THE EUROPEAN
LIQUID BIOPSY
SOCIETY

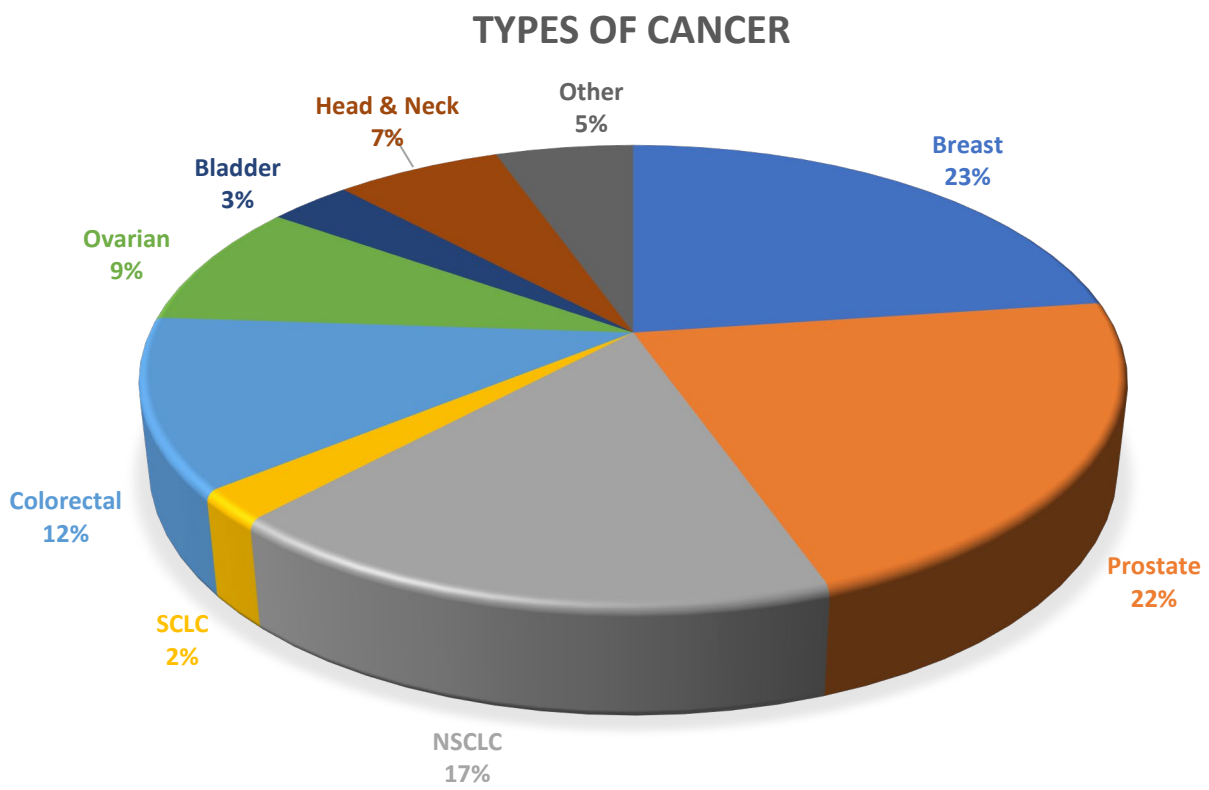
Agenda

CTC Technology Meeting, February 9th 2022

Work Package Leads: Evi Lianidou and Nikolas Stoecklein

THE EUROPEAN LIQUID BIOPSY SOCIETY

Types of cancer



CTC enumeration / CellSearch
14/35 Laboratories 40%

- FRANCE: Institut Claudius Regaud, Toulouse University Cancer Institute - Oncopole, Prospective Biology Unit **(Anne Pradines, Gilles Favre)**
- FRANCE: Institut Curie, Paris **(Jean-Yves Pierga)**
- FRANCE: Laboratory Rare Human Circulating Cells (LCCRH), University Medical Center of Montpellier, France **(Catherine ALIX-PANABIERES)**
- FRANCE: Rare circulating cell translational laboratory, Gustave Rousy, Paris, France (**Françoise Farace)**
- GERMANY: Institute of Tumor Biology, University Medical Center Hamburg-Eppendorf, **(Klaus Pantel)**
- GERMANY: University Hospital and Medical Faculty of the Heinrich Heine University Duesseldorf, Liquid Biopsy Center Düsseldorf **(T. Fehm/W.T. Knoefel/H. Neubauer/N. Stoecklein)**
- GREECE: Analysis of Circulating Tumor Cells (ACTC) Lab, University of Athens, **(Evi Lianidou)**
- ITALY: Menarini Silicon Biosystems SpA, **(Nicolò Manaresi, Tim Pitfield, Andrea Lasagni, Cecilia Simonelli)**
- ITALY: SAFU and ONCOGENOMIC AND EPIGENETIC Labs., IRCCS REGINA ELENA NATIONAL CANCER INSTITUTE, 00144 Rome, **(Sabrina Strano)**
- ITALY: Veneto Institute of Oncology, IOV-IRCCS, CTC LAB **(Antonella Facchinetti, Maria Chiara Scaini, Cristina Poggiana, Elisabetta Rossi)**
- NORWAY: Dept of Cancer Genetics, Institut of Cancer Research, Oslo University Hospital, **(Björn Naume, Hege Russness, Gry Geitvik)**
- SPAIN: Traslational Medical Oncology Group (Oncomet), Instituto de Investigación Sanitaria de Santiago de Compostela **(Clotilde Costa, Laura Muinelo Romay)**
- THE NETHERLANDS: Medical Cell BioPhysics / University of Twente, **(Leon Terstappen)**
- THE NETHERLANDS: Translational Cancer Genomics, **(Stefan Sleijfer, JWM Martens, Jaco Kraan)**

CTCs/Parsortix
8/35 Laboratories 25.7%

- AUSTRIA: Molecular Oncology Group, University of Vienna, (R. Zeillinger)
- FRANCE: Rare circulating cell translational laboratory, Gustave Rousy, Paris, France (Françoise Farace)
- GERMANY: Institute of Tumor Biology, University Medical Center Hamburg-Eppendorf, (Klaus Pantel)
- GERMANY: University Hospital and Medical Faculty of the Heinrich Heine University Duesseldorf, Liquid Biopsy Center Düsseldorf (T. Fehm/W.T. Knoefel/H. Neubauer/N. Stoecklein)
- GREECE: Analysis of Circulating Tumor Cells (ACTC) Lab, University of Athens, (Evi Lianidou)
- SPAIN: Traslational Medical Oncology Group (Oncomet), Instituto de Investigación Sanitaria de Santiago de Compostela (Clotilde Costa, Laura Muinelo Romay)
- UK: ANGLE S2A Laboratories, (Michael O‘Brien)
- UK: Centre for Biomarker and Biotherapeutics, Barts Cancer Institute, University of London, (Yong-Jie Lu)

CTCs/Single Cells
19/35 Laboratories 54.3%
DEPArray 8/35 (22.8%)

- FRANCE: Laboratory Rare Human Circulating Cells (LCCRH), University Medical Center of Montpellier, France (Catherine ALIX-PANABIERES)
- GERMANY: Institute of Tumor Biology, University Medical Center Hamburg-Eppendorf, (Klaus Pantel)
- GERMANY: University Hospital and Medical Faculty of the Heinrich Heine University Duesseldorf, Liquid Biopsy Center Düsseldorf (T. Fehm/W.T. Knoefel/H. Neubauer/N. Stoecklein)
- ITALY: Menarini Silicon Biosystems SpA, (Nicolò Manaresi, Tim Pitfield, Andrea Lasagni, Cecilia Simonelli)
- ITALY: SAFU and ONCOGENOMIC AND EPIGENETIC Labs., IRCCS REGINA ELENA NATIONAL CANCER INSTITUTE, 00144 Rome, (Sabrina Strano)
- NORWAY:, Dept of Cancer Genetics, Institut of Cancer Research, Oslo University Hospital, (Björn Naume, Hege Russness, Gry Geitvik)
- SPAIN: Traslational Medical Oncology Group (Oncomet), Instituto de Investigación Sanitaria de Santiago de Compostela (Clotilde Costa, Laura Muinelo Romay)
- THE NETHERLANDS: Medical Cell BioPhysics / University of Twente, (Leon Terstappen)

CTCs VyCAP / Single Cell
5/35 Laboratories 14.3%

- GERMANY: University Hospital and Medical Faculty of the Heinrich Heine University Duesseldorf, Liquid Biopsy Center Düsseldorf (T. Fehm/W.T. Knoefel/H. Neubauer/N. Stoecklein)
- THE NETHERLANDS: Translational Cancer Genomics, (Stefan Sleijfer, JWM Martens, Jaco Kraan)
- GREECE: Metastasis signaling Lab/University of Patras, Greece, Galatea Kallergi
- THE NETHERLANDS: Medical Cell BioPhysics / University of Twente, (Leon Terstappen)
- SPAIN: Traslational Medical Oncology Group (Oncomet), Instituto de Investigación Sanitaria de Santiago de Compostela (Clotilde Costa, Laura Muinelo Romay)

CTCs/Single Cells
19/35 Laboratories 54.3%
CellSelector ALS: 3/35, 8.6%

- **CellSelector ALS: GERMANY:** University Hospital and Medical Faculty of the Heinrich Heine University Duesseldorf, Liquid Biopsy Center Düsseldorf (T. Fehm/W.T. Knoefel/H. Neubauer/N. Stoecklein)

- **CellSelector ALS: GERMANY:** ALS Automated Lab Solutions GmbH, (Jens Eberhardt)

- **CellSelector ALS: AUSTRIA:**Molecular Oncology Group, University of Vienna, (R. Zeillinger)

CTCs/Single Cells
19/35 Laboratories 54.3%
Other technologies

- **CytePicker®**RareCyte: **(Alexandre Darmoise)**
- **EPIDROP**: FRANCE: Laboratory Rare Human Circulating Cells (LCCRH), University Medical Center of Montpellier, France **(Catherine ALIX-PANABIERES)**
- **FACS:** Medical Cell BioPhysics / University of Twente, **(Leon Terstappen)**
- **FACS**: FRANCE: Rare circulating cell translational laboratory, Gustave Rousy, Paris, France **(Françoise Farace)**
- **FACS:** University Hospital and Medical Faculty of the Heinrich Heine University Duesseldorf, Liquid Biopsy Center Düsseldorf **(T. Fehm/W.T. Knoefel/H. Neubauer/N. Stoecklein)**
- **Fluidigm**: Centre for Biomarker and Biotherapeutics, Barts Cancer Institute, University of London, **(Yong-Jie Lu)**
- **Genesis/Immunodetection**: Bio-Rad, Adam Corner, Alexandre Brandt, Brandan Walters –
- **ISET**: Analysis of Circulating Tumor Cells (ACTC) Lab, University of Athens, **(Evi Lianidou)**
- **ISET:** Metastasis signaling Lab/University of Patras, Greece, **(Galatea Kallergi)**
- **LMD (patented):** ITALY: Veneto Institute of Oncology, IOV-IRCCS, CTC LAB **(Antonella Facchinetti, Maria Chiara Scaini, Cristina Poggiana)**

CTCs/Imaging biomarkers
12/35 Laboratories 34.3%

- AUSTRIA: Graz, Research Group El-Heliebi, (Amin El-Heliebi): AR-V7, ??
- FRANCE: Institut Curie, Paris (Jean-Yves Pierga)
- FRANCE: Laboratory Rare Human Circulating Cells (LCCRH), University Medical Center of Montpellier, France (Catherine ALIX-PANABIERES), PD-L1, HER-2
- FRANCE: Rare circulating cell translational laboratory, Gustave Rousy, Paris, France (Françoise Farace): HER2, HER3, ALK, EGFR, gH2AX, PSMA
- GERMANY: ALS Automated Lab Solutions GmbH, (Jens Eberhardt) : ???
- GERMANY: Institute of Tumor Biology, University Medical Center Hamburg-Eppendorf, (Klaus Pantel): PD-L1
- GERMANY: University Hospital and Medical Faculty of the Heinrich Heine University Duesseldorf, Liquid Biopsy Center Düsseldorf (T. Fehm/W.T. Knoefel/H. Neubauer/N. Stoecklein)
- GERMANY: University Hospital and Medical Faculty of the Heinrich Heine University Duesseldorf (names???) - PD-L1, AXL
- GREECE: Metastasis signaling Lab/University of Patras, Greece, Galatea Kallergi : CK-7, anti-PD-L1, VIM, CD45, JUNB, CXCR4
- UK: ANGLE S2A Laboratories, Michael O‘Brien: CKs, Mesenchymal markers, exclusion markers, nuclear stain
- UK: Bio-Rad, (Adam Corner, Alexandre Brandt, Brandan Walters): ???
- UK: Centre for Biomarker and Biotherapeutics, Barts Cancer Institute, University of London, (Yong-Jie Lu):

CTCs: gene expression-RNA analysis
10/35 Laboratories 28.6%

- AUSTRIA: Graz, Research Group El-Heliebi, (Amin El-Heliebi): AdnaTest Prostate cancer, RT-PCR, AR-V7 prostate cancer
- AUSTRIA: Molecular Oncology Group, University of Vienna, (R. Zeillinger), RT-qPCR
- FRANCE: Rare circulating cell translational laboratory, Gustave Rousy, Paris, France (Françoise Farace):WES, Targeted PCR, NGS
- GERMANY: QIAGEN GmbH, (Siegfried Hauch, Thorsten Singer): AdnaTest, All Prep Nano CTC, RNA profiling
- GERMANY: University Hospital and Medical Faculty of the Heinrich Heine University Duesseldorf, Liquid Biopsy Center Düsseldorf (T. Fehm/W.T. Knoefel/H. Neubauer/N. Stoecklein): Ampli1, QIAseq Transcriptome
- GREECE: Analysis of Circulating Tumor Cells (ACTC) Lab, University of Athens, (Evi Lianidou): RT-qPCR, RT-ddPCR, Multiplex RT-qPCR, Multiplex RT-ddPCR
- ITALY: Veneto Institute of Oncology, IOV-IRCCS, CTC LAB (Antonella Facchinetti, Maria Chiara Scaini, Cristina Poggiana, Elisabetta Rossi), Nanostring
- NORWAY: Stavanger University Hospital, Department of hematology and oncology (Oddmund Nordgård): ??
- SPAIN: Traslational Medical Oncology Group (Oncomet), Instituto de Investigación Sanitaria de Santiago de Compostela (Clotilde Costa, Laura Muinelo Romay): RT-qPCR,
- UK: Centre for Biomarker and Biotherapeutics, Barts Cancer Institute, University of London, (Yong-Jie Lu): Fluidigm, Multiplex RT-PCR, TaqMan RT-PCR

CTCs/ddPCR
4/35 Laboratories 11.4%

- GERMANY: QIAGEN GmbH, (Siegfried Hauch, Thorsten Singer)
- GREECE: Analysis of Circulating Tumor Cells (ACTC) Lab, University of Athens, (Evi Lianidou): ddPCR, Multiplex ddPCR, RT-ddPCR, Multiplex RT-ddPCR, dMSP, Multiplex dMSP
- ITALY: Veneto Institute of Oncology, IOV-IRCCS, CTC LAB (Antonella Facchinetti, Maria Chiara Scaini, Cristina Poggiana, Elisabetta Rossi)
- SPAIN: Traslational Medical Oncology Group (Oncomet), Instituto de Investigación Sanitaria de Santiago de Compostela (Clotilde Costa, Laura Muinelo Romay)

CTCs/Methylation studies
3/35 Laboratories 8.6%

- GERMANY: University Hospital and Medical Faculty of the Heinrich Heine University Duesseldorf, Liquid Biopsy Center Düsseldorf (T. Fehm/W.T. Knoefel/H. Neubauer/N. Stoecklein)

- GREECE: Analysis of Circulating Tumor Cells (ACTC) Lab, University of Athens, (Evi Lianidou): MSP, dMSP,, multiplex MSP, multiplex dMSP, NGS

- SPAIN: Traslational Medical Oncology Group (Oncomet), Instituto de Investigación Sanitaria de Santiago de Compostela (Clotilde Costa, Laura Muinelo Romay)

CTCs/NGS
9/35 Laboratories 25.7%

- FRANCE: Rare circulating cell translational laboratory, Gustave Rousy, Paris, France (Françoise Farace): Targeted PCR-NGS
- GERMANY: Institute of Tumor Biology, University Medical Center Hamburg-Eppendorf, (Klaus Pantel): NGS, Mass spectrometry
- GERMANY: University Hospital and Medical Faculty of the Heinrich Heine University Duesseldorf, Liquid Biopsy Center Düsseldorf (T. Fehm/W.T. Knoefel/H. Neubauer/N. Stoecklein):
- ITALY: Menarini Silicon Biosystems SpA, (Nicolò Manaresi, Tim Pitfield, Andrea Lasagni, Cecilia Simonelli):
- ITALY: SAFU and ONCOGENOMIC AND EPIGENETIC Labs., IRCCS REGINA ELENA NATIONAL CANCER INSTITUTE, 00144 Rome, (Sabrina Strano): NGS-ILLUMINA-NovaSeq
- ITALY: Veneto Institute of Oncology, IOV-IRCCS, CTC LAB (Antonella Facchinetti, Maria Chiara Scaini, Cristina Poggiana, Elisabetta Rossi)
- RareCyte, Inc., Alexandre Darmoise: RareCyte, Single Cell targeted sequences, Paragon Genomics
- SPAIN: Traslational Medical Oncology Group (Oncomet), Instituto de Investigación Sanitaria de Santiago de Compostela (Clotilde Costa, Laura Muinelo Romay): NextSeq, Illumin
- THE NETHERLANDS: Translational Cancer Genomics, (Stefan Sleijfer, JWM Martens, Jaco Kraan): NGS-Single CTCs

ACCREDITATION ACCORDING TO ISO 15189:2012 STANDARD

- procedure by which an authoritative body gives formal recognition that a clinical laboratory is performing in accordance with the requirements of ISO 15189:2012 standard and is competent to carry out specific analyses

- Useful tool for clinical laboratories that allows them to provide qualitative services to clinicians and patients by ensuring the generation of accurate and reliable results.

- A key step to obtain accreditation according to ISO 15189:2012 is the constant and periodic participation in External Quality Assessment (EQA) schemes.

ICS › 03 › 03.120 › 03.120.10

ISO 15189:2012

Medical laboratories — Requirements for quality and competence

- **Scope:** to guide laboratories to develop a quality management system that regulates the whole testing procedure, thus ensuring constant quality services of patient care.

- The ISO15189:2012 describes which procedures and aspects must be included regarding pre-analytical, analytical and post-analytical phase, in order to design a proper quality management system.

- This International Standard can be used by medical laboratories in developing their quality management systems and assessing their own competence. It can also be used for confirming or recognizing the competence of medical laboratories by laboratory customers, regulating authorities and accreditation bodies.

ISO 15189: 2012 REQUIREMENTS

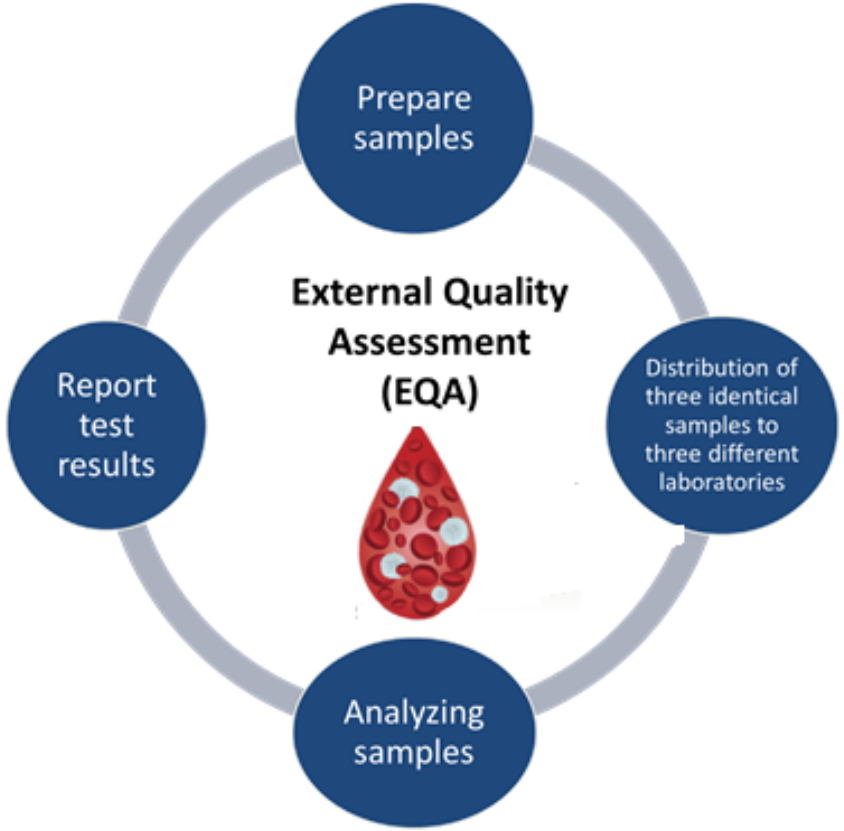
Management requirements

Organization and management responsibility
Quality management system
Document control
Service agreements
Examination by referral laboratories
External services and supplies
Advisory services
Resolution of complaints
Identification and control of nonconformities
Corrective action
Preventive action
Continual improvement
Control of records
Evaluation and audits
Management review

Technical Requirements

Personnel
Accommodation and environmental conditions
Laboratory equipment, reagents, and consumables
Pre-examination processes
Examination processes
Ensuring quality of examination results
Post-examination processes
Reporting of results
Release of results

ISO-15189 ACCREDITATION
CTC analysis main issues



Identification of CTC assays to be ISO-15189 certified (clinical utility), eg:

- **CTC enumeration**
- **AR-V7,**
- **PD-L1,**
- **HER-2, ….**

Identification of ELBS labs that want to be ISO-15189 certified for specific assays and use identical technologies (ELBS questionnaires)

Preparation of spiked samples (CANCER ID Experience!!)

Organize ring trials providing the same blood samples to all in a coded format

Use of ISO certified couriers to ship the samples

Analysis and report test results (all documentation needed)

Ask for ISO-15189 certificate from the local agencies in European Countries

Main goal for ELBS!
Reimbursement of liquid biopsy tests by the National Health System of each country

CTC enumeration / CellSearch
14/35 Laboratories 40%

- FRANCE: Institut Claudius Regaud, Toulouse University Cancer Institute - Oncopole, Prospective Biology Unit **(Anne Pradines, Gilles Favre)**
- FRANCE: Institut Curie, Paris **(Jean-Yves Pierga)**
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- ITALY: Veneto Institute of Oncology, IOV-IRCCS, CTC LAB **(Antonella Facchinetti, Maria Chiara Scaini, Cristina Poggiana, Elisabetta Rossi)**
- NORWAY: Dept of Cancer Genetics, Institut of Cancer Research, Oslo University Hospital, **(Björn Naume, Hege Russness, Gry Geitvik)**
- SPAIN: Traslational Medical Oncology Group (Oncomet), Instituto de Investigación Sanitaria de Santiago de Compostela **(Clotilde Costa, Laura Muinelo Romay)**
- THE NETHERLANDS: Medical Cell BioPhysics / University of Twente, **(Leon Terstappen)**
- THE NETHERLANDS: Translational Cancer Genomics, **(Stefan Sleijfer, JWM Martens, Jaco Kraan)**

ACTC lab : ISO-15189 ACCREDITATION
For CellSearch CTC enumeration

Εθνικό Σύστημα Διαπίστευσης

ΕΣΥΔ

ACCREDITATION CERTIFICATE
No. 1108

The Hellenic Accreditation System S.A. (ESYD), as the national accreditation body of Greece in accordance with the Law 4468/2017

ACCREDITS

of the

Analysis of Circulating Tumor Cells lab

of the

Department of Chemistry

of the

National and Kapodistrian University of Athens

in Zografou, Attiki, Greece

under the terms of the ELOT EN ISO 15189: 2012 Standard and the ESYD Criteria, to carry out tests, as specified in the attached Scope of the Accreditation, which may be revised by decisions of ESYD.

This Certificate is valid until 28.12.2021, provided that the accredited body will comply with the above Standard and the ESYD Criteria.

Athens, December 28th, 2017

Konstantinos Voutsinas
Managing Director, ESYD

ESYD is a signatory of the European co-operation for Accreditation (EA) Multilateral Agreement for the activities covered by this certificate

Hellenic Accreditation System

ΕΣΥΔ

Annex G1/1 to the Certificate No. **1108**

SCOPE of ACCREDITATION
of the
Analysis of Circulating Tumor Cells lab
of the
Department of Chemistry,
of the
National and Kapodistrian University of Athens

Tested materials/ products	Types of test/ Properties to be measured	Applied Standards/ Techniques to be used
Biological testing		
1. Peripheral blood, plasma (EDTA), (Liquid Biopsy)	1. Detection of somatic mutations in exons 18, 19, 20, και 21 of the EGFR gene in patients with Non-Small Cell Lung cancer (NSCLC) CE-IVD approved FDA cleared assay EGFR MUTATIONS **G719X** (2156G>C, 2155G>A, 2155G>T) **Ex19Del:** 2240_2251del12, 2239_2247del9, 2238_2255del18, 2235_2249del15, 2236_2250del15, 2239_2253del15, 2239_2256del18, 2237_2254del18 2240_2254del15, 2240_2257del18, 2239_2248TTAAGAGAAG>C, 2239_2251>C, 2237_2255>T, 2235_2255>AAT, 2237_2252>T, 2239_2258>CA, 2239_2256>CAA, 2237_2253>TTGCT, 2238_2252>GCA, 2238_2248>GC, 2237_2251del15, 2236_2253del18, 2235_2248>AATTC, 2235_2252>AAT, 2235_2251>AATTC, 2253_2276del24, 2237_2257>TCT, 2238_2252del15, 2233_2247del15	**Cobas z 480 Analyzer, ROCHE DIAGNOSTICS*** Real time PCR for the semi-quantitative measurement of mutations. COBAS® EGFR Mutation Test v2 CE-IVD approved FDA cleared assay

Tested materials/ products	Types of test/ Properties to be measured	Applied Standards/ Techniques to be used
1. Peripheral blood, plasma (EDTA), (Liquid Biopsy) cont.	**S768I** (2303G>T) **T790M** (2369C>T) **Ex20Ins** 2307_2308ins9GCCAGCGTG, 2319_2320insCAC, 2310_2311insGGT, 2311_2312ins9GCGTGGACA, 2309_2310AC>CCAGCGTGGA **L858R** 2573T>G, 2573_2574TG>GT **L861Q** (2582T>A)	
2. Peripheral blood (Cellsave Preservative Tubes) (Liquid Biopsy)	Identification and enumeration of Circulating Tumor Cells (CTCs)	**CELLSEARCH SYSTEM CIRCULATING TUMOR CELL KIT, Menarini*** CE-IVD approved FDA cleared assay

**Reference to the commercial name of a specific analyzer/kit, refers to a specific analytical method and protocol*

Site of assessment: **Laboratory permanent premises: Department of Chemistry, National and Kapodistrian University of Athens, University Campus, 157 71 Zografou, Attiki, Greece.**
Approved signatory: **E. Lianidou**

The Accreditation Certificate No. **1108**, to ELOT EN ISO 15189:2012, is valid until 28.12.2021.

Athens, December 28th, 2017

Konstantinos Voutsinas
Managing Director, ESYD

ISO-15189 ACCREDITATION
CellSearch CTC enumeration

- **ACTC lab is accredited for the CTC enumeration using the CellSearch system according to ISO 15189:2012**

An example when EQA schemes for liquid biopsy analyses are not available

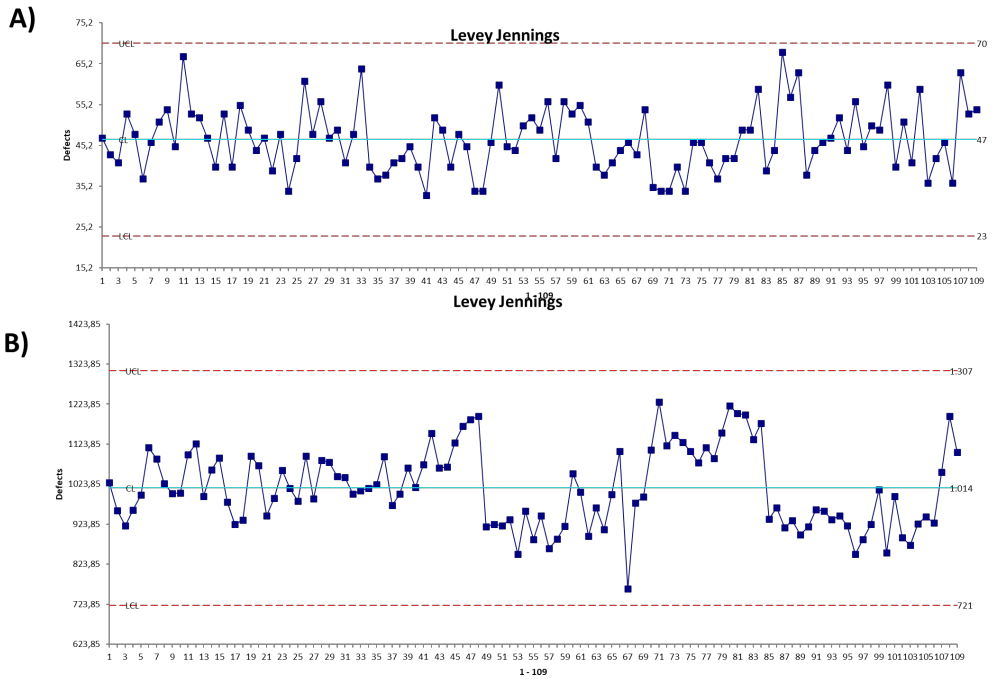
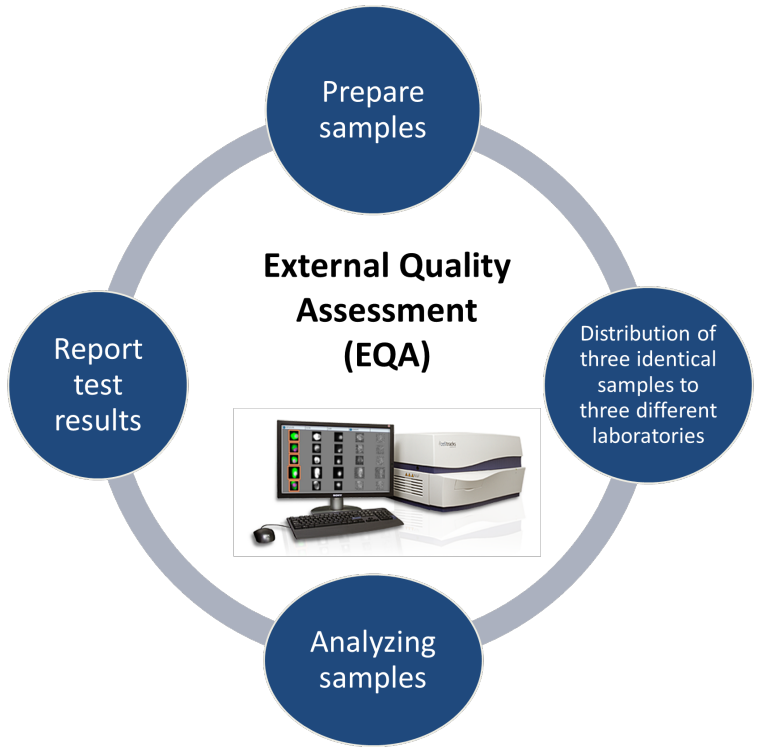


Figure 3. Quality control of CellSearch analysis (Levey–Jennings graphs): A) Low control, B) High Control



Day to day repeatability:
CellSearch® Circulating Tumor Cell Control

	Low CTC numbers			High CTC numbers		
Day	median	range	result	median	range	result
1	48	25-71	36	971	819-1123	927
2	48	25-71	46	971	819-1123	943
3	48	25-71	42	971	819-1123	925
4	48	25-71	36	971	819-1123	871
5	48	25-71	59	971	819-1123	890
6	48	25-71	41	971	819-1123	993
7	48	25-71	51	971	819-1123	853
8	48	25-71	40	971	819-1123	1010
9	48	25-71	60	971	819-1123	923
10	48	25-71	49	971	819-1123	886
11	48	25-71	50	971	819-1123	849
12	48	25-71	45	971	819-1123	920
13	48	25-71	56	971	819-1123	945
14	48	25-71	44	971	819-1123	935
15	48	25-71	52	971	819-1123	957
16	48	25-71	47	971	819-1123	960
Median value			47			924
Standard deviation (SD)			7,4			46
(CV%)			16			5,0

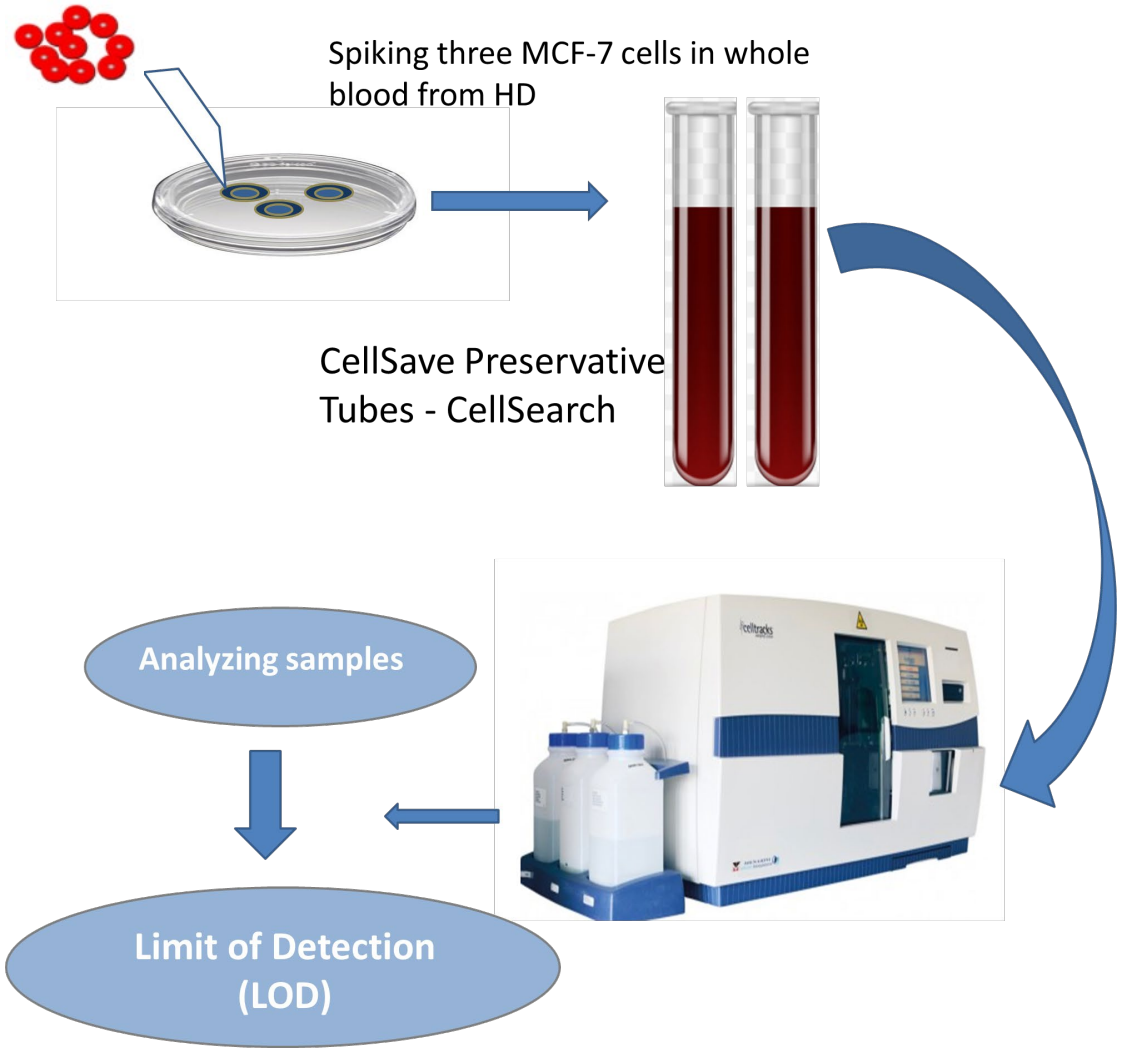


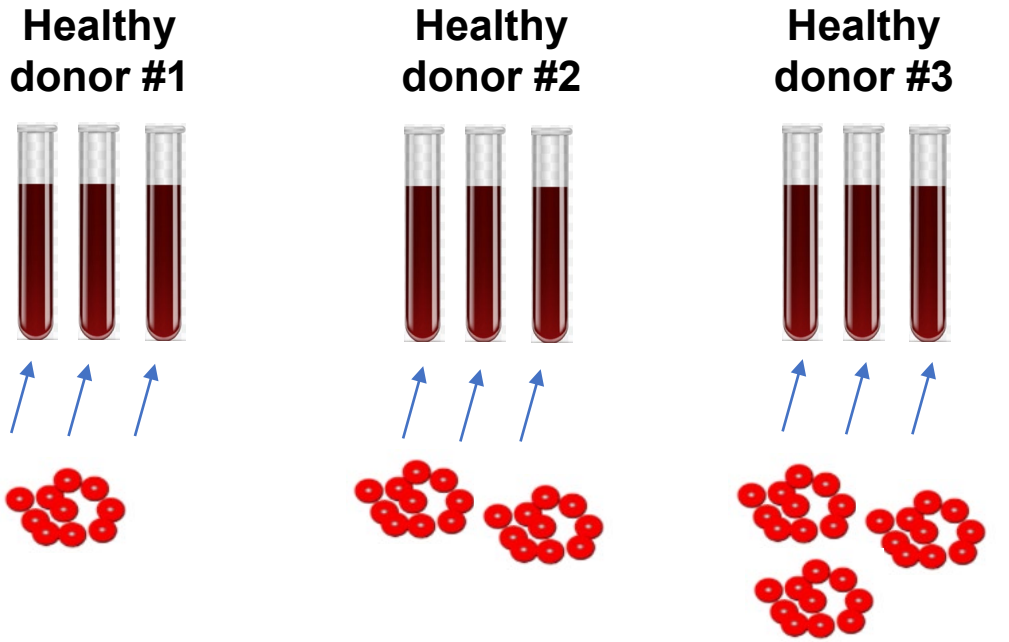
Table 1. Intra-operator repeatability for CTC enumeration

Number of spiked MCF-7 cells	Enumeration of CTCs	Recovery (%)
100	92	92%
100	80	80%
100	74	74%
Mean	82	
SD	9,2	
CV	11%	

Table 2. Validation of the LOD of the CellSearch System

Number of spiked MCF-7 cells	Enumeration of CTCs	Recovery (%)
3	3	100%
3	3	100%
Mean	3	

Preparation of samples: Spiking MCF-7 cells in healthy donors blood samples



EQA 2017 RESULTS

Participating labs: E. Lianidou, K. Pantel, L. Terstappen

Table 3. External Quality Assessment Schemes for CellSearch testing for the year 2017

Samples	Lab#1	Lab#2	Lab#3	Mean	SD	CV%
Sample 1	51	37	34	41	9,1	22,3
Sample 2	12	12	3	9	5,2	57,7
Sample 3	0	0	0	0	0,0	
Sample 4	90	64	62	72	15,6	21,7

EQA 2019 RESULTS

Participating labs: E. Lianidou, R. Zamarchi, N. Stoecklein

Table 4. External Quality Assessment Schemes for CellSearch testing for the year 2019

Samples	Lab#1	Lab#2	Lab#3	Mean	SD	CV%
Sample 1	7,0	0,0	4,0	3,7	3,5	95,8
Sample 2	3,0	2,0	1,0	2,0	1,0	50,0
Sample 3	9,0	7,0	8,0	8,0	1,0	12,5

BENEFITS OF ISO-15189 ACCREDITATION

- **Harmonization of results for the same liquid biopsy assays in different labs that use exactly the same technology**
- **Highly important to establish a network of ISO-15189 certified labs for specific liquid biopsy tests within ELBS**
- **Validation of liquid biopsy protocols and their implementation in the clinical setting**
- **Reimbursement of liquid biopsy tests by the National Health System (example: AR-V7, Epic assay, USA)**
- **Evaluation of novel technologies by comparing results for the same samples in ISO-15189 labs**

CTC analysis, CLIA / ISO-15189 certification
2/35 Laboratories 5.7%

1. GREECE: Analysis of Circulating Tumor Cells (ACTC) Lab, University of Athens, (Evi Lianidou)

2. ITALY: Menarini Silicon Biosystems SpA, (Nicolò Manaresi, Tim Pitfield, Andrea Lasagni, Cecilia Simonelli), ISO-13485, ISO-15189 in progress

Utilization of CT(A)C:
From therapy management towards screening

DATAR
CANCER GENETICS
UNITED KINGDOM | GERMANY | INDIA

Datar Cancer Genetics

Current status:

- Based in India/UK/Germany
- ISO 15189, CLIA, CAP accredited, UKAS
- CE mark
- Foundation in 2013
- Staff : 280

Certificate No.: MC-2309

ISO 9001

ISO 27001

CAP ACCREDITED
COLLEGE of AMERICAN PATHOLOGISTS
Accreditation No.: 1790695

CLIA
Accredited Laboratory
Reg. No.: 99D2171983

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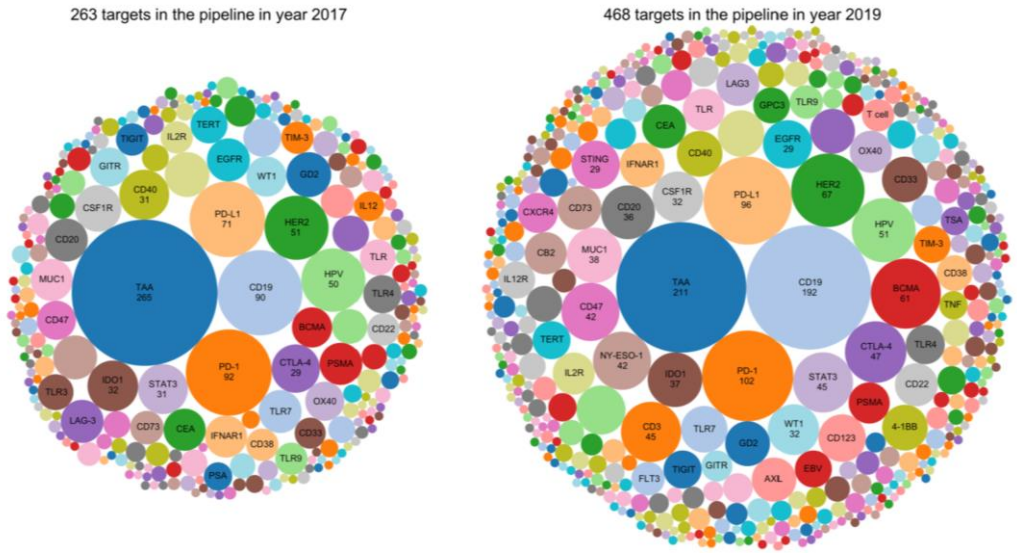
Clinical Needs Therapy Management

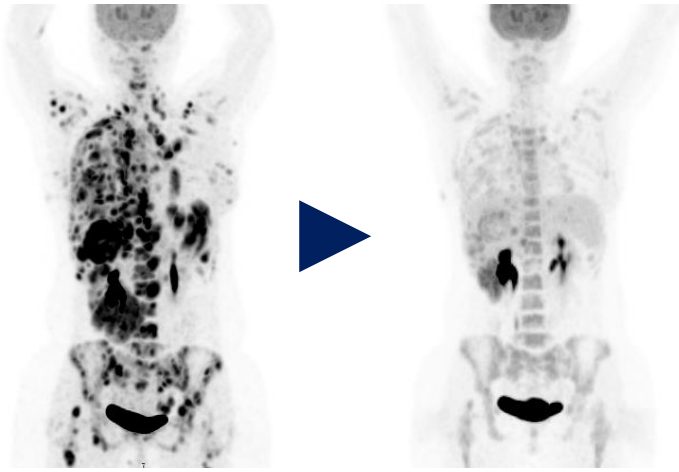
Increase of drug targets in oncology



The increasing number of drugs is only sensible, when the diagnostic tools cover all targets

Advanced Salivary Duct Carcinoma

Stage IV Invasive Ductal Triple Negative Breast Cancer



Cancer had progressed following **5** lines of therapy.

Administration of DCGL-recommended therapy led to regression.

Immuno-oncology drug development goes global
Jia Xin Yu, Vanessa M. Hubbard-Lucey and Jun Tang
https://doi.org/10.1038/d41573-019-00167-9

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Cancer Screening in Germany

Reimbursed Screening	What	Who	Age	How often	How many do participate
Skin	Visual	Men/Women	Starting at 35	Every two years	**Unknown**
Colon	Faeces blood / coloscopy	Men/Women	50/55	Every two years/1 coloscopy in 10 years	**58%**
Cervix	Smear	Women	20	Yearly	**Unknown**
Breast	Palpation of the breast/ mammography	Women	30 yearly/50-69 every two years	yearly/every two years	**Unknown/74%**
Prostate	Palpation	Men	45	yearly	**40%**

The road towards Cancer Screening

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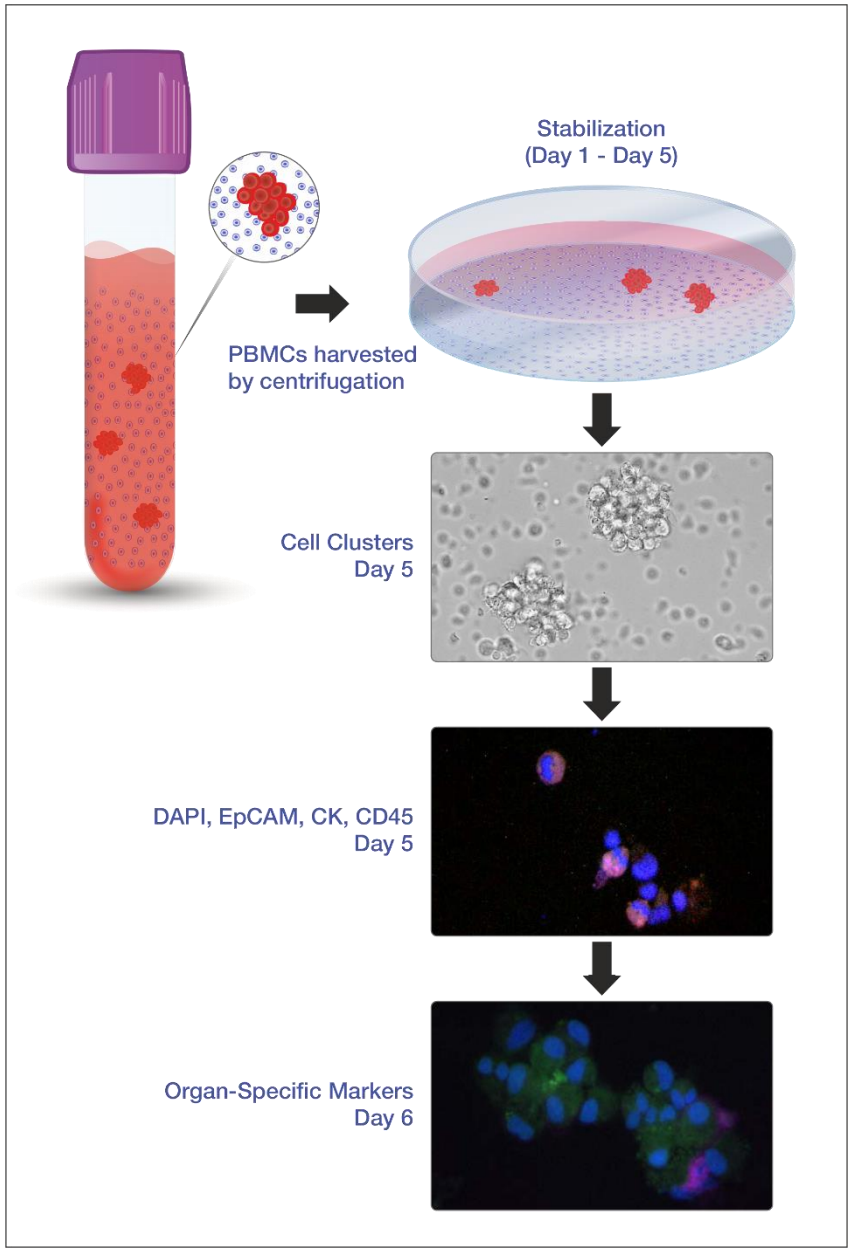
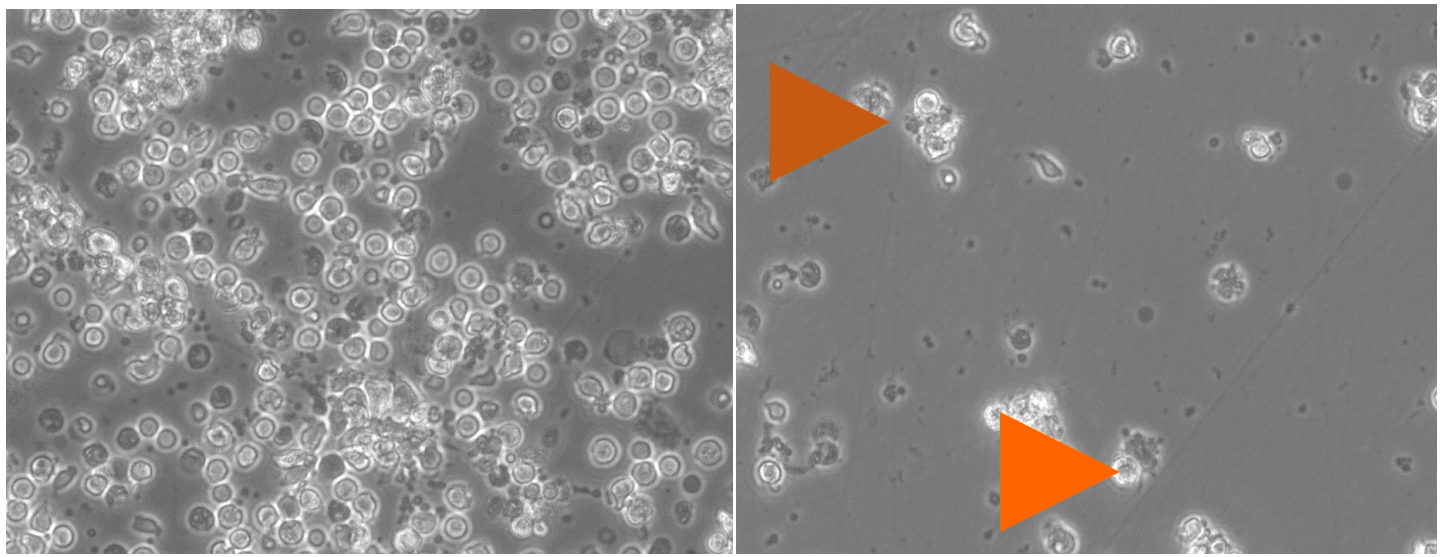
Relevant Clinical Trials

	TrueBlood	ProState	GlioLENS	RESOLUTE
Study Type	Prospective, Observational			
Cancer Types	All solid organ	Prostate cancers	CNS malignancies	None
WHO ICTRP ID	CTRI/2019/03/017918	CTRI/2019/02/017863	CTRI/2019/02/017663	CTRI/2019/01/017219
Population	9,276 known solid organ cancers, 325 known benign cases, 5,784 suspected cancers	140 known prostate cancers, 71 known benign prostate conditions, 347 suspected prostate cancers,	130 suspected of CNS malignancies	13,919 asymptomatic individuals
Sample	15-20 mL venous blood	15-20 mL venous blood	15-20 mL venous blood	15-20 mL venous blood
Study Procedures (post blood collection)	Suspected individuals underwent biopsy	Suspected individuals underwent biopsy	Suspected individuals underwent biopsy	LDCT, Mammography, Pap smear, CA125, PSA, CEA, CA19-9, AFP
Notes	Findings of Histopathological examination (following biopsy) were initially masked.			-

METHOD: Cell Wizard ™





Day 1 **Post Cell Wizard™**

Cell Wizard uses medium with paradoxical cytotoxicity to kill cells with functional apoptotic mechanism while holding harmless apoptosis resistant cells and clusters in the PBMC milieu.

Any emboli / cluster like assemblages of cells surviving after 5 days of exposure to the special media were aspirated for characterization with immunofluorescent (IF) antibodies i.e. DAPI, EPCAM, CK and CD45.

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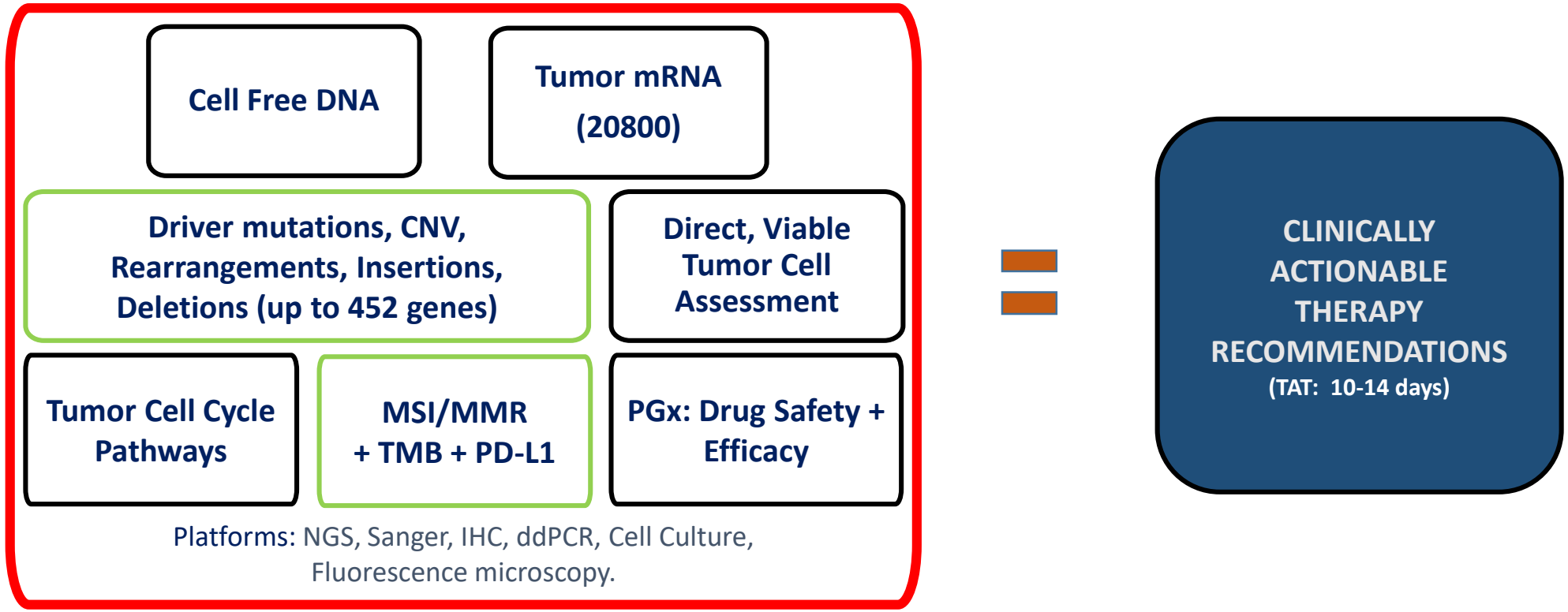
(US Patent Office Application Numbers 62849840 and 62796098)

exacta®
ENCYCLOPEDIC TUMOR ANALYSIS

ABSOLUTE IMPACT
ABSOLUTE SCIENCE
ABSOLUTE COMMONSENSE



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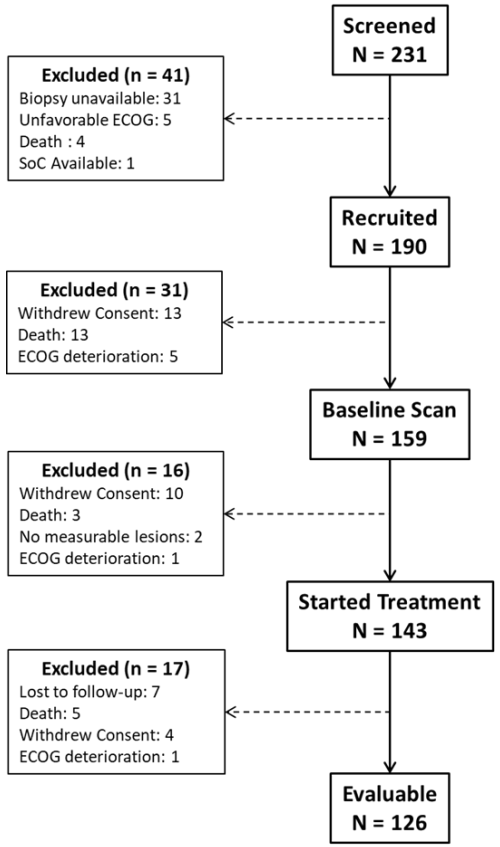
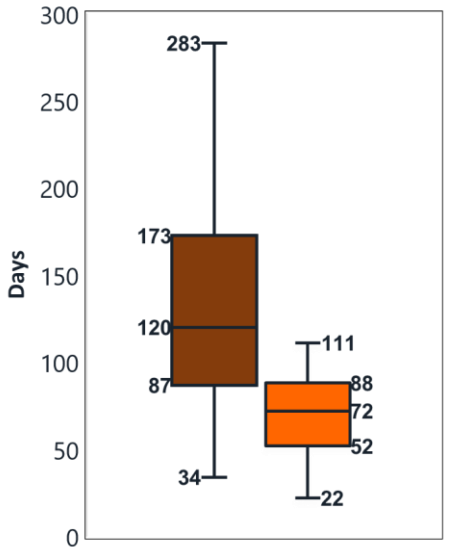
RESILIENT Study 2019

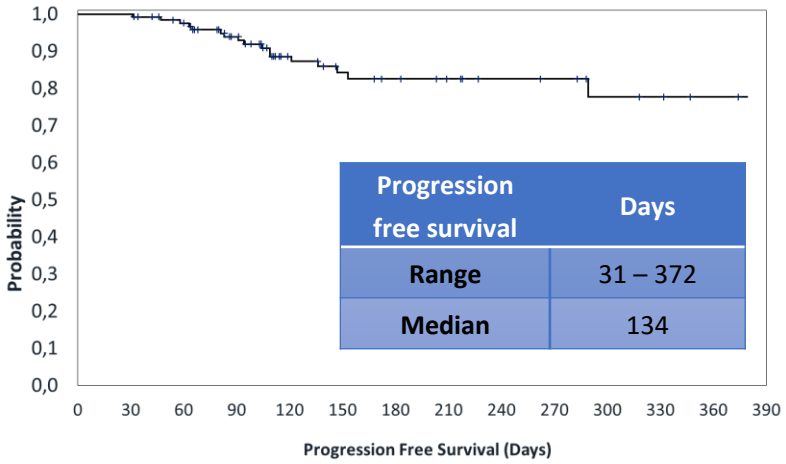


- All patients underwent baseline ^{18}F-FDG PET-CT scan.
- Each patient received individualized therapy (unique combination of agents + dosage).
- Treatment response evaluated ^{18}F-FDG PET-CT scan.
- First follow-up scan: after 2 cycles of therapy OR 60 days after initiation of therapy.

Progression free survival (PFS) **vs last line** ***time to progression (TTP)***



PFS : TTP	Patients	%
≥2.5	22	35.5%
≥1.3	47	75.8%



Progression free survival	Days
Range	31 – 372
Median	134

Best Response	Value
Evaluable patients	126 Patients
Partial Response + Complete Response	54 Patients
Objective Response Rate	42.9 % (CI 34.3 -51.5%)

Nagarkar R., Patil D., Crook T., Datta V., Bhalerao S., Dhande S., Palwe V., Roy S., Pandit P., Ghaisas A., Page R., Kathuria H., Srinivasan A., et al Encyclopedic tumor analysis for guiding treatment of advanced, broadly refractory cancers: results from the RESILIENT trial. Oncotarget. 2019; 10: 5605-5621.

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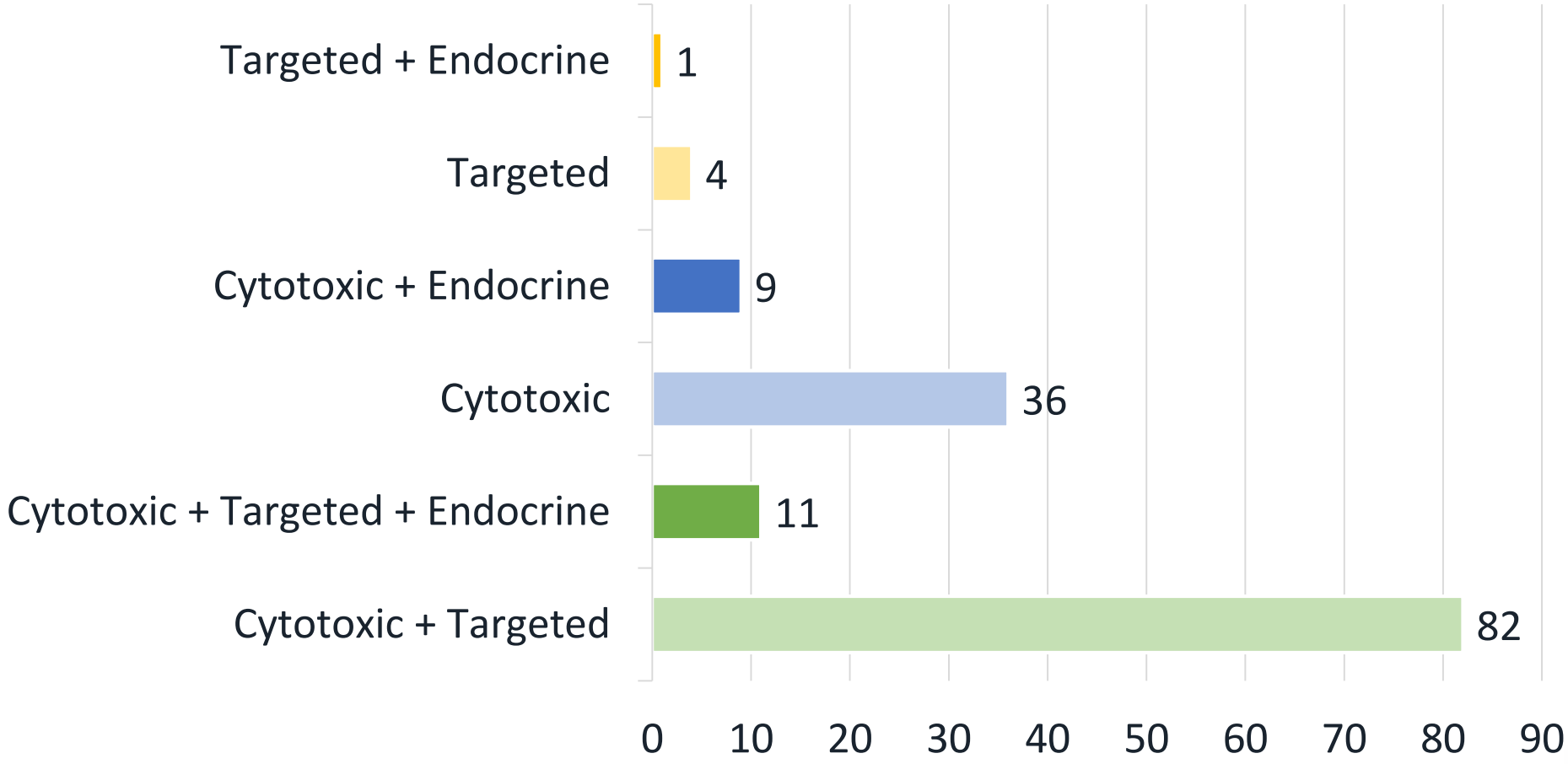
Exacta-Guided Treatment Profile (ITT)



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Study: Activation of mTOR signaling pathways 2021

Treatments:

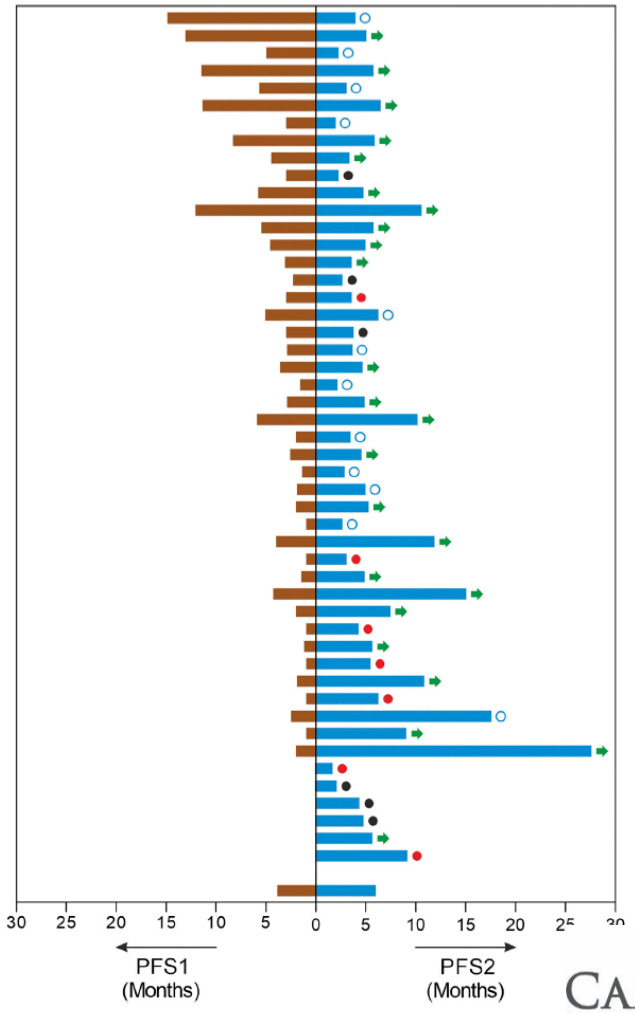
- Individualized combination regimens with mTOR inhibitors and other targeted, endocrine or cytotoxic drugs on the basis of ETA findings

Study cohort:

- 49 patients (23males, 26 females, median age: 49)
- 46 patients had refractory cancers

Results:

- Objective Response Rate = 57.1%
- **Disease Control Rate = 91.8%**
- Median Progression Free Survival = 4.9 months
- Median Overall Survival = 9.4 months
- No treatment related adverse events or treatment related deaths



Crook T, Patil D, Gaya A, et al. Improved Treatment Outcomes by Using Patient Specific Drug Combinations in Mammalian Target of Rapamycin Activated Advanced Metastatic Cancers. *Front Pharmacol*. 2021;12:631135. Published 2021 Apr 16. doi:10.3389/fphar.2021.631135

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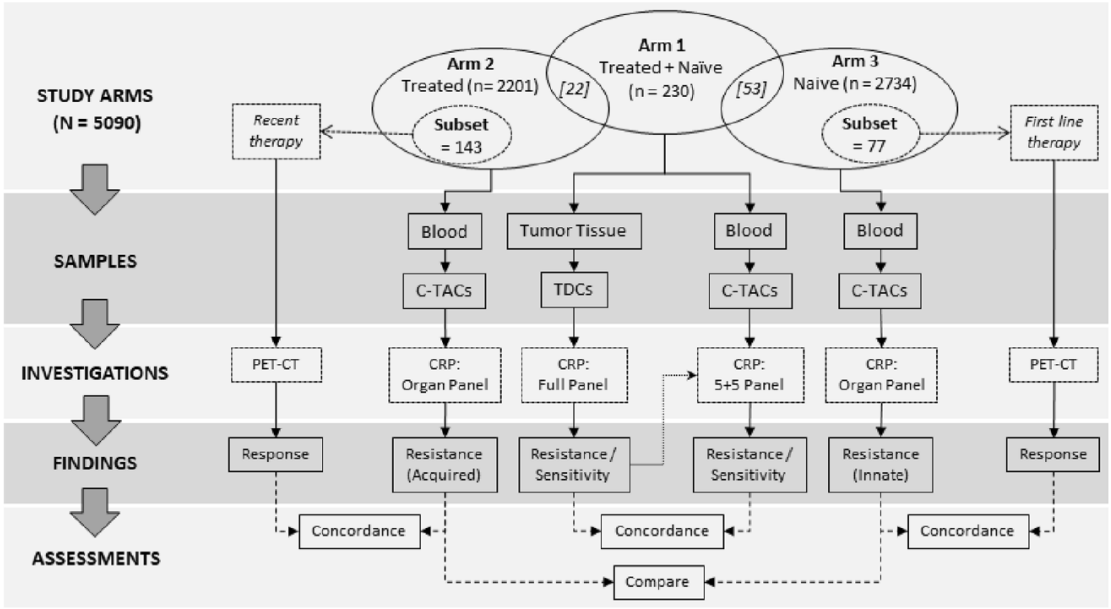
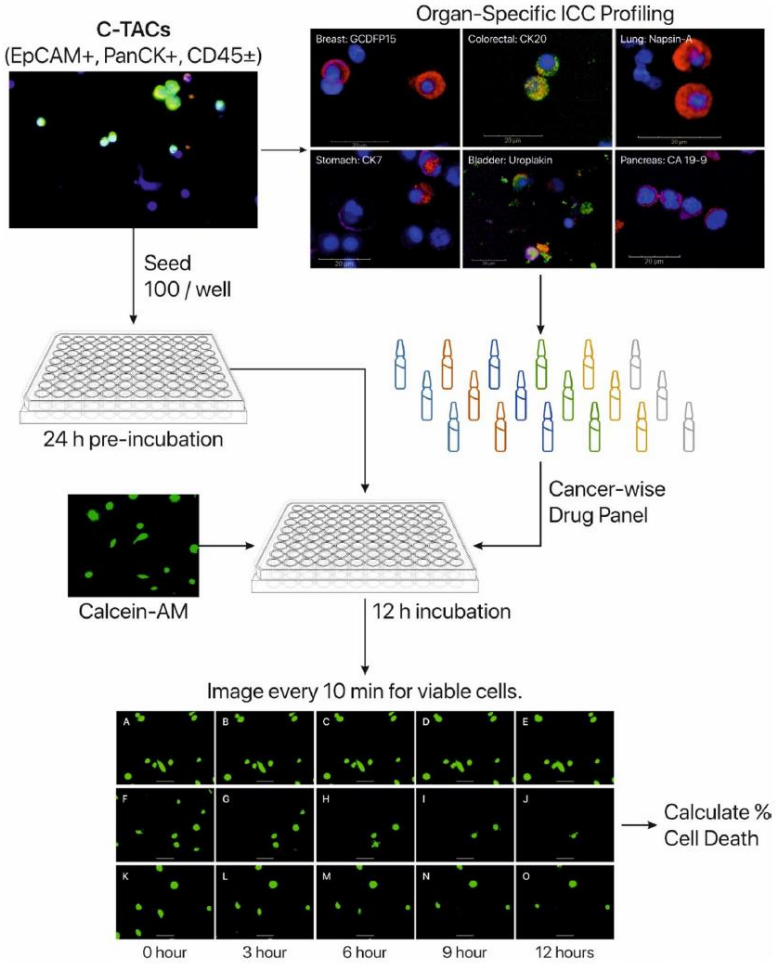
Therapy Management 2021
(Chemosensitivity Testing on CTACs)





93,7% Concordance between tissue and CTC

In pretreated patients the chemo profile of C-TACs was in 87% concordant with in vivo treatment failure

Crook, T., Gaya, A., Page, R. *et al.* Clinical utility of circulating tumor-associated cells to predict and monitor chemo-response in solid tumors. *Cancer Chemother Pharmacol* **87,** 197–205 (2021). https://doi.org/10.1007/s00280-020-04189-8

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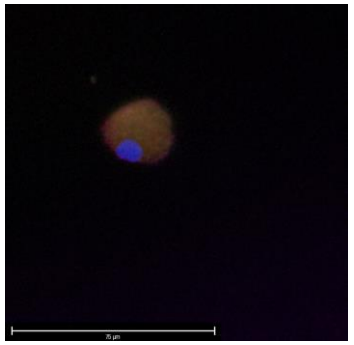
Concordance of IHC:ICC → Tissue:CTACs 2021

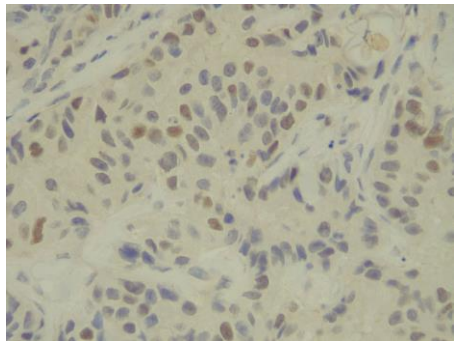
Scope:

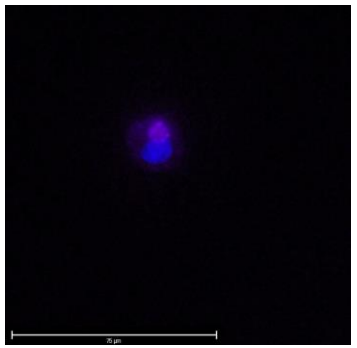
- Comparison of Tissue IHC and CTAC ICC
- 743 matched pairs

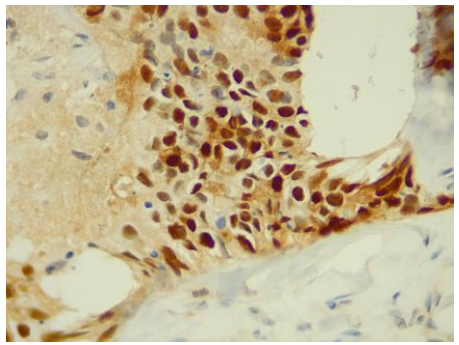
Results:

- 87,6% overall concordance
  - ER 82,9%
  - PR 100%
  - Her2 90,2%
  - AR 93,8%
  - PD-L1 85,1% and 87,6% (two clones)
  - MMR 85,6%









Concordancy of immunocytochemistry profiling of circulating tumor cells with immunohistochemistry for analysis of therapeutically relevant biomarkers.
Dadasaheb B Akolkar, Darshana Patil, Sudha S Murthy, Kiran Bendale, Revati Patil, Vineet Datta, Pradip Fulmali, Pooja Fulmali, Archana Adhav, Shoeb Patel, Rohit Chougule, Vishal Ranjan, Shabista Khan, Raja Dhasarathan, Sachin Apurwa, Stefan Schuster, Ajay Srinivasan, and Rajan Datar
Journal of Clinical Oncology 2021 39:15_suppl, 3047-3047

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Key Publications for Diagnostic Triaging 2020

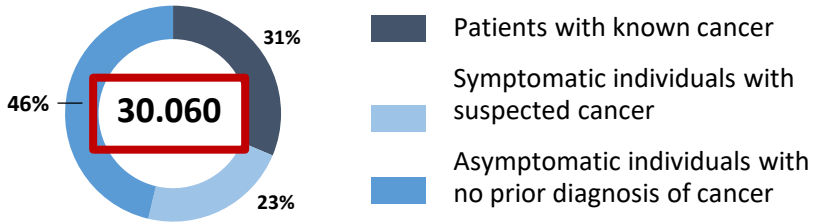
Observance of C-ETACs in cancer patients confirms the possibility of noninvasive diagnosis of cancer with high accuracy

1

American Cancer Society®

Cancer Cytopathology

Evaluation of Circulating Tumor Cell Clusters for Pan-Cancer Non-invasive Diagnostic Triaging



C-ETACs detected in 91.8% cancer cases

C-ETACs provided diagnostically relevant information >93% of the cases

First Comprehensive report on CTC based diagnosis with accuracy comparable to tissue-based diagnosis

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Cancer Screening

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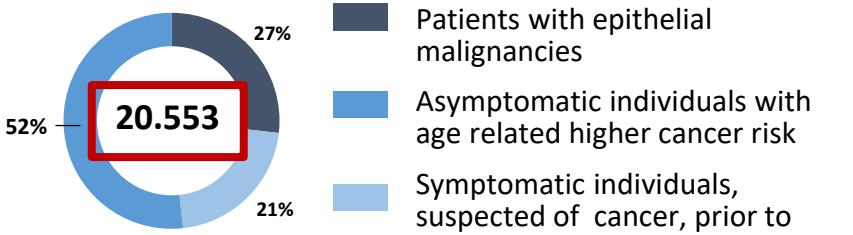
Key Publications for Cancer Screening Applications 2020-21

Presence of C-ETACs helps in confirming cancer and in identifying asymptomatic individuals who are at risk

IJC

1 DCG proposed Circulating Ensembles of tumor-associated cells (C-ETACs) as a new Hallmark of Cancer



AACR American Association for Cancer Research

2 Hallmark Circulating Tumor Associated Cell Clusters Signify 230 Times Higher One-Year Cancer Risk

Overall, C-ETACs detected in **90%** of cancer cases

230 - fold higher cancer risk in asymptomatic individuals

C-ETACs were detected in **93%** of newly diagnosed cancer patients

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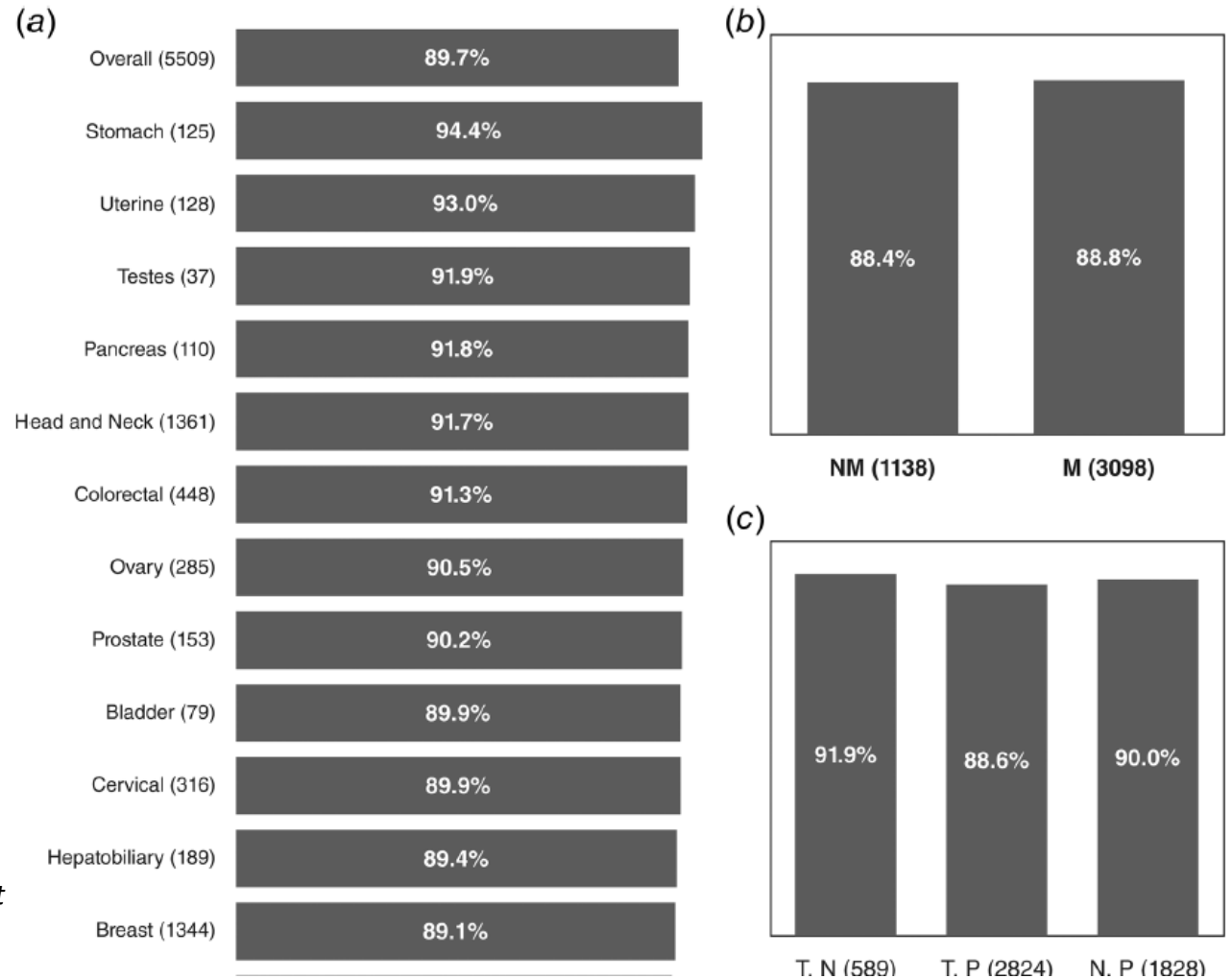
Ubiquity of CETACs



Circulating ensembles of tumor-associated cells: A redoubtable new systemic hallmark of cancer Dadasaheb Akolkar,Darshana Patil,Timothy Crook,Sewanti Limaye,Raymond Page,Vineet Datta,Revati Patil,Cynthe Sims,Anantbhushan First published: 30 November 2019 https://doi.org/10.1002/ijc.32815

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Early Diagnostics/Screening – Cell Clusters

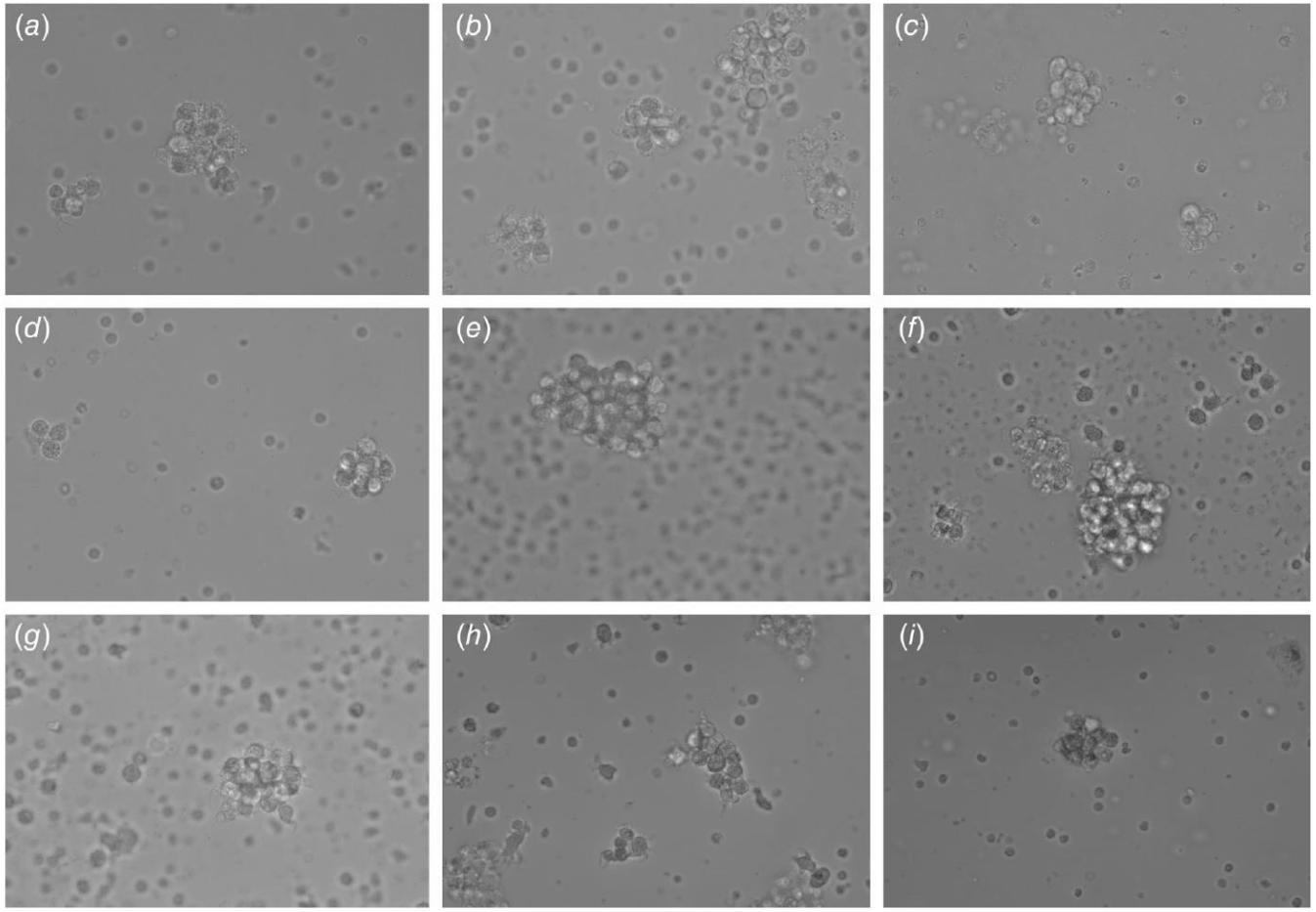

Figure 1. Cell assemblages on Day 5. Viable intact cell assemblages (white arrow) were imaged under a phase contrast microscope at 40× magnification. Samples from various cancer types are depicted (*a*) breast, (*b*) lung, (*c*) prostate, (*d*) stomach, (*e*) gallbladder, (*f*) kidney, (*g*) bladder, (*h*) buccal mucosa and (*i*) pancreas. Field width is ~160 μm.

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Staining of the Cell Clusters

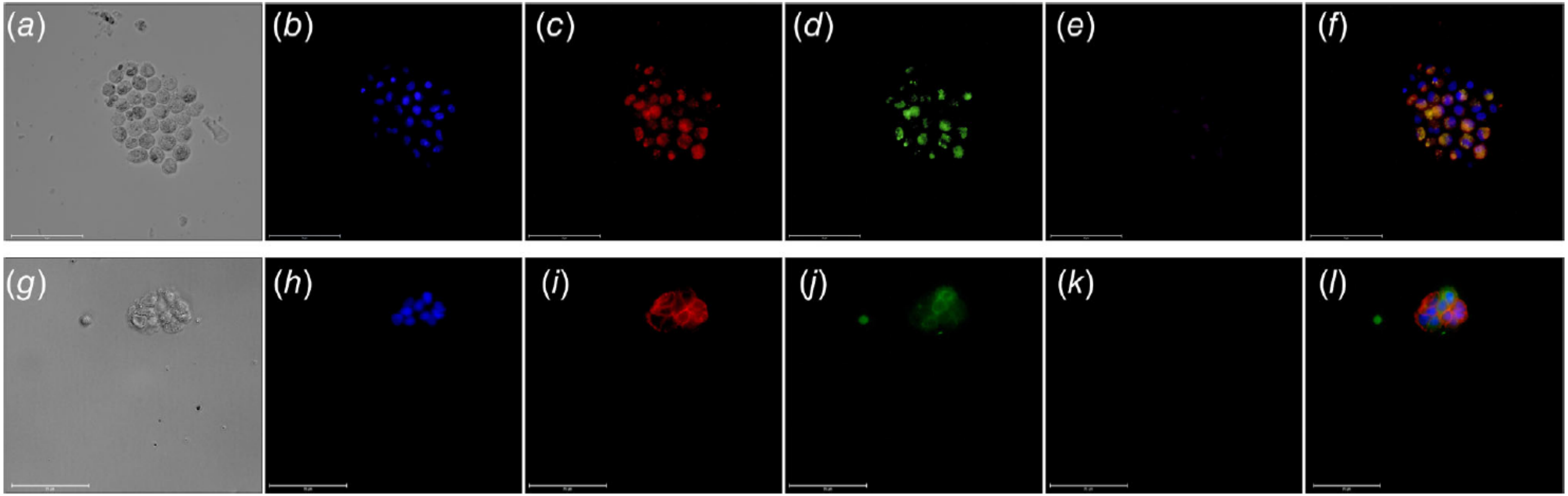
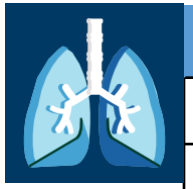


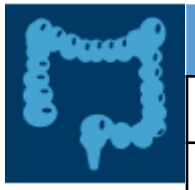
Figure 2. Immunostaining of C-ETACs. Cytospin smears prepared from cell-assemblages obtained on Day 5 from a case of Ca lung (*a*–*f*) and Ca endometrium (*g*–*l*) were stained with DAPI, anti-EpCAM, anti-CK and anti-CD45. (*a*, *g*) Bright field; (*b*, *h*) DAPI; (*c*, *i*) EpCAM; (*d*, *j*) panCK; (*e k*) CD45; (*f*, *l*) composite overlay (without bright field).

Screening Data

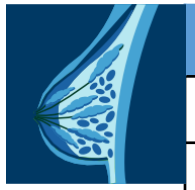
	Trucheck	PSA
Sensitivity	85.6%	90.0%
Specificity	99.9%	19.7%
PPV	99.9%	33.9%
NPV	99.9%	81.0%



	Trucheck	Lung RADS	NLST
Sensitivity	87.9%	84.9%	93.5%
Specificity	99.9%	87.2%	73.4%
PPV	99.9%	6.9%	3.8%
NPV	99.9%	99.8%	99.9%



	Trucheck$_T$	Colonoscopy / CT Colongraphy
Sensitivity	82.6%	73%-98%
Specificity	99.9%	89%
PPV	99.9%	-
NPV	99.9%	-



	Trucheck	Mammography
Sensitivity	88.2%	86.9%
Specificity	99.9%	88.9%
PPV	99.9%	69.6%
NPV	99.9%	95.9%

1 Lehman CD et al. National Performance Benchmarks for Modern Screening Digital Mammography: Update from the Breast Cancer Surveillance Consortium. Radiology. 2017 Apr; 283(1): 49-58. doi: 10.1148/radiol.2016161174.
2 PPV and NPV Calculated from data reported by Lehman et al (above).
3 Pinsky P et al Performance of Lung-RADS in the National Lung Screening Trial: a retrospective assessment. Ann Intern Med. 2015; 162(7): 485-491. doi: 10.7326/M14-2086
4 USPSTF reported data.
5 McKiernan J et al. A Prospective Adaptive Utility Trial to Validate Performance of a Novel Urine Exosome Gene Expression Assay to Predict High-grade Prostate Cancer in Patients with Prostate - specific Antigen 2-10 ng/ml at Initial Biopsy. Eur Urol. 2018 Dec; 74(6):731-738. doi: 10.1016/j.eururo.2018.08.019.

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Take Home Messages

- **CTC and CTACs**
- **Highly comprehensive** therapy management
- **Blood based** Cancer Screening
- We can distinguish and **localize** more than **70 different solid tumors**
- More than **57.000 indivduals/patients** stand for clinical relevance

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