FINAL REPORT ON THE MEETING OF EXTERNAL EXPERTS FOR THE PREPARATION OF A DOCUMENT CONSENSUS on development priorities IN LIQUID BIOPSY:

EXAMINATION OF THE EXTERNAL EXPERTS OF THE PROPOSAL OF THE LOCAL GROUP OF LIQUID BIOPSY - GRANADA

INTRODUCTION

The Andalusian Health Service (SAS), within the framework of the innovation policies developed by the Ministry of Health of the Andalusian Government, intends to develop a corporate initiative for Public Procurement of Innovation in the field of "Diagnosis and Precision Treatment in Infectious Diseases and Cancer".

This initiative is supported by an agreement signed by the SAS together with the Ministry of Knowledge, Innovation and Universities, in the context of the Program for the Promotion of Innovation from Demand in the Health Sector (FID-Health), which aims to develop innovative solutions from the industrial sector based on the needs and priorities of the public health system.
One of the lines of this initiative pursues the development of non-invasive solutions for the diagnosis, monitoring and control of cancer patients, through the detection of peripheral blood biomarkers: liquid biopsy (LB).

Defined the needs in a generic way as biomarkers (Circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), exosomes, etc. for breast, colon, lung and prostate cancer, it has been estimated as relevant by part of the promoters of the initiative (General Secretary of R+D+i of the Andalusian Ministry of Health, Direction Management of the SAS, Direction of the Oncology Plan and the Local Group of Research and Clinical Development constituted by the UGC

of Oncology of the Hospitals of Granada and the GENyO Center) holding a Consensus Meeting of experts, of international scope and external to the SAS, on development priorities according to the state of the art and the needs identified by the care system.

To this end, they were summoned and met in Granada at the headquarters of GENyO on May 14, 2018, the panel of external experts (experts listed in ANNEX I, and meeting program in ANNEX III) and met to discuss the methodological innovation that the model of the CPI projects represents as engine of a research of solutions in the sustainable and dynamic bio-sanitary area. This consideration is, above all, the most important in terms of the pioneering nature of this initiative, which requires all our efforts to achieve the desired objectives. On the other hand, it is an extraordinarily important aspect the close cooperation with the industry so that a new way of working is going to be started, through which the bio-sanitary professionals can be the necessary co-operators to promote and test innovations, contrasting with the usual way until now, of having to accept the industry's offer.

A. DOCUMENT DEVELOPMENT

For the correct development of the consensus, a questionnaire was previously sent to each expert (ANNEX II) with different sections that address different clinical and technical aspects for the implementation of the LB in public hospitals. The questionnaire served as the basis for the development of the meeting.

The document addressed the main types of Liquid Biopsies.

Peripheral blood offers several sources of cancer-derived material, such as circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), platelets, or microRNAS (miRNAS).

Circulating tumor DNA (ctDNA): Cell-free DNA (cfDNA) refers to short fragments of acellular nucleic acids detectable in body fluids, including blood, and which is involved in various physiological and pathological phenomena such as immunity, coagulation, aging and cancer. **In patients with cancer**, the haematogenous cfDNA fraction originates from tumors and is called **circulating tumor DNA (ctDNA),** and may have the same mutations and genetic alterations as those of the primary tumor.

Circulating tumor cells (CTCs): Circulating tumor cells were first found in the blood under the microscope almost 150 years ago. They are tumor cells from the tumor that are poured into the bloodstream and can circulate around the body. As a result, these cells can serve as precursors of metastasis.

MicroRNAs: MicroRNAs (miRNAs) are a class of small endogenous RNA molecules (synthesized in the cell itself) with a length between 19 and 22 nucleotides, single-stranded and non-coding (do not contain information that gives rise to proteins) that act as posttranscriptional regulators, mainly inhibiting gene expression.

The importance of miRNAs in cancer biology by controlling the expression of their target mRNAs to facilitate tumor growth, invasion, angiogenesis and immunological evasion has been demonstrated. The tumor miRNA profiles can define relevant subtypes, patient survival and response to treatment.

Tumor Educated Platelets (TEPs). TEPs are considered as local and systemic markers for the presence of cancer, since they are carriers of genetic information from the tumor, such as RNA biomolecules derived from extracellular vesicles, proteins or RNA variants.

After discussing the different aspects contained in the questionnaire, a consensus is reached in the following terms:

B. TYPE OF DETERMINATION, TUMOR and PRELIMINARY TECHNICAL DETAILS

1. ***In reference to circulating free DNA*** for breast, colon and lung cancer

It was concluded that:

- The sample to analyze should be the plasma fraction starting from whole blood (Plasma).
- That the analysis of this LB should be applied to patients with colon, breast and lung tumors.
- That the mutations and biomarkers to be analyzed should focus on:
    - Case of LUNG cancer: detection of T790M EGFR, ALK, BRAF ROS1, RET, NTRK, ERB2 and common EGFR mutations in exon 18 and 19 should be included.
    - Case of COLON cancer: detection of KRAS, NRAS, BRAF and ERB2 should be included.
    - Case of BREAST cancer: detection of EGFR and ERB2 should be included.
- It is recommended to incorporate in every determination the frequency in which each mutation occurs.

2. ***In reference to circulating tumor cells (CTCs)***

- It is recommended only for breast cancer
- The use of specific preservation tubes is recommended.
- The samples should be analized within 48h after their extraction.
- Phenotypic analysis on CTCs of the erb2 and ESR1 markers is recommended.

3. ***In referencie to miRNA and PLATELETS***

- Recommendation of the experts is not to include them in the program as there is still not enough clinical evidence for their implementation.

C. HIGHLIGHTED TECHNICAL ASPECTS

- About ctDNA analyses platforms: A threshold sensitivity of 0.05% should be required.
- Origin of the DNA must be identified (tumor vs. non-tumor).
- Final report to be sent to the specialist, the result of the most important mutation should be included, as well as that of the less frequent mutations.
- Response time should not exceed 1 week.
- Cost of the process must be 30% or less of the average cost of determination in the market (determined by the competent body).
- Clinical report has to be adequate to the needs of clinical information (providing the most relevant, scientifically evidenced). This report should incorporate pathological tumor data when available.
- About the platform: it must incorporate multiplexing capacity.
- About the platform: it must include mutations, amplifications and rearrangements determinations.
- About the platform: it must incorporate the ability to analyze multiple samples simultaneously.

D. APLICABILITY INTENDED WITHIN THE PROJECT

- Project procedures should have prognostic and predictive applicability regarding treatment. For this same reason it should be used in the follow-up of patients.
- Determinations will be requested by the specialists who depend for their clinical decision on the information of this technology (oncologists basically).

E. MARGINAL APPLICATIONS

- It is recommended to know the mutational spectrum of patients in Andalusia.
- It is recommended to design documents for the use by protocol determinations.
- It is recommended to also design a mandatory form to request determinations by physicians.
- It is recommended to renew, to automatically update and to auto-feed. Networking
- It is recommended to consider the procedure as a tool allowing to design studies on the cost / benefit binomial.
- Replacement of kits by companies is recommended when the expiration period is very short.

In Granada on May 16th, 2018

ANNEX I. Expert Panel

Masimo Cristofanilli, MD, FACP, (Northwestern University | Northwestern Medicine CHICAGO).

Medical oncologist. Pioneer in precision medicine within the molecular context and liquid biopsy. He is currently associate director of the translational research program at the Lurie Cancer Center.

Expert in translational research and treatment of patients with inflammatory breast cancer, Massimo Cristofanilli, MD, is a professor of Medicine in the Division of Hematology / Oncology at the Feinberg School of Medicine at Northwestern University.

Cristofanilli has led the development of new diagnostic and prognostic markers in primary and metastatic breast cancer. His research focuses on the advancement of a patient-centered and biologically oriented cancer care model that combines molecular diagnostic technologies based on sophisticated blood and tissue and innovative treatments. G. Morris Dorrance Jr. Endowed Chair in Medical Oncology.

Christian Rolfo. Amberes (Bélgica)

Since October 2012 he is a senior member of the Department of Oncology as an associate professor at the University Hospital of Antwerp, University of Antwerp in Belgium, led by Professor Marc Peeters. He is currently Head of Phase I - Early Clinical Trials Unit, and Director of the Clinical Path Management Program. He is focused on Clinical Research, Drug Development and Resistance.

He is actively working in a research program of Liquid Biopsies in Lung Cancer, specifically in the isolation of exosomes and circulating tumor DNA.

He completed his organizational training in Phase I at the Department of Therapeutic Cancer Research, Division of Cancer Medicine, MD Anderson Cancer Center at the University of Texas, Houston, TX (Visiting Professor Program) with Prof. David Hong. Dr. Rolfo is author of numerous articles

published in several journals, including New England Journal of Medicine, Annals of Oncology, Lancet Oncology, Oncotarget, translational oncology and Journal of Clinical Oncology, among others. He is also a speaker at national and international forums for lung cancer.

Ignacio Duran. Santander

Department of Medical Oncology at the Marqués de Valdecilla University Hospital in Santander (Spain), where he heads the oncology section of GU. He is actively involved in the promotion of clinical and translational research in the department.

He obtained his M.D. in 1997 at the University of Salamanca (Spain) and the Ph.D. in 2005 at the Complutense University of Madrid (Spain), with the highest recognition. He completed his training in Medical Oncology at the Son Dureta University Hospital in Palma de Mallorca (Spain) and then at the Princess Margaret Hospital in Toronto, Canada, as a fellow in Development of Medicines and Genitourinary Oncology between 2004-2007. Along with clinical training in Canada, he completed a two-year Master's Program in Teaching Medicine at the University of Toronto.

Author of numerous publications in scientific journals he has received some prizes, such as the Merit Award of the ASCO Foundation and the Outstanding Research Presentation Award of the Health Network of the University. In general, his main interests are clinical research in the area of genitourinary oncology and development of anticancer drugs along with education in medicine and equipment management.

Joan Carles. Barcelona.

Clinical genitourinary oncology, sarcoma and first clinical phases and translational investigations.

Associate Professor of Medicine of the Autonomous University of Barcelona and coordinator of the optional subject of Medical Oncology (UDIMAS) from 1995 to 2008.

Associate professor of oncology at the International University of Catalonia (UIC) since 2011.

Active member of the European Society of Medical Oncology, the American Society of Clinical Oncology and the Spanish Research Group in Sarcoma (GEIS).

Vice President of the Spanish Genitourinary Tumors Group from November 2009 to November 2011

Miquel Tarón. Sevilla

Biologist, Doctor in internal medicine and surgery. Senior researcher at the Institute of Biomedicine of Seville (IBIS) in the GROUP: ADVANCED THERAPIES AND BIOMARKERS IN ONCOLOGY. International expert in liquid biopsy markers related to lung cancer. 25 years of experience in diagnosis and R+D+i in oncology and molecular biology of cancer.

He has co-authored of more than 150 scientific publications in international journals; More than 400 presentations at national and international conferences. He is president of the Spanish Society of Pharmacogenetics and Pharmacogenomics (SEFF). He is member of GECP, GETHI and GEIS.

Experience in the coordination and management of biological samples and molecular analysis in clinical trials with cooperative groups of more than 140 national and international hospitals. He has business experience as co-founder of Pangea Biotech SL (Laboratory Director and EVP Diagnostics 2007-2014), and as Development and Regulatory Director in Amadix SL (2014-2016). Experience in ISO regulations, quality assurance programs, biobank and patent management.

Carlos Camps. Valencia

Head of the Medical Oncology Service of the General Hospital and of the Onco-Hematological Disease Clinical Area of the General Hospital of Valencia. In addition, he is a tenured professor at the University of the Department of Medicine of the University of Valencia.

He is president of the Continuous Care Section of the Spanish Society of Medical Oncology, president of the ECO Foundation (Excellence and Quality in Oncology). He is member of the Board of Directors of the Spanish Lung Cancer Group and member of ETOP (European Thoracic Oncology Platform). He is reviewer of international journals in the area of Oncology (J Thorac Oncol, Int J Cancer, Lung Cancer, Clin Transl Oncol, Plos Medicine, Annals Oncology, among others). He is president of the scientific society: ASEICA, Member of the Scientific Committee of the Ministry of Health of France and the National Cancer Institute of France (INCa). He is member of the Scientific Committee of the Ministry of Health for the genome and precision medicine program of the Senate.

Jesus Garcia Foncillas

Jesús García-Foncillas López is the director of the Oncology Institute "OncoHealth" that brings together the University Hospitals Jiménez Díaz Foundation, King Juan Carlos, Infanta Elena as well as the General Hospital of Villalba and the Hospital of Albacete, and director of the Department of Oncology of the University Hospital "Fundación Jiménez Díaz" -Universidad Autónoma de Madrid, and director of the Division of Translational Oncology of the Health Research Institute FJD-UAM.

He has developed his scientific work aimed at the application of basic research to the clinical field in the treatment and diagnosis of cancer as well as in the search for prognostic and predictive biomarkers, Co-coordinator of the Genomics and Proteomics Program of the Cancer Centers Network of the Health Institute Carlos III, Member of the Board of Directors of the Hereditary Cancer Section of the SEOM and of the Board of Directors of the Spanish Federation of Oncology Societies (FESEO).

He has been vice president of the National Commission of Medical Oncology of the Council of Specialties of the Ministry of Health and Consumption from 2007 to 2014. Since 2012 he is the President of the Mediterranean Society of Oncology (MOS).

Luis Paz –Ares. Madrid

Dr. Luis Paz-Ares is currently the Head of the Medical Oncology Service of the University Hospital 12 de Octubre (Madrid). He is also an associate professor at the Faculty of Medicine of the Complutense University of Madrid.

He graduated in Medicine from the Autonomous University of Madrid and took the specialty of Medical Oncology at the University Hospital 12 de Octubre. Subsequently, he completed a Master's Degree in Clinical Pharmacology (University of Glasgow) and another in Clinical Unit Management (UNED). He has been Head of Service of the Virgen de Rocío University Hospital (Seville), Deputy Director of the Institute of Biomedicine of Seville (IBIS), also serving as Director of the onco-hematology group program. Previously, he was a Physician Assistant (FEA) in charge of the Unit of Pharmacology and Early Clinical Studies, Thoracic and Genitourinary Tumors of the Medical Oncology Service, Doce de Octubre University Hospital of Madrid. Dr. Paz-Ares was a guest researcher at the Prostate Cancer Program at the Dana-Farber Cancer Institute of the Massachusetts General Hospital (Boston, United States).

He is a member of the Spanish Society of Medical Oncology (SEOM), Spanish Federation of Oncological Societies (FESEO), European Society on Medical Oncology (ESMO) and American Society of Clinical Oncology (ASCO). His main interests are the assistance and clinical and translational research in lung cancer and new treatment strategies.

He has participated in more than 200 scientific publications and media such as New England Journal of Medicine, Lancet, Lancet Oncology, Journal of Clinical Oncology, etc ... He is an active member of numerous scientific committees (including ASCO, ISALC, ESMO and many others) and other collaborating groups (European Organization for Research and Treatment of Cancer [EORTC], Spanish Lung Cancer Group, and the International Germ Cell Cancer Collaborative Group).

He participates as a member in numerous Foundations and organizations (AECC, CNIO, RTICC, ECO Foundation, Carolina Foundation..., being since 2016 President of the ONCOSUR Foundation).

Eloisa Jantus. Valencia

Eloisa Jantus is the Head of the Molecular Oncology Laboratory (Carlos Camps group) and Professor of Molecular Biology of Cancer in the Biotechnology Department of the Polytechnic University of Valencia. The Molecular Oncology group is strongly involved in translational research in cancers of the lung and upper respiratory tract and is characterized by its multidisciplinary nature.

She has been working on the search for new prognostic biomarkers (related to the immune system) in resectable lung cancer, and recently the goal of her laboratory is to isolate, characterize and analyze lung CSC populations (isolated from patients). Dr. Jantus also has extensive experience in the analysis of biomarkers in liquid biopsies (saliva, plasma, CTC).

Ignacio Gil-Bazo. Pamplona

Ignacio Gil-Bazo is MD and PhD from the Faculty of Medicine of the University of Navarra in Pamplona, Spain. He is a medical oncologist and associate professor in oncology, with special interest in the study and treatment of lung cancer and molecular oncology. He completed his training as a postdoctoral researcher at the Memorial Sloan-Kettering Cancer Center in New York, USA. UU., In the Cancer Biology and Genetics Program. He received the Medical Oncology Certificate in 2012 from the European Society of Medical Oncology (ESMO) and has a master's degree in immuno-oncology (University of Navarra, October 2016). He directs the Department of Oncology of the University of Navarra and the laboratory of New Therapeutic Targets at the Center for Applied Medical Research (CIMA), with special interest in liquid biopsy, resistance to drugs, mechanisms of metastasis in lung cancer and new targets in immunotherapy.

ANNEX II. Questionnaire

CUESTIONNAIRE CPI .
PRECISION MEDICINE BASED ON LIQUID BIOPSY

PART A CLINICAL ASSESSMENT OF THE PROPOSAL

About DNA

PREANALYTICAL VARIABLES	**VALUE*** YES NO INCOMPLETE
1.1 Type of sample	
Plasma	Other: Pleural liquid, … Central Nervous System (SNC), Bronchoalveolar lavage (BAL),
1.2 Sample origin Peripheral blood	Otras: L Pleural, SNC, BAL…
1.3 PRESERVATION TUBES	
EDTA collection tubes	
Citrate collection tubes	
1.4 Transport Room temperature	
1.5 Preservation time 6 hours maximum	
Storage -80ºC	

ANALYTICAL VARIABLES	**VALUE** YES NO INCOMPLETE
Mutations known and validated as targets to treatment response Lung: T970 ALK BRAF	RET, ROS, NTRK, Her2, MET EGFR (other except for T790M)

Mutations known and validated as targets to treatment response COLON: KRAS NRAS BRAF	

Mutations known and validated as targets to treatment response BREAST: HER2 TP53 PIK3CA EGFR KRAS ESR1	Is it worth it nowadays to evaluate all of them?

ABOUT CTCs

PREANALYTICAL VARIABLES	**VALUE** **YES** **NO** **INCOMPLETE**
1.1 **Type of sample**	
Whole blood	
1.2 Type of extraction Venopunction	
1.3 Preservation tubes	
EDTA collection tubes	
Preservation tubes	
1.4 Transport Room temperature	
1.5 Preservation time 4 hours maximum (EDTA TUBES) 24 hours maximum (PRESERVATION TUBES)	
No Preservation	

PREANALYTICAL VARIABLES	**VALUE** **YES** **NO** **INCOMPLETE**
Mutations known and validated as targets to treatment response **COLON:** KRAS NRAS BRAF	
BREAST HER2 TP53 PIK3CA EGFR KRAS ESR1	
LUNG: T970 ALK BRAF	Other mutations and fusions

PREANALYTICAL VARIABLES	**VALUE** **YES** **NO** **INCOMPLETE**
Protein markers known and validated as targets for response to treatment or markers of progression **Colon** Citokeratins VEGF PDGFR FGFR	

Breast Citokeratins CD44/CD24 EGFR RE RP PD-1/PDL-1	

ABOUT MIRNAS

PREANLAYTICAL VARIABLES	VALUE YES NO INCOMPLETE
1.1 Type of sample	
Plasma Serum	
1.2 PRESERVATION TUBES	
EDTA collection tubes	
Preservative collection tubes	
1.4 Transport Room temperature	
1.5 Preservation time 6 hours maximum	
Storage -80ºC	

ANALYTICAL VARIABLES	VALUE YES NO INCOMPLETE
NO VALID MIRNAS SHOULD BE INCORPORATED	

ABOUT PLATELETS

PREANALYTICAL VARIABLES	VALUE YES NO INCOMPLETE
1.1 Type of sample	
Whole blood	
1.2 Preservation tubes	
EDTA Collection tubes	
Heparin Collection tubes	
1.4 Transport Room temperature	
1.5 Preservation time 4 hours maximum	
Storage of the GENETIC MATERIAL **-80ºC**	

ANALYTICAL VARIABLES	VALUE YES NO INCOMPLETE
PLATELETS ARE NOT VALIDATED IN THE CLINICS **THEY SHOULD BE INCORPORATED**	EFGR, ALK, others...

PART B

ON THE PROPOSED PROPOSAL

VARIABLES	**VALUE** **YES** **NO** **INCOMPLETE**
1.1 Functional requirements of the devices	
Incorporation of a barcode for each patient	
Time of manual handling (from the collection of the sample to the final result) should be less than 6 hours	
Results should be provided in a format that can be easily interpreted by the clinician	
The system must provide exhaustive information of the process, if required by the user	
Detection and quantification of mutated DNA molecules with a high sensitivity (in the 1% range).	
Multiplexing capabilities: Analysis of multiple mutations depending on the tissue	
The devices should include the possibility of detecting genetic rearrangements, genetic variants of the gene, as well as genetic amplifications	
The system must allow analysis of multiple samples simultaneously	
Short analysis time (30-60 min).	
The cost of the analysis process must not exceed 50€ per patient	

PART C

ON THE LOGISTICS OF INCORPORATION TO CLINICAL PRACTICE

VARIABLES	**VALUE** **YES** **NO** **INCOMPLETE**
1.1 Requirements for the incorporation to the clinical routine	
It should be included within the standard request repertoire	
Requirements of the request - Type of liquid biopsy - Type of tube - Type of molecular marker / cytogenetic / phenotypic - Type of patient (diagnosis / follow-up)	
Requirements of the report to be sent to the clinical specialist - Type of liquid biopsy - State of the analyzed sample - Annex with request of Access to the complete genetic and phenotypic report of the result of the liquid biopsy, if necessary.	

*** Assess on the basis of these three possibilities, justify at the work table on the 14th.**

ANNEX III. Program

LIQUID BIOPSY-PUBLIC PROCUREMENT OF INNOVATION WORKSHOP

14th June 2018. GRANADA.

GENyO CENTRE. PTS. Av. de la Ilustración, 114. CP 1816. Granada

FIRST SESSION 10:00- 10:30

10.00:10:10 WELCOME

José Antonio Lorente. *Director of the Health Research and Innovation Strategy Andalucia*
Mª José Serrano. *Senior Research Oncology Unit. University Hospital Virgen de las Nieves / Genyo Centre*

10:10-10:20 INTRODUCTION TO PUBLIC PROCUREMENT OF INNOVATION (PPI)

José María de la Higuera. *Andalusian Public Health Care System PPI Program Coordinator.*

10:20-10:30 OBJECTIVES AND PLANNING OF PPI: "New diagnostic system for liquid biopsy (LIQUID BIOPSY)"

Mª José Serrano. *Senior Research Oncology Unit. University Hospital Virgen de las Nieves / Genyo Centre*
Juan José Díaz Mochón. *Associate professor. University of Granada*

SECOND SESSION 10:30- 14:00

Chairperson Mª José Serrano / Juan José Díaz Mochón

10.30:11:30 ABOUT DNA. INTRODUCTION AND DISCUSSION OF THE QUESTIONAIRE
All Researches

11.30:12:30 ABOUT CTC. INTRODUCTION AND DISCUSSION OF THE QUESTIONAIRE
All Researches

12.30:14:00 ABOUT PLATELETS AND mRNA. INTRODUCTION AND DISCUSSION OF THE QUESTIONAIRE
All Researches

Lunch 14:00 – 15:00

THIRD SESSION 15:00 - 17:00

15.00:17:00 CONCLUSIONS AND DEVELOPMENT OF THE CONCENSUS DOCUMENT

José Luis García Puche. Researcher. *President of the International Society of Liquid Biopsy.*
Mª José Serrano. *Senior Research Oncology Unit. University Hospital Virgen de las Nieves / Genyo Centre*
Juan José Díaz Mochón. *Associate professor. University of Granada*

CLOSING OF THE WORKSHOP

José Antonio Lorente. *Director of the Health Research and Innovation Strategy Andalucia*
José Expósito. *Director of the Oncology Unit. University Hospital Virgen de las Nieves. Andalusian Health Service. Granada.*