. At least one mutation was detected in 80% of patients (8/10); median mutations per sample was lower compared with plasma at progression: 1.8 (range 1-4). All APC and TP53 mutations were present baseline. 12/15 (80%) of KRAS mutations and all NRAS, MAP2K1 and EGFR mutations were not detected baseline, and acquired during treatment. As a novelty, the SMAD4 mutation was not detected baseline and emerged after treatment with a 5.25% MAF.

Best response was: PR 8 patients, SD 6 patients and PD 2. In both patients with PD all mutations detected in plasma at progression were already detected in pre-treatment sample, with no new acquired mutations detected at progression (TP53 p.R175H + KRAS p.A146T and TP53 p.[V274F.;R273L;G245D] + PIK3CA p.H1047R respectively).

Conclusions:

ctDNA analysis captured inpatient heterogeneity which developed as a result of EGFR inhibition. All EGFR ECD mutations emerged in the left colon and always co-existed with several other mechanisms of acquired resistance, reflecting genomic complexity.

11- ANALYSIS OF SINGLE CIRCULATING TUMOR CELLS (CTCS) TO INFER PHENOTYPE AND GENOME CHANGES IN RESPONSE TO THERAPEUTIC PRESSURES IN BILIARY TRACT CANCER

C. Reduzzi¹, L. Celio², M. Vismara¹, M. Silvestri¹, R. Motta¹, P. Miodini¹, A. Martinetti², F. De Braud², M.G. Daidone¹ and V. Cappelletti¹.

¹Department of Experimental Oncology and Molecular Medicine,

²Department of Medical Oncology,

Fondazione IRCCS Istituto Nazionale dei Tumori, Milano, Italy.

Introduction:

Biliary tract cancer (BTC) is a rare disease with poor prognosis and limited therapeutic options. Access to tissue biopsies for genotype analysis is often impossible hampering this way the use of targeted therapies. Circulating tumor cells (CTCs) could function as surrogate of tumor material and could also allow monitoring the disease in response to treatment. However, when using conventional methods based on the expression of epithelial markers, CTC are detected only in a low proportion of BTC patients.

Objectives:

Being aware of the limitations in conventional CTC detection, we aimed at the development of a novel and unbiased CTC detection and characterization approach, allowing the analysis of different subpopulation of CTC at single-cell level. The specific objective was to obtain information regarding i) presence, ii) peculiar phenotype and iii) molecular profile of CTCs which could inform on treatment options thus giving prognostic and predictive advice.

Methodology:

Ten mL of peripheral whole blood were processed for CTC enrichment by the Parsortix which selects cells based on size and deformability. CTCs were then identified and recovered with the DEPArray by positive selection for epithelial markers (eCTCs) and negative selection based on lack of epithelial and leukocyte markers (non conventional CTCs, ncCTC). After whole genome amplification, single CTCs were used for molecular characterization (copy-number alterations (CNA) and mutations) at single-cell level.

Results:

Forty-one blood samples were longitudinally collected from 16 patients and analyzed. Overall, 36/41 samples were CTC-positive, with ncCTCs being more frequent than eCTCs (71% vs. 39% of samples respectively). At baseline all patients presented at least 1 CTC, with the two CTC populations being equally represented. During treatment we observed a switch towards the non-conventional phenotype and a decrease in global CTC positivity (100% at baseline vs 80% during treatment), which could be linked to resistance or response to therapy, respectively. Overall, CTC-status correlated with clinical outcome of patients. From a biological point of view, molecular characterization of different CTCs from the same samples showed a high intra-patient heterogeneity both at CNA and mutation level. CNA data were used both to estimate chromosomal instability of CTCs in relation to response to treatment and to identify recurring alterations across patients and alterations arising during disease progression in single patients.

Findings:

The developed protocol allows the isolation of CTC in 100% of patients and their molecular characterization at single-cell level, supporting their value as valuable surrogate when tissue biopsies are not available. CTC phenotype seems to mirror (sometimes anticipate) clinical outcome of patients. A combined characterization at phenotype and genotype (mutations, CNA and chromosomal instability) level can be used to monitor the emergence of CTC subclones with peculiar phenotypic-genotypic patterns, related to response/resistance to treatment thus improving clinical management in BTC patients.

12- FAST AND EFFICIENT ISOLATION OF CIRCULATING TUMOR CELLS FROM UNPROCESSED WHOLE BLOOD USING A MICROFLUIDIC CELL FILTER

Thais Pereira-Veiga¹, Silvina Ribeiro-Samy², Marta I Oliveira², Laura Muinelos-Romay^{3,4}, Rafael López-López^{1,3,4}, Lorena Diéguez² and Clotilde Costa^{1,3}

1. Roche-Chus Joint Unit. University Hospital of Santiago. Travesía da Choupana s/n 15706 Santiago de Compostela, Spain.
2. INL - International Iberian Nanotechnology Laboratory, Av. Mestre José Veiga s/n, 4715-330 Braga, Portugal.
3. CIBERONC, Centro de Investigación Biomédica en Red Cáncer, Madrid, Spain.
4. Liquid Biopsy Analysis Unit, Oncomet, Health Research Institute of Santiago (IDIS), Complejo Hospitalario Universitario de Santiago de Compostela (SERGAS); Trav. Choupana s/n, Santiago de Compostela 15706, Spain.

Introduction:

Metastatic disease is directly responsible for most of cancer-related deaths worldwide. Circulating tumour cells (CTCs) shedding from the primary tumour have the ability to invade other organs, causing metastasis. Therefore, the study of CTCs might provide continuous and real-time valuable information for the clinical management of cancer patients¹.

However, these cancer cells are extremely rare – presenting ratios as low as 1 to 10 CTCs per a billion blood cells –, which makes their isolation a very hard task². Unravelling the phenotypic and molecular profile of CTCs can provide key information about the biology of tumour cells and highly contribute to personalized therapy. Isolation and characterization of these exceptionally scarce entities is still a challenge, mainly due to CTCs heterogeneity.

Objective:

To overcome this limitation, the CTC+ chip, a microfluidic cell filter for label-free isolation of CTCs based on size and deformability, was developed and its performance was compared to the CellSearch® system.

Methods:

We have developed and fabricated, by Silicon Deep Reactive Ion Etching, the CTC+ chip: a size-based rare cell capture device, which comprises several isolation areas containing size exclusion filters.

Efficiency of the device was measured with spiked samples using SW480 colon cancer cell line. Blood samples from nine advanced colorectal cancer patients were analysed in parallel with the CTC+ chip and with CellSearch as the gold standard technique. Following isolation, cells retained in the CTC+ chip were immunostained with fluorescently labelled antibodies to identify specific phenotypes and molecular characterization was performed by droplet digital PCR (ddPCR).

Results:

Spiking experiments with cultured tumour cells run through the CTC+ chip demonstrated a high capture efficiency, 70% in average.

The CTC+ chip was able to rapidly (i.e., less than one hour) process whole blood samples from a set of metastatic colorectal cancer patients and capture more CTCs than the current gold standard CellSearch® in all individuals analysed with high efficiency and purity. To check the tumoral origin of the CTCs we performed ddPCR, with APC mutations being detected in CTC+ chip-isolated CTCs, confirming the versatility of the technology for downstream applications.

Since the isolation efficiency of the CTC+ chip is higher, and considering the established cut off for bad prognosis in colorectal cancer used by the CellSearch® technology (≥ 3 CTCs/7.5 ml of whole blood), by enumerating the CTCs isolated in the CTC+ chip, it was possible to stratify patients with different prognosis.

Conclusion:

The CTC+ chip allows the fast and efficient isolation of unlabelled CTCs. Furthermore, after CTCs isolation with this device, downstream analysis can be performed to achieve prognosis utility.

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13- CLINICAL UTILITY OF LIQUID BIOPSY IN ADVANCED LUNG CANCER.

Main author:

Name: Dra. Ana Reguera Arias

Servicio de Anatomía Patológica, Complejo Hospitalario Universitario A Coruña (CHUAC)

Other authors:

Hermida Romero T, Escalante Pérez M, López Solache L, Álvarez Martínez M, Antón Aparicio L*, Concha López A.

Servicio de Anatomía Patológica

*Servicio de Oncología Médica

Complejo Hospitalario Universitario A Coruña (CHUAC)

Tutor:

Dr. Ángel Concha López, Jefe de Servicio de Anatomía Patológica, Complejo Hospitalario Universitario. A Coruña (CHUAC)

Introduction:

Liquid biopsy is a non-invasive technique that allows circulating biomarkers to be identified in peripheral blood and recognize the genetic profile of tumours and metastasis in order to offer the patient targeted therapy.

It also allows to monitorize treatment response and to detect possible resistance to it.

Objective:

To determine through liquid biopsy the T790M resistance mutation to tyrosine kinase inhibitors in lung cancer patients in advanced stages (III- IV) carrying epidermal growth factor receptor (EGFR) mutations.

Methodology:

The patient cohort was formed by 53 patients with an average age of 61 years old (38-86), 32 women and 21 males. Out of the 53, 35 presented the EGFR gene mutation in the tissue, determined by a commercial Real-Time PCR kit, being the most common mutation EGFR Exon 19 deletion (57% of the total).

A liquid biopsy has been carried out in those patients with a clinical or radiological progression and with a clinical suspicion to tyrosine kinase inhibitors resistance or with not enough tissue available to determine the mutational profile. The liquid biopsy was carried out extracting of 10cc of plasma to determine the circulating ctDNA.

Results:

Out of the 35 patients with an EGFR gene mutation, it has been determined that the T790M resistance mutation was present in 14 cases. 11 of them were detected in the first blood extraction and the other 3 in the second extraction, that took place a few days later. In addition and due to clinical insistence, there were 3 other cases in which the T790M was eventually determined through a surgical biopsy after several negative results of the liquid biopsy.

Out of the 18 remaining cases, in which the mutational profile was unknown, the EGFR gene mutation was determined in three of them after the first liquid biopsy.

Conclusions:

Liquid biopsy is an extremely helpful tool to determine the EGFR T790M secondary mutation, especially in cases in which the obtention of a tissue sample is difficult, although surgical biopsies are sometimes needed when the clinical and radiological results indicate a progression of the illness

14- MOLECULAR CHARACTERIZATION OF CTCs FROM PATIENTS WITH ADVANCED NSCLC IS A VALUABLE STRATEGY TO PREDICT FIRST LINE CHEMOTHERAPY RESPONSE

Silvia Calabuig-Fariñas^{1,2,3,#}, Laura Muínelo Romay^{2,4,#}, Alicia Abalo^{2,4}, Héctor Amado Labrador¹, Ramón Lago^{2,4}, Marais Mosqueda¹, Carmela Rodríguez^{4,5}, Eva Escorihuela², Jorge García^{4,5}, Eloisa Jantus-Lewintre^{1,2,6}, Rafael López^{2,4,5*}, Carlos Camps^{2,7,8,*}

1 Molecular Oncology Laboratory, Fundación Investigación, Hospital General Universitario de Valencia, Valencia, Spain

2 CIBERONC

3 Department of Pathology, Universitat de València, Valencia, Spain

4 Liquid Biopsy Analysis Unit, Oncomet, Health Research Institute of Santiago (IDIS), Complejo Hospitalario Universitario de Santiago de Compostela (SERGAS); Santiago de Compostela, Spain.

5 Medical Oncology Department, Health Research Institute of Santiago (IDIS), Complejo Hospitalario Universitario de Santiago de Compostela (SERGAS); Santiago de Compostela, Spain.

6 Department of Biotechnology, Universitat Politècnica de València, Valencia, Spain

7 Department of Medical Oncology, Hospital General Universitario de Valencia, Valencia, Spain

8 Department of Medicine, Universitat de València, Valencia, Spain # co-first authors *equal contribution

Introduction:

Non-small cell lung carcinoma (NSCLC) represents the 85% of lung cancer cases. At diagnosis, approximately 70% of patients present advanced disease. Although targeted therapies have improved their prognosis, there is still a clear need of more specific tumour biomarkers that allow a better treatment selection and monitoring. Circulating Tumour Cells (CTCs) represent a principal component of the dissemination process. Importantly, CTCs evaluation allows readily “liquid biopsy” meanwhile access to tissue specimens is often insufficient and fails to reflect tumour dynamics, heterogeneity or even drug sensitivity.

Objective:

The present study was conducted to validate the characterization CTCs from patients with advanced NSCLC as a valuable tool for anticipating the disease evolution and the therapy response. Materials and Methods: 78 patients with advanced NSCLC were enrolled in the study at Hospital General Universitario de Valencia and Complejo Hospitalario de Santiago de Compostela. EpCAM positive CTCs from these patients were isolated and analysed using both CellSearch technology and a qRT-PCR based approach at baseline and within treatment. For the gene expression analysis we selected a panel of genes with a relevant role for NSCLC aggressiveness and the resistance to platinum-based treatments.

Results:

From all patients included in the study 46% were positive for CTCs using CellSearch system at baseline, showing only 12% of patients ≥ 5 CTCs. In accordance with previous studies in NSCLC, patients with ≥ 5 CTCs showed poor PFS (3,2 vs 10,5 months, $p=0,015$) and OS (3,3 vs 13,9 months, $p<0,001$) rates compared with those patients with CTCs <5 . In addition patients with high CTCs levels during treatment also had a more aggressive disease evolution in terms of PFS and OS. Importantly, from the analyzed gene panel in the CTC-enriched fraction we found low ERCC1 levels as a powerful marker to discriminate patients with a good response to chemotherapy from those that progressed during treatment administration (11,6 vs 4,86 months, respectively, $p=0,001$). In addition, patients with high ERCC1 expression in the CTCs population at baseline showed also lower OS (7,1 vs 14,6 months respectively, $p<0,001$).

Conclusions:

We demonstrated that gene expression analyses in CTCs represents an adequate strategy to identify biomarkers with prognostic value and potential applicability in the management of NSCLC patients.

15- LIQUID BIOPSIES ALLOW EARLY DETECTION IN EGFR RESISTANCE

Silvia Calabuig-Fariñas^{1,2,3}, Eloisa Jantus-Lewintre^{1,2,4}, Amaya Fernández Díaz⁵, Albert Junquero⁵, Ana Blasco^{2,5}, Cristóbal Aguilar-Gallardo^{1,2}, Eva Escorihuela^{1,2}, Marais Mosqueda¹, Francisco Aparisi⁵, Carlos Camps^{1,2,5,6}

1Molecular Oncology Laboratory, Fundación Investigación Hospital General Universitario de Valencia; 2Centro de Investigación Biomédica en Red de Cáncer, 2Department of Pathology, Universitat de València; 4Department of Biotechnology, Universidad Politécnica de Valencia; 5Department of Medical Oncology, Consorcio Hospital General Universitario de Valencia; 6 Department of Medicine, Universitat de València.

Introduction:

Liquid biopsies appear a reliable alternative to conventional biopsies that can provide both useful precise molecular data to improve the clinical management of lung cancer patients and invasive way to monitor tumor behaviour. These advances are supported by important biotechnological developments in the fields of circulating tumor cells (CTCs) and circulating tumor DNA (ctDNA). An analysis of CTCs and ctDNA may be useful in treatment selection, for response monitoring, and for studying resistance mechanisms.

Methods:

Review and report a case of a female patient with lung adenocarcinoma EGFR exon 19 deletion with non-invasive mutational monitoring. Blood samples were collected at diagnosis and repeated sampling was performed during follow-up and at progression in Streck cell-free DNA BCT® or EDTA tubes. Circulating cell-free DNA genotyping was performed with the OncoBEAM™ EGFR kit. The results were compared to those obtained from the DNA extracted from tissue at diagnosis and at progression.

Results:

Case report of a 73 year old female nonsmoker patient, with a clinical 4-month evolution of cough, dyspnea and weight loss. A thoraco-abdominal CT scan showed a 65 mm mass in the right upper lobe, pleural effusion, bilateral multiple pulmonary micronodules and metastatic bone lesions (iliac, sacro, lumbar spine). Bronchofibroscopy and a histopathological exam showed adenocarcinoma, with an EGFR mutation (exon 19 deletion). Lung adenocarcinoma stage IV (EGFR exon 19 deletion) diagnosis was made and the patient started treatment with erlotinib 150 mg/day. The EGFR mutation was confirmed on cfDNA at diagnosis, and monitoring was performed with early undetectable blood mutations after EGFR-TKI predicted a good clinical response. Subsequent imaging revealed that the lesions in her chest and bone had stabilized or reduced in size in the following for next months. At 10 months, of the analyzed blood-based EGFR mutation status assessment revealed a T790M resistance mutation, however therapy with erlotinib continued. Thoracic and bone progression was observed at 17 months, and new tissue biopsy positive results for the T790M mutation were confirmed. The patient was started on osimertinib and remained stable for 8 months until progression was exhibited in TACs. According to a liquid biopsy, exon 19 deletion reappeared, which suggested that osimertinib had failed. The patient died 3 months later from disease complications. When we compare our methodology with the conventional approach to detect mutations in liquid biopsy, we confirm anticipated detections. Our results confirm that early detected mutations can provide relevant outcome information.

Conclusions:

The purpose of this case report is to confirm the clinical utility of ctDNA to detect epidermal growth factor receptor mutations by BEAMING methodology, which is feasible and effective monitoring treatment response to EGFR TKIs, and to detect the underlying mechanism of resistance. This work was supported by Astra Zeneca (ISSRES0110), the RD12/0036/0025 ISCIII and grants from the Fondo Europeo de Desarrollo Regional (FEDER).. Keywords: Circulating free DNA, EGFR mutation, NSCLC



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