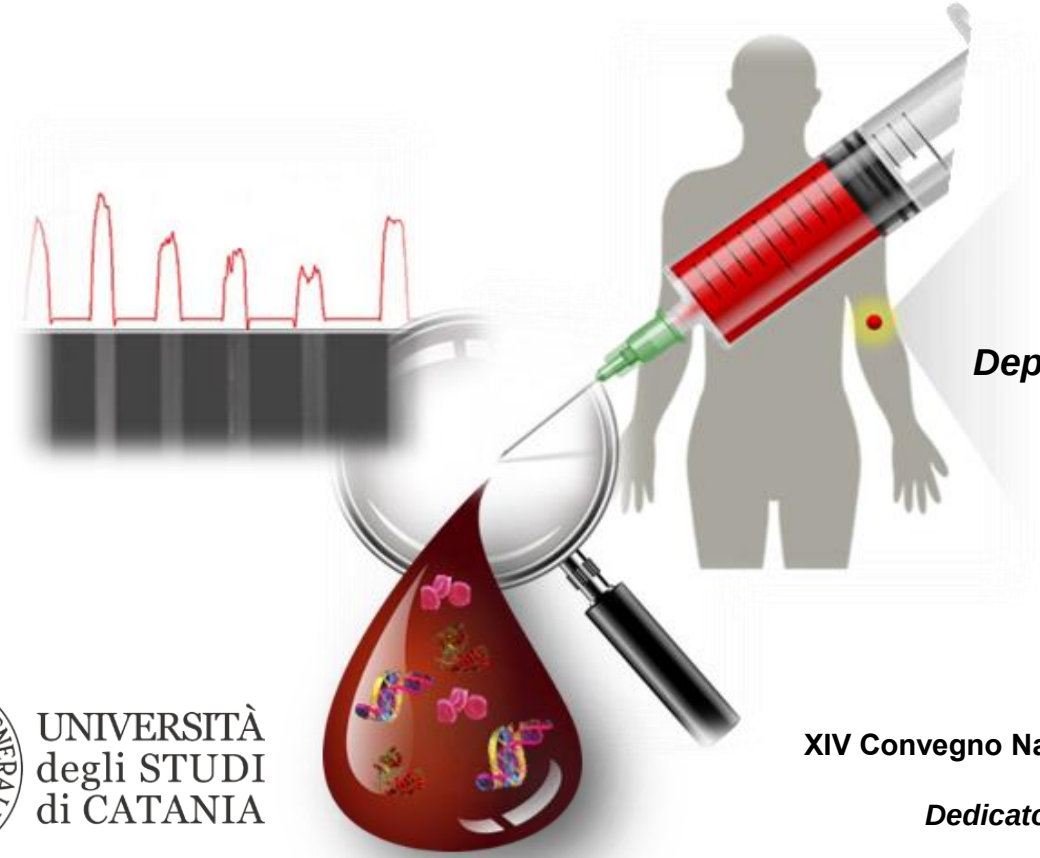
HIGH-PERFORMANCE PLASMONIC IMAGING SENSOR TO REVEAL ONCOGENIC DNA WITH A LIQUID BIOPSY APPROACH



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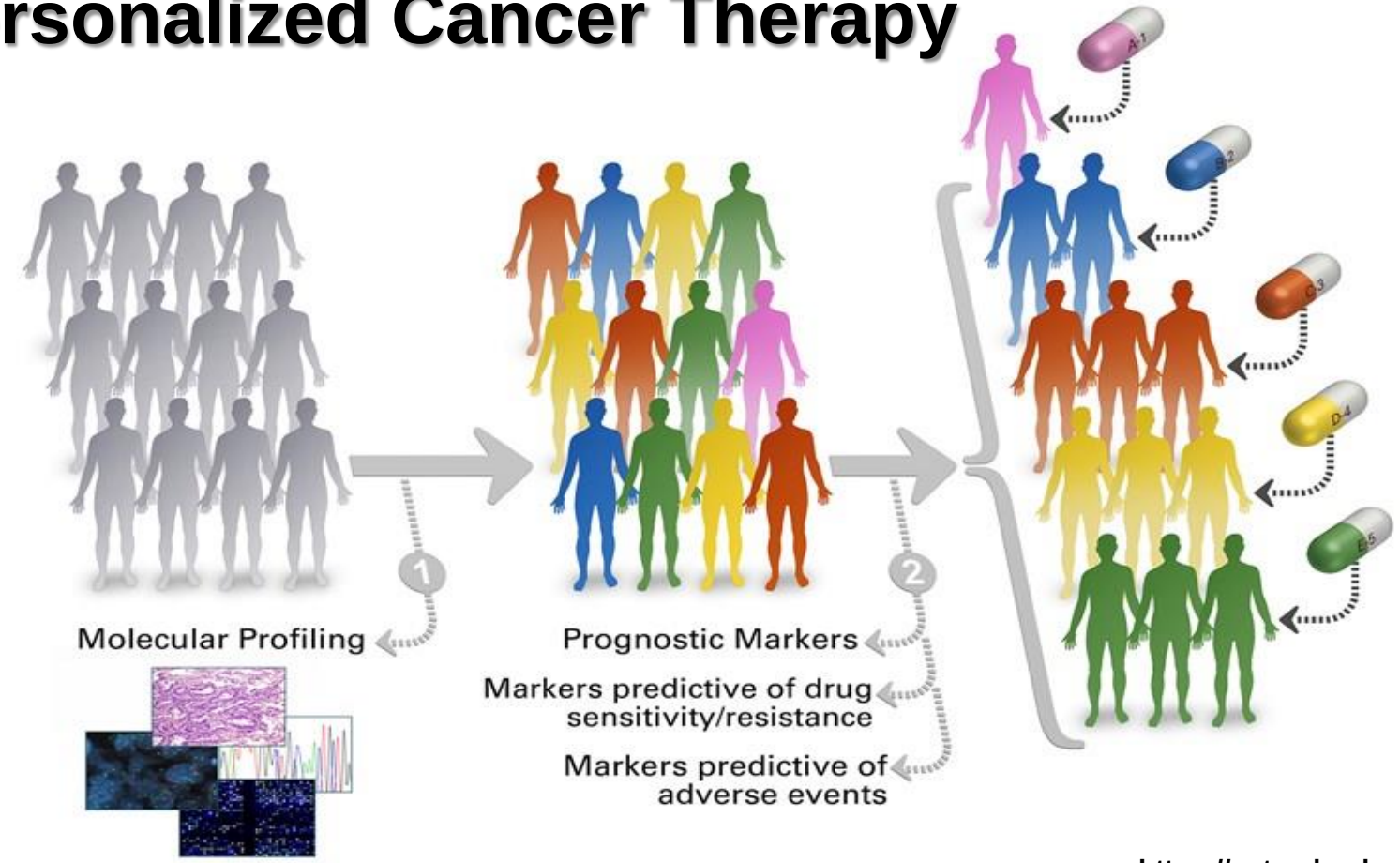
UNIVERSITÀ degli STUDI di CATANIA

I.N.B.B Consorzio Interuniversitario

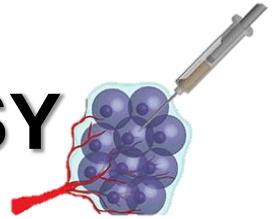
XIV Convegno Nazionale "Ricerca e Innovazione per Ambiente, Salute ed Alimentazione"
***Dedicato alla memoria del Prof. Gustavo Mita*,**
17-18 Novembre 2022, Roma

Personalized Cancer Therapy

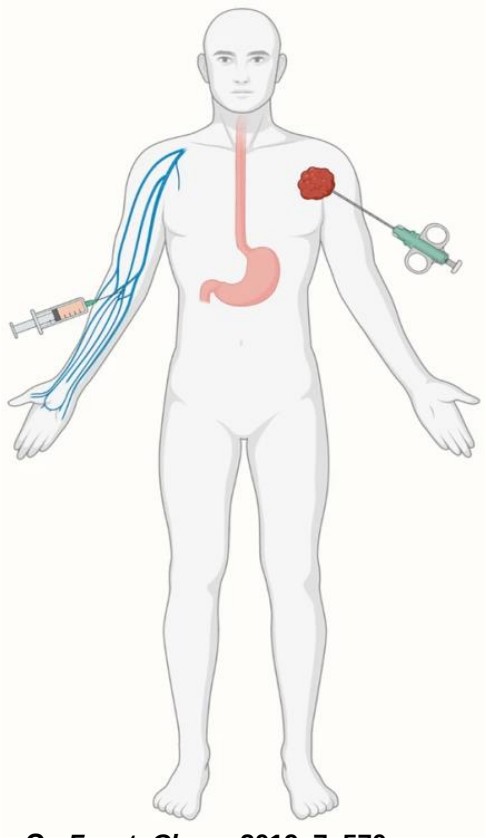


https://pct.mdanderson.org/

LIQUID BIOPSY Vs TISSUE BIOPSY



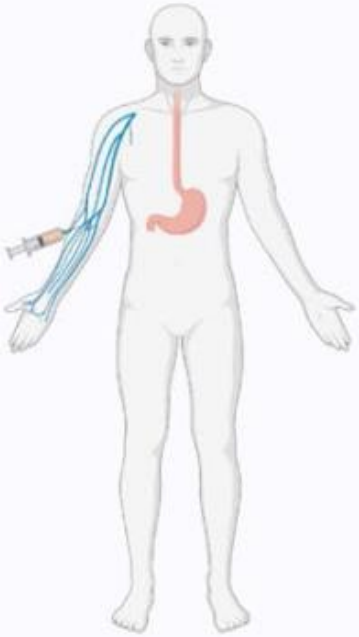
- *Body fluids (usually blood)*
- *Non invasive*
- *Assessment of tumor*
- *Heterogeneity*
- *No subject to tumor accessibility and patient condition*
- *Frequent monitoring*
- *Faster and cheaper than tissue biopsy*
- *Lack of standardization*



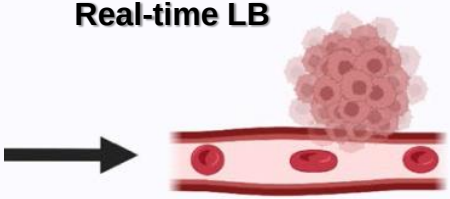
- *Invasive*
- *Potential clinical complications*
- *Snapshot: difficulty in accounting for inter and intra tumor heterogeneity*
- *Subject to tumor accessibility and patient condition*
- *No frequent monitoring*
- *Costly*
- *Gold standard in clinical practice*

Bellassai, N., D'Agata, R., Jungbluth, V., and Spoto, G., *Front. Chem*. 2019, 7, 570.

Circulating Biomarkers & Liquid Biopsy (LB)



Real-time LB



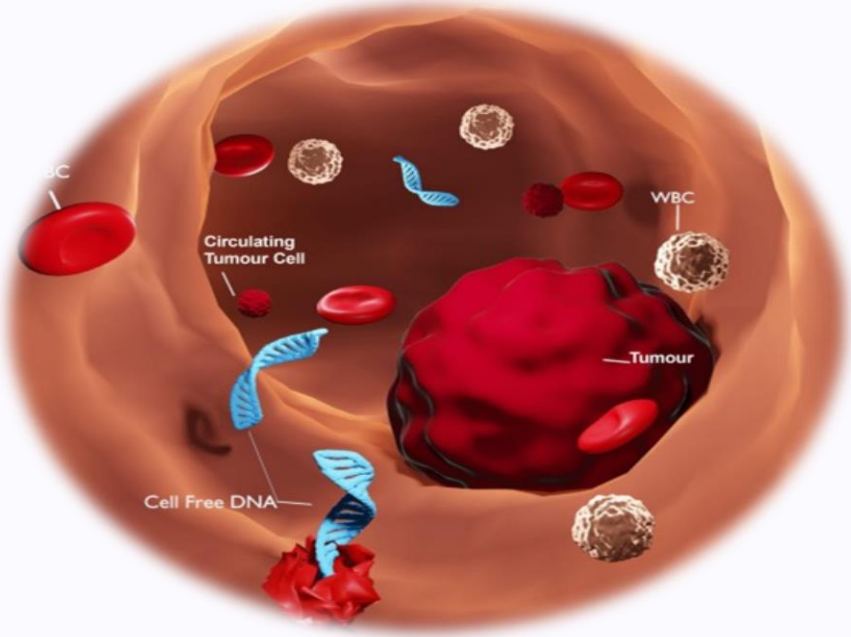
ctDNA

miRNAs

Proteins

CTCs

EVs

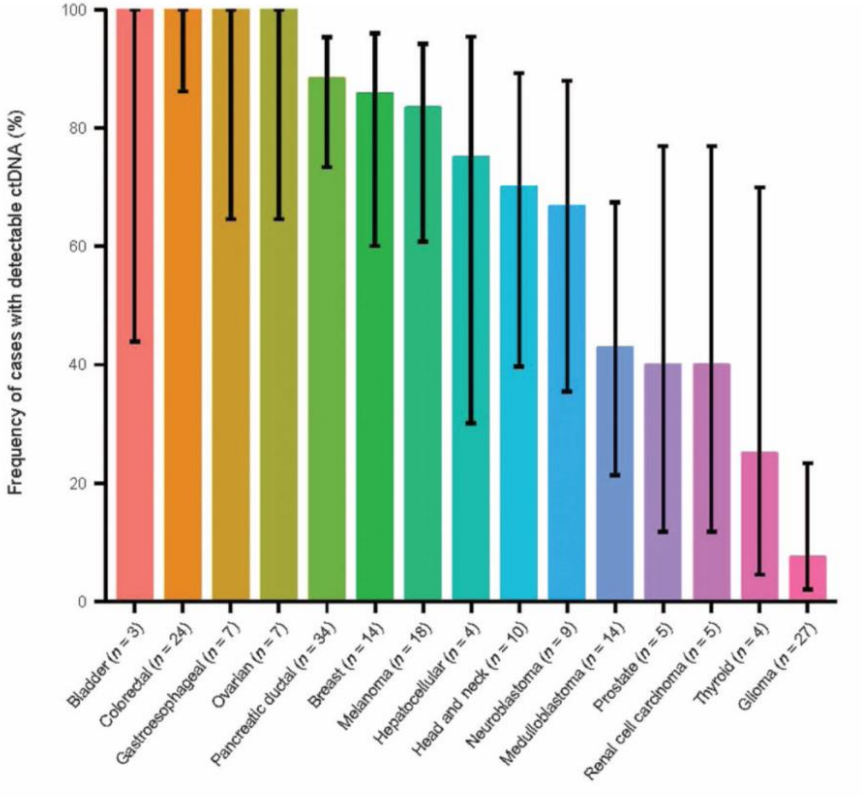


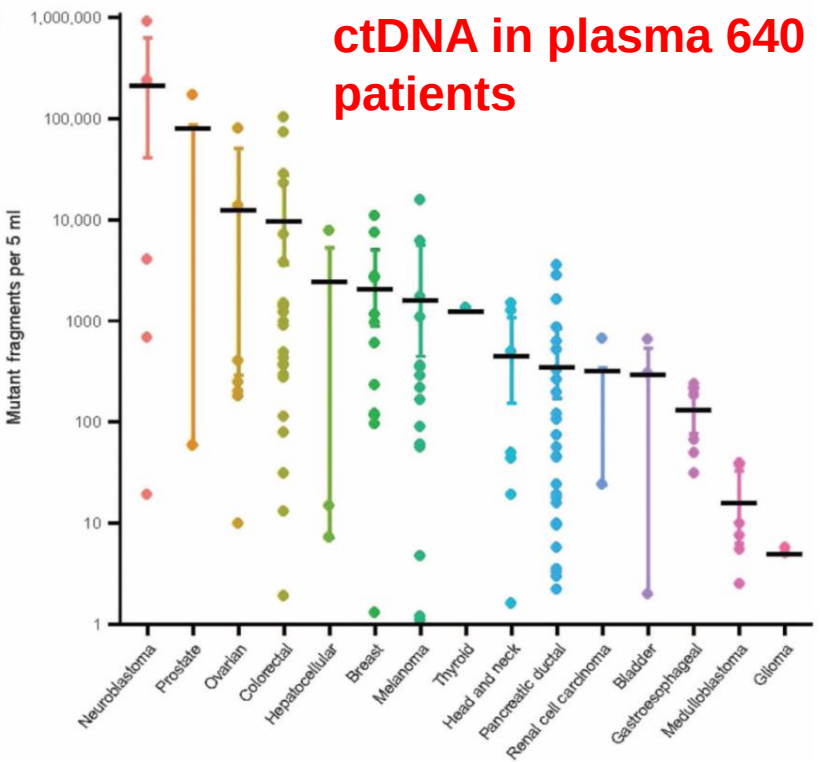
Cell-free DNA(cfDNA) exists as fragments of ~ 180 base pairs in length; the tumor-derived fraction of cfDNA is termed circulating tumor DNA (ctDNA)

Lone et al. *Molecular Cancer*, 2022, 21, 79.

Detection of cfDNA and ctDNA: challenges

Concentration

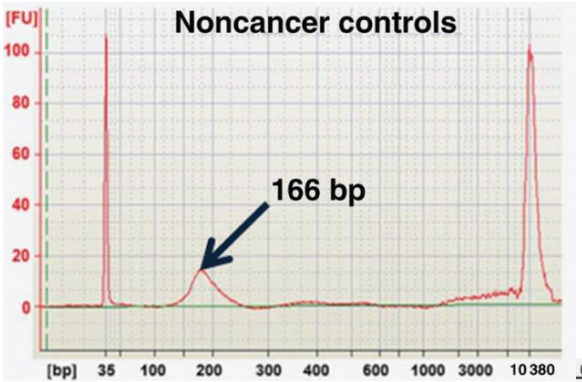


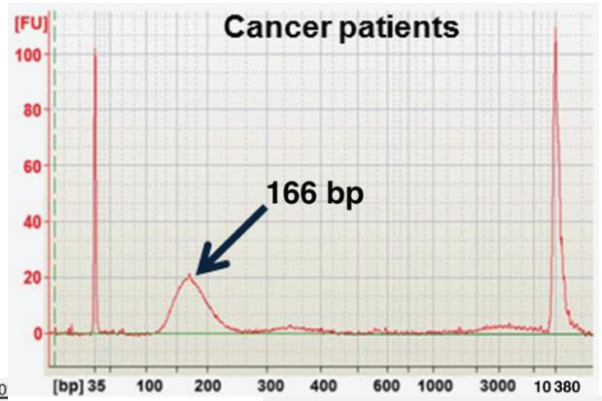


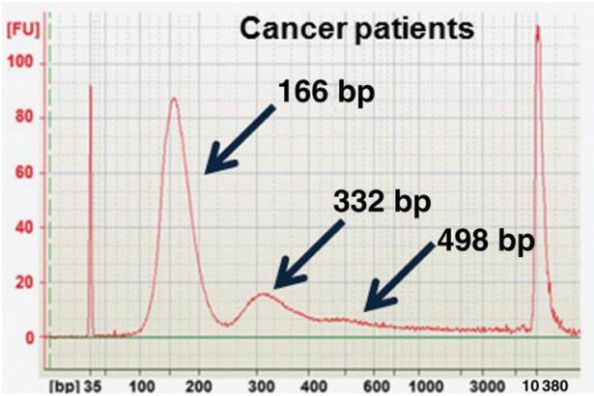
Bettegowda et al. *Sci Transl Med.* 19, 2014, 6(224)

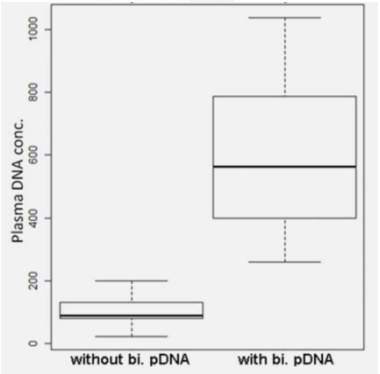
Detection of cfDNA and ctDNA: challenges

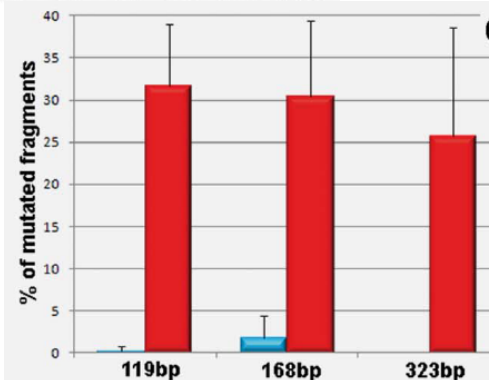
Highly fragmented











Heitzer et al.
Int. J. Cancer: 133, 2013, 346–357

Integration of LB into clinical practice

- Ensure analytical validity
- Establish clinical validity
- Demonstrate clinical utility#

- Obtain sufficient level of evidence for the assay in a specific clinical indication
- Evaluate cost-effectiveness

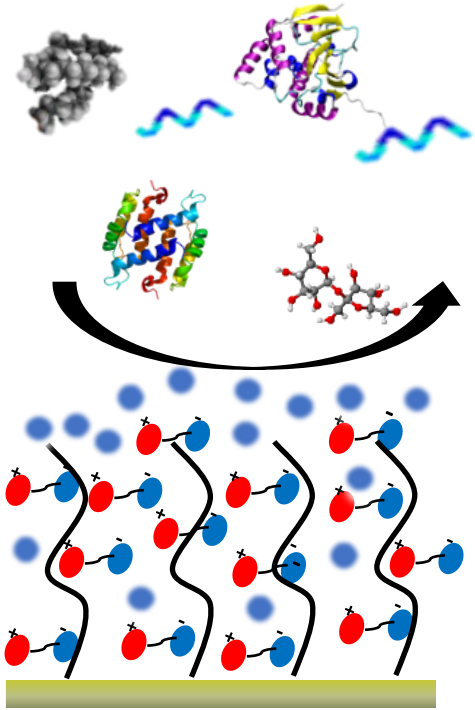
- Invest in laboratory and human resources
- Train physician in application of the test and interpretation of the findings
- Create standard operating procedures for application in different clinical scenarios

*** Needs for standardization in pre-analytical procedures**
 # Large clinical validation studies are now mandatory

Ignatiadis et al. *Nat. Rev. Clin. Oncol.* 2021.

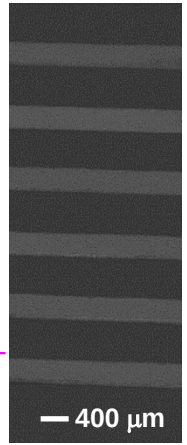
Surface Plasmon Resonance (SPR) Biosensing in Clinical Diagnostics

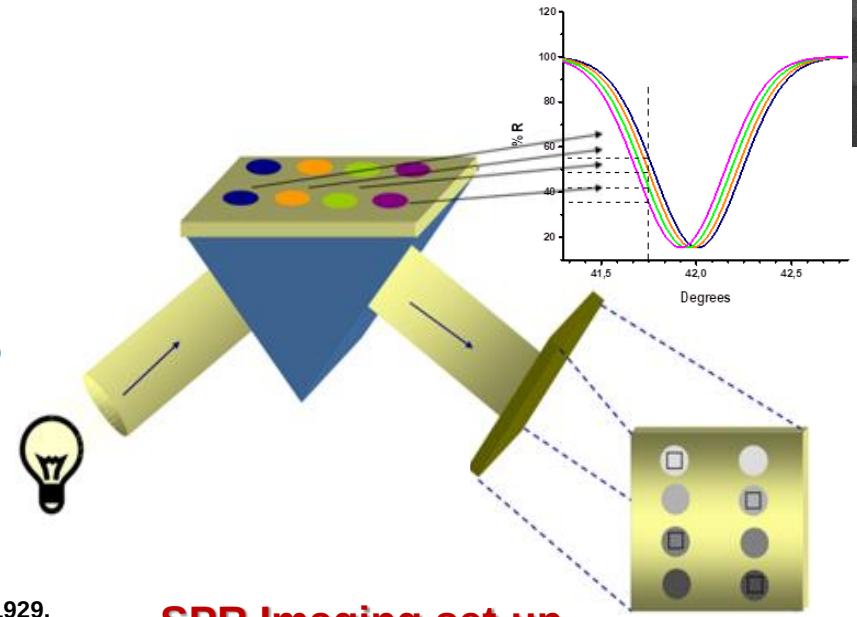
Patient's sample



SPR sensor surface

- Real-time analysis
- High sensitivity
- No labeling requirements
- Multiplexing capability





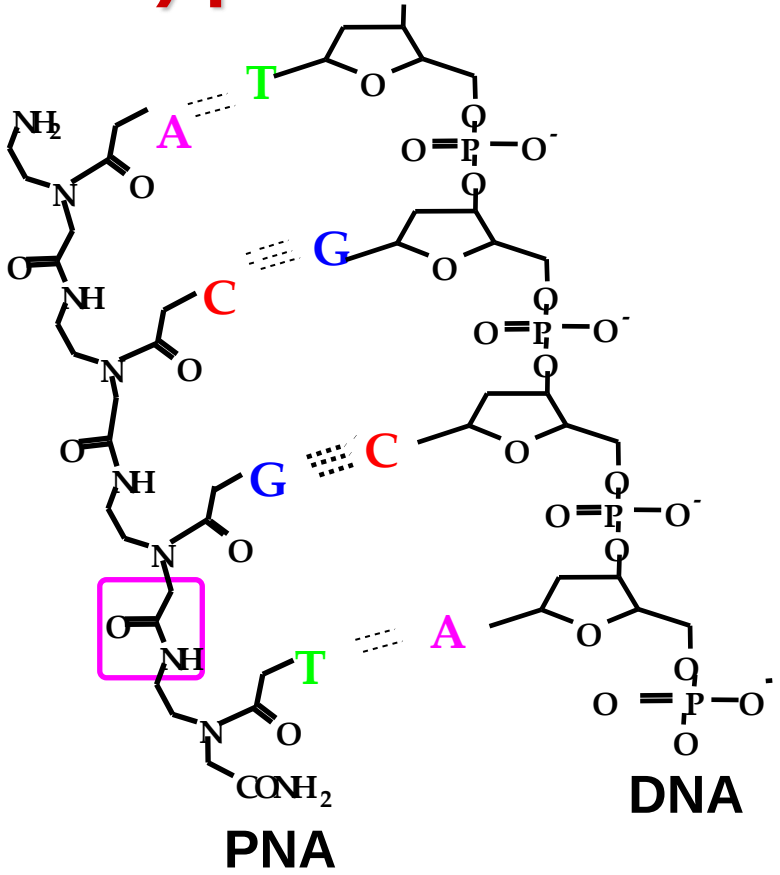
SPR Imaging set-up

D'Agata, R. Bellassai, N. Jungbluth, V. Spoto, G. *Polymers* 2021, 13, 1929.
Spoto G., Minunni M., *J Phys Chem Lett.* 2012, 3, 2682.

Peptide nucleic acid (PNA) probe

PNA vs DNA

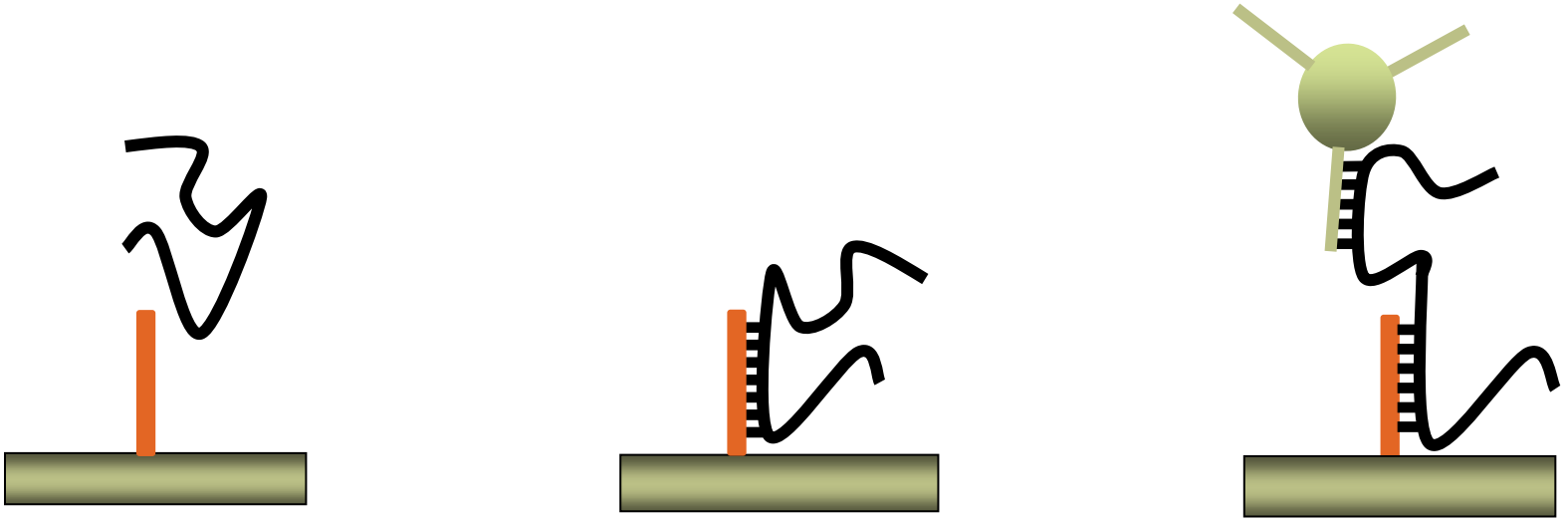
- **High affinity to complementary DNA or RNA**
- **High sequence-specificity**
- **Stable. Resistant to nucleases**



Scaffold: N-(2-aminoethyl)-glycine

P.E. Nielsen et al. *Science* 1991, 254, *1497-1500.*
R. D'Agata, G. Spoto, Artificial DNA: PNA & XNA 2012, 3, 45-52.
D'Agata R., Giuffrida MC, Spoto G. *Molecules* 2017, 22, 1951.

Nanoparticle-enhanced SPR Imaging



Signal enhancement:

-Surface mass loading of NPs

-Dielectric constant of AuNPs

-EF enhancement

Calcagno M., et al. *Anal. Chem*. 2022, 94, 2, 1118–1125.

R. D'Agata, P. Palladino, G. Spoto. *Beilstein J. Nanotechnol*. 2017, 8, 1–11.

R. D'Agata, G. Spoto, *Anal. Bioanal. Chem*. 2013, 405, 573–584.

Liquid biopsy technologies Vs Nanoparticle-enhanced SPRI

DNA isolation from patient's plasma

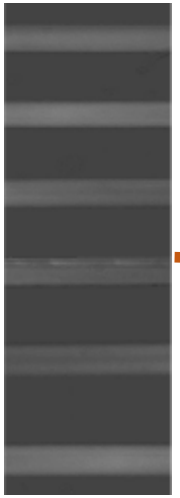
Next generation sequencing

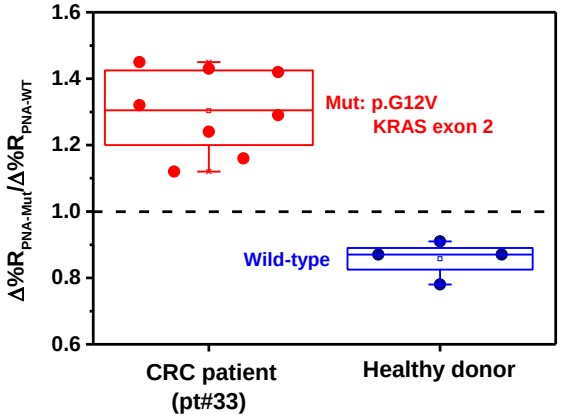
- Input 1-3 mL → Input 40 μL
- Blood waste → No blood waste
- Time, costly → 1.5 hours (10-12 min. hands-on)
- Risk of sample contamination → Reduced risk

Polymerase chain reaction (PCR)

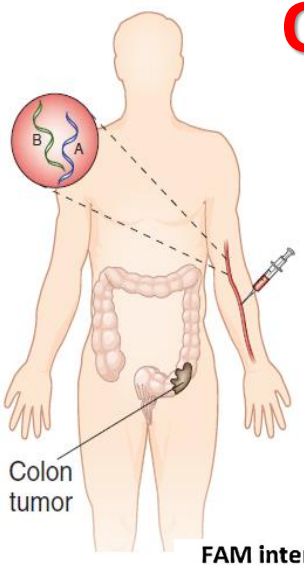
- Prone to sample contamination
- Preferential amplification of short template sequences
- Artifact generated by recombination between homologous regions of DNA
- Subject to errors (~1:10^6 bp)

Digital PCR





Colorectal (CRC) cancer clinical samples

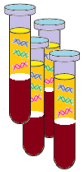


Blood draw

4 x 6mL whole blood samples

2000g x 20', 4°C centrifugation

Plasma isolation



~5-7x 2mL plasma samples

16000g x 10', 4°C centrifugation

Plasma collection and banking

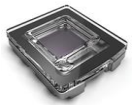
~5-7x 1.8mL plasma samples

Storage at -80°C

Extraction of cfDNA by Qiamp CNA kit

~ 30-50µL eluate

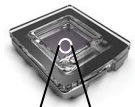
~ 7µL



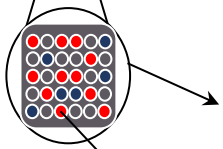
~ 15µL final volume

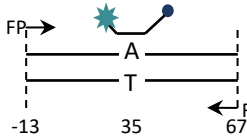
PCR reaction

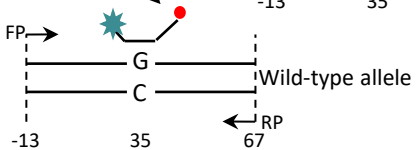
digital PCR

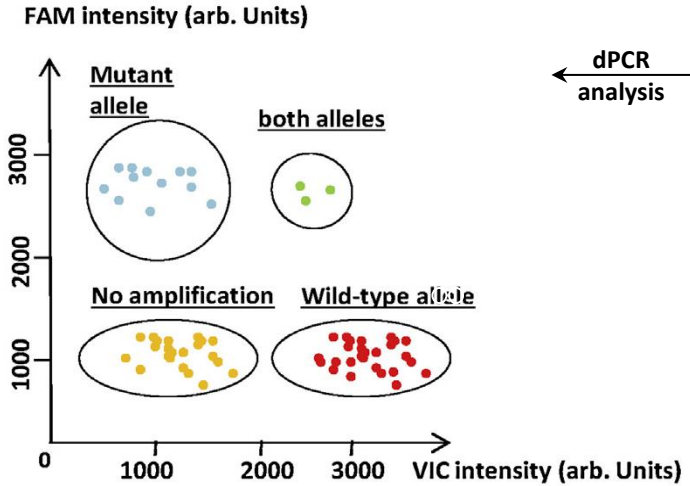


dPCR analysis





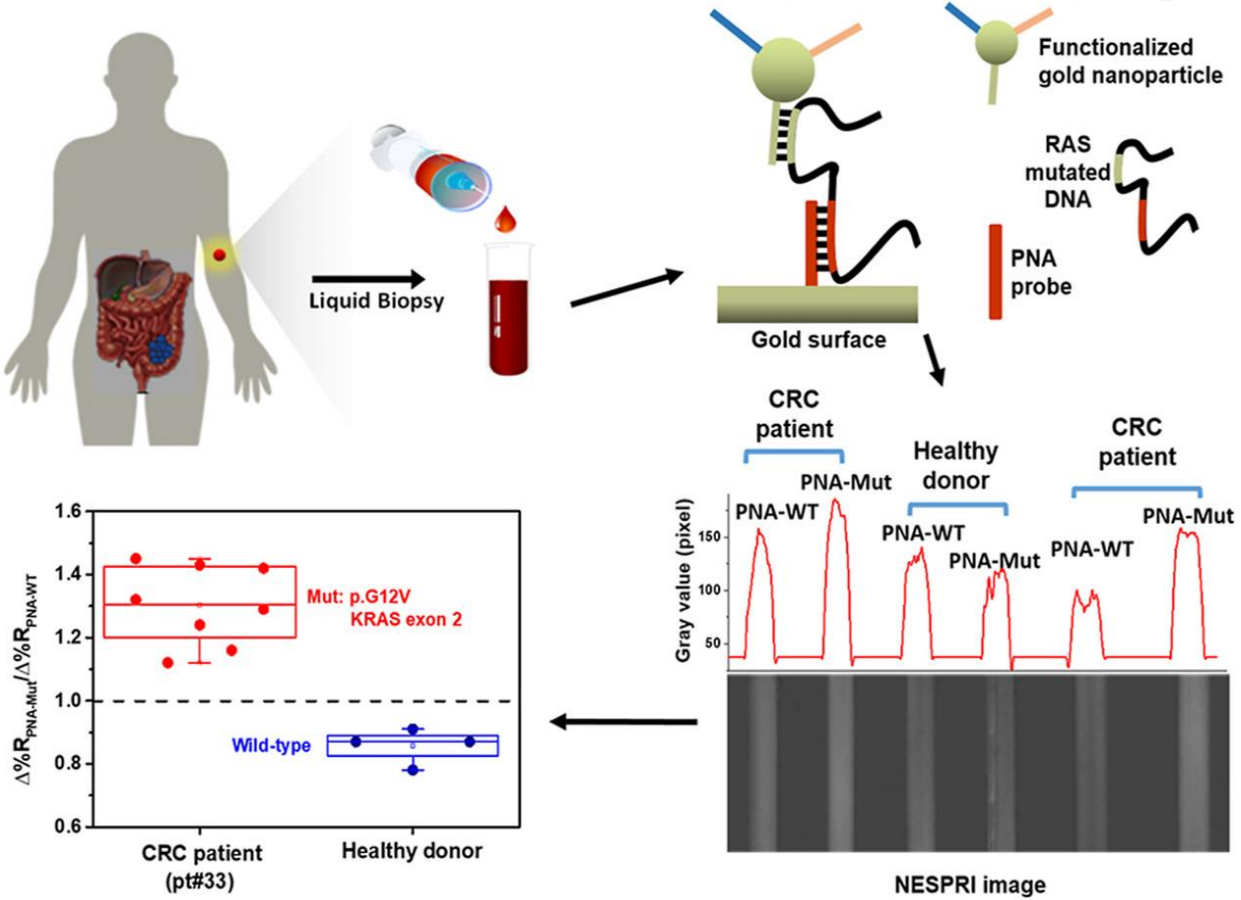




IRE ISTITUTO NAZIONALE TUMORI REGINA ELENA

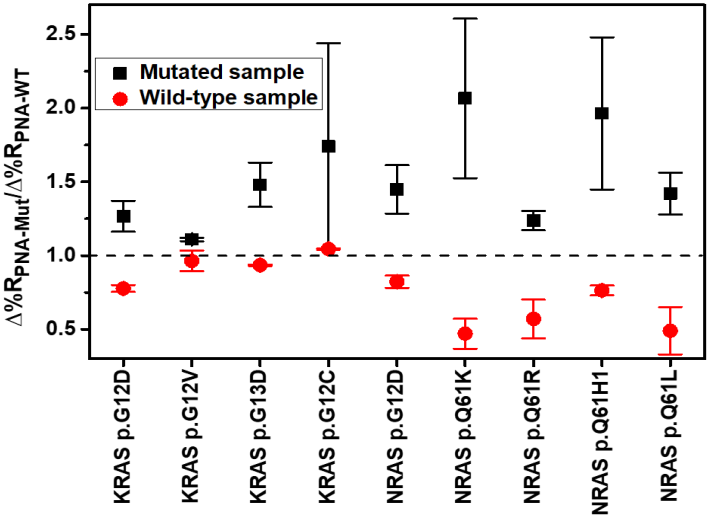
Nanoparticle-enhanced SPRI for liquid biopsy

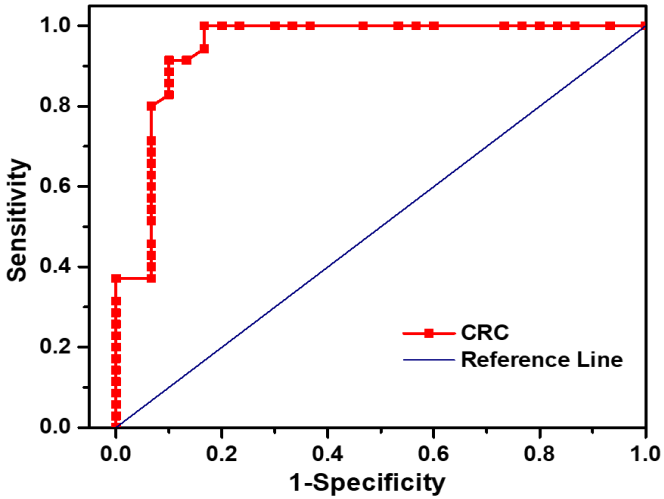
- ✓ Label and PCR-free assay
- ✓ Fast and real-time analysis
- ✓ Multi-analyte monitoring
- ✓ Small sample volume (< 40 µL)



D'Agata R. et al., *Biosens. Bioelectron.* 2020, *170*, 112648.

SPRI detection of oncogenic DNA from tissue biopsy of colorectal cancer (CRC) patients





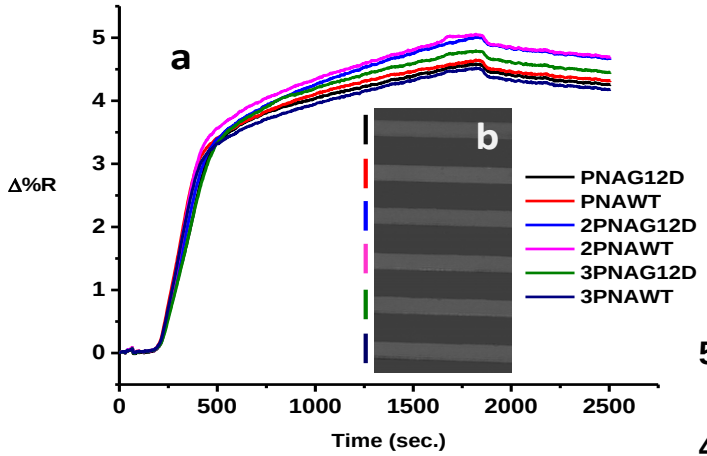
Targeting 11 selected KRAS (exon 2) and NRAS (exons 2 and 3) mutations.

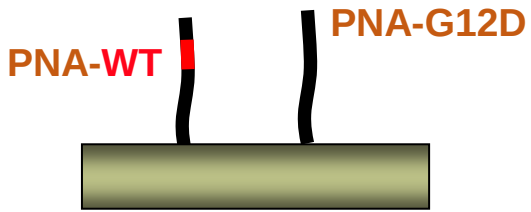
AUC= 0.947; sensitivity = 100%; specificity = 83.33%

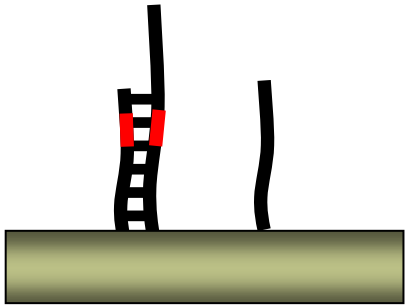
Isolated tDNA, no PCR,
wild-type = isolated gDNA

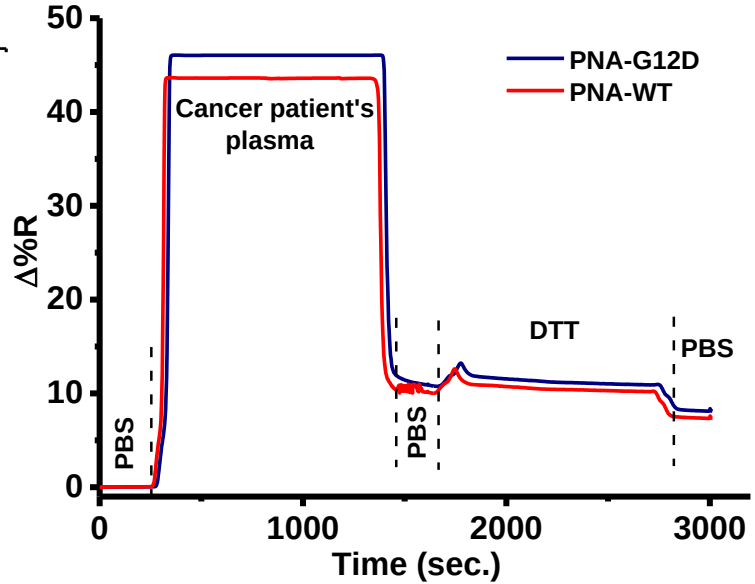
D'Agata R. et al., *Biosens. Bioelectron.* 2020, *170*, 112648

SPRI detection of oncogenic DNA in blood





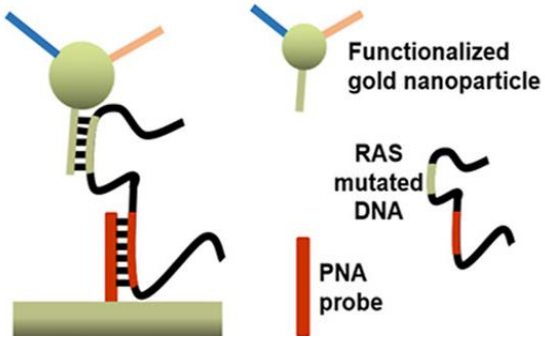




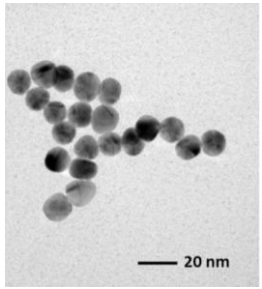
- ✓ **Pre-analytical treatment 1.5 h (Proteinase K)**
- ✓ **Dithiothreitol treatment 20 min.**

D'Agata R. et al., *Biosens. Bioelectron.* 2020, *170*, 112648

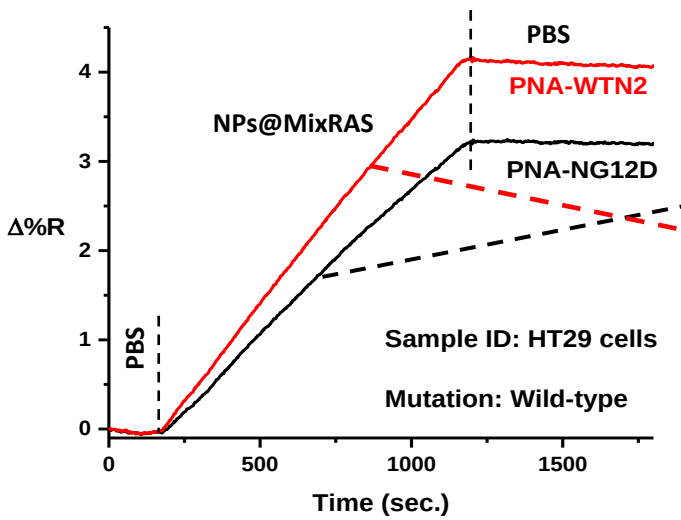
SPRI detection of oncogenic DNA in blood



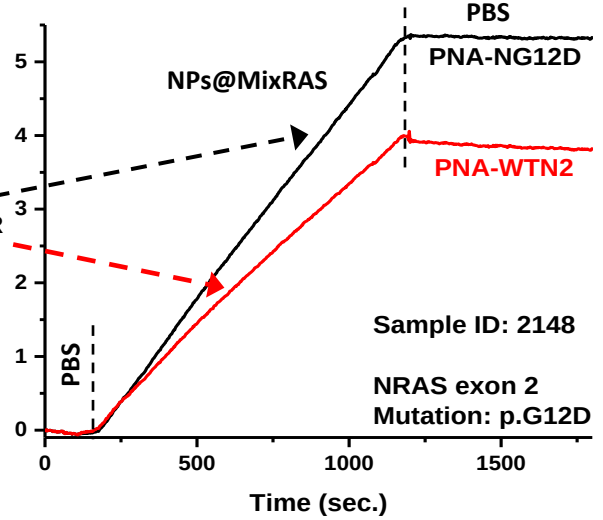
Optimized a protocol to produce one single set of shelf-stable functionalized AuNPs for NESPRI detection of all the selected *RAS* mutations.



Healthy donor



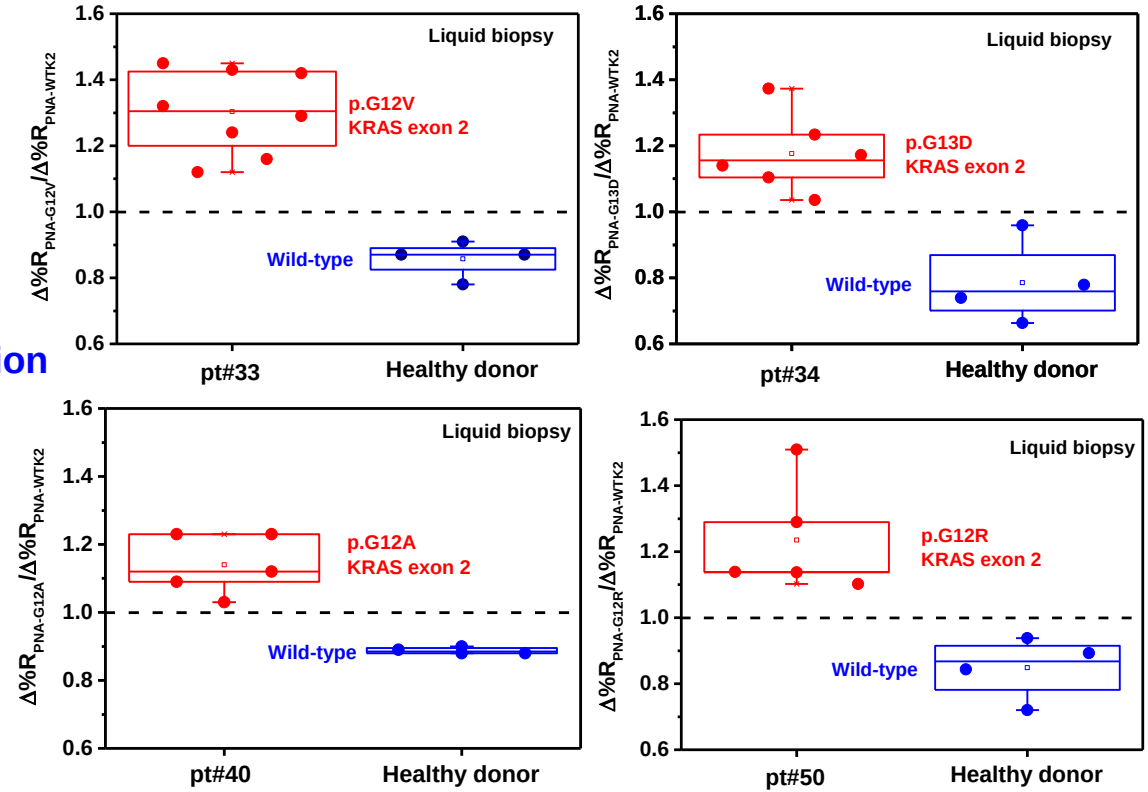
Colorectal cancer patient

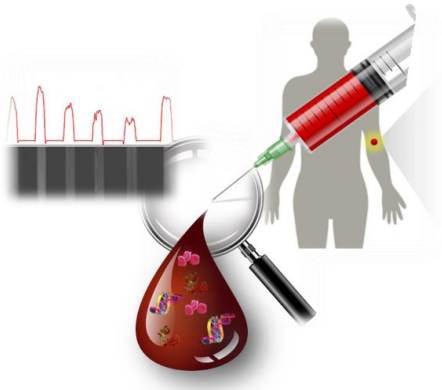


D'Agata R. et al., *Biosens. Bioelectron.* 2020, *170*, 112648

Liquid biopsy CRC patients

No PCR
No DNA isolation





Reduced:

- ✓ **Sample contamination**
- ✓ **Analysis time**
- ✓ **Assay costs**

All genomic DNAs (gDNAs) and cfDNAs were KRAS genotyped by targeted NGS and confirmed by specific digital PCR (dPCR) assays using as templates either gDNA (20 ng) or ctDNA (from 0.5 mL of plasma).

D'Agata R. et al., *Biosens. Bioelectron.* 2020, *170*, 112648

European Union's Horizon 2020 research and innovation programme under grant agreement n. 764281

ULTRAPLACAD

Bimodal industrial prototype

integrating surface plasmon resonance imaging (NESPRI) and plasmon-enhanced fluorescence (PEFSI) sensing technologies

ULTRAsensitive PLAsmonic devices for early CAncer Diagnosis

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MESA+ Institute for Nanotechnology
GINOLIS
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I.N.B.B.
Universität Siegen
IRE Istituto Nazionale Tumori Regina Elena
future diagnostics
AMIRES
Università degli Studi di Ferrara - Ex Labore Fructus -
HORIBA Scientific
SCRIBA Nanotechnologie
VTT
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ULTRAPLACAD as success story!

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Project Team

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Thank you!

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University of Ferrara
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Dr Jessica Gasparello

VTT- Oulu (Finland)
Dr Sanna Aikio
Prof Jussi Hiltunen
Dr Christina Liedert

European Innovation Council
Funded by the European Union
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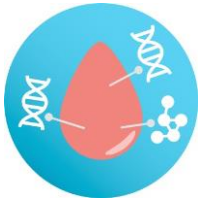
PRIN 2017
STARTING GRANT 2020 Project PATmiREC
ULTRAPLACAD

Didida: detecting multiple diseases at once

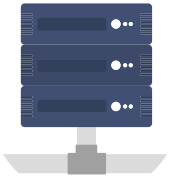
A cost-effective mobile based solution in Africa

User-friendly paper-based test



Detecting multiple diseases from one sample

Mobile application for data transmission



Connected platform for data collection and analysis

Reporting to health care workers

Aggregated collected data to make informed decisions in a specific region

Funded by the European Union

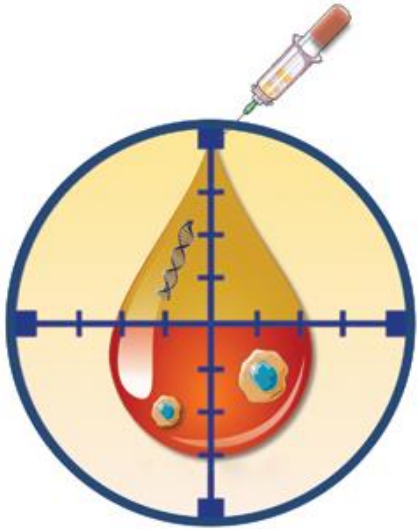
UK Research and Innovation

The consortium includes 14 partners from 8 countries: Kenya, Senegal, Tanzania, Uganda, United Kingdom, France, the Netherlands and Italy.

LB supports clinical decision-making and guides therapeutic choices for CRC patient care

Therapeutic targets (KRAS and NRAS genes)

Assessing plasma for the presence of specific mutations that can direct patient management- is clinically actionable.



Resistance mechanisms

Identifying resistance mutations that occur when patients first respond to therapy and then progress remarkably frequent mutations at codon 61 of NRAS and of KRAS, representing 46% of the detected mutations in patients resistant to EGFR blockade (e.g. cetuximab)

May 2021 FDA approved AMG510 (sotorasib) as the first treatment for adult patients with KRAS p.G12C mutated non-small cell lung cancer.