



Con il patrocinio di



## 11<sup>a</sup> EDIZIONE Progetto CANOA

# CARCINOMA MAMMARIO:

### QUALI NOVITA' PER IL 2021?

"Saper leggere" uno studio clinico per migliorare la pratica clinica

26 Marzo 2021

ore 14.00

FAD SINCRONA - WEBINAR

in diretta da  
Negrar di Valpolicella



Coordinatori scientifici: Stefania Gori - Giovanni L. Pappagallo



*University of Palermo*

Dipartimento di Discipline Chirurgiche,  
Oncologiche e Stomatologiche

UOC di Oncologia Medica  
(Dir.: Prof. Antonio Russo)



I SESSIONE: Il carcinoma mammario  
HR+/HER-2 negativo

Biopsia liquida: quale ruolo in questo  
setting?

Antonio Russo

# DISCLOSURE INFORMATION



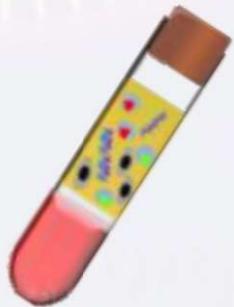
All financial reports made in the last two years

- Honorarium for advisory boards: Bristol, Pfizer, Bayer, Kyowa Kirin, Ambrosetti
- Speaker honorarium: Roche Diagnostic



# Liquid biopsy in BC

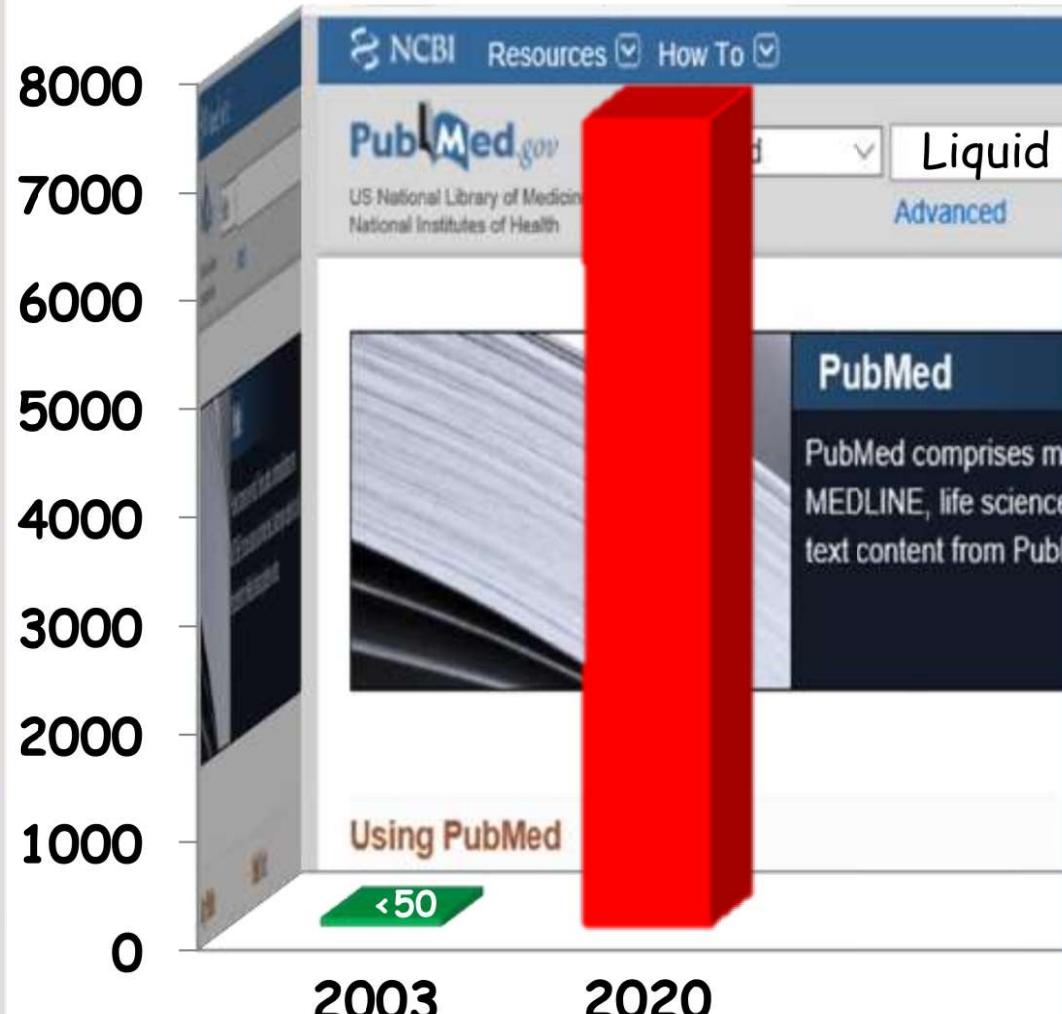
## OUTLINE



- **Introduction to liquid biopsy**
- **Potential clinical applications in Breast Cancer**

# Liquid Biopsy

## The extent of the problem



### Liquid biopsy in cancer

Gut, 915-16, 2003

Mutant K-ras2 in serum

H. J. N. Andreescu,<sup>1</sup> R. Benamouzig,<sup>2</sup> M. Beranek,<sup>3</sup> P. Clarke,<sup>4</sup> D. Cunningham,<sup>5</sup> A. R. Norman,<sup>6</sup> W. Giaretti,<sup>7</sup> A. F. P. M. de Goeij,<sup>7</sup> B. J. Iacopetta,<sup>8</sup> E. Julian,<sup>9</sup> K. Krtolica,<sup>10</sup> J. Lee,<sup>11</sup> S. T. Wang,<sup>11</sup> N. Lee,<sup>12</sup> F. Al-Mulla,<sup>13</sup> D. Muir,<sup>11</sup> M. Pauli,<sup>14</sup> V. V. Prichard,<sup>10</sup> A. Russo,<sup>17</sup> C. Tringali,<sup>18</sup> N. Umarovit,<sup>19</sup> P. and R. Wang<sup>20</sup>

Ann Oncol. 84-90, 2006

**Molecular detection of TP53, Ki-Ras and p16<sup>INK4A</sup> promoter methylation in plasma of patients with colorectal cancer and its association with prognosis. Results of a 3-year GOIM (Gruppo Oncologico dell'Italia Meridionale) prospective study**

V. Bazzan<sup>1\*</sup>, L. Bruno<sup>1#</sup>, C. Augello<sup>1</sup>, V. Agnese<sup>1</sup>, V. Calò<sup>1</sup>, S. Corsale<sup>1</sup>, G. Gargano<sup>1</sup>, M. Temasi<sup>1</sup>, V. Schiro<sup>1</sup>, G. Di Fede<sup>1</sup>, V. Adamo<sup>2</sup>, C. Intrivici<sup>1</sup>, A. Crosta<sup>1</sup>, G. Rinaldi<sup>1</sup>, F. Latteri<sup>2</sup>, G. Dardanoni<sup>6</sup>, N. Grassi<sup>4</sup>, M. R. Valerio<sup>1</sup>, G. Colucci<sup>6</sup>, M. Macaluso<sup>5</sup> & A. Russo<sup>1</sup>

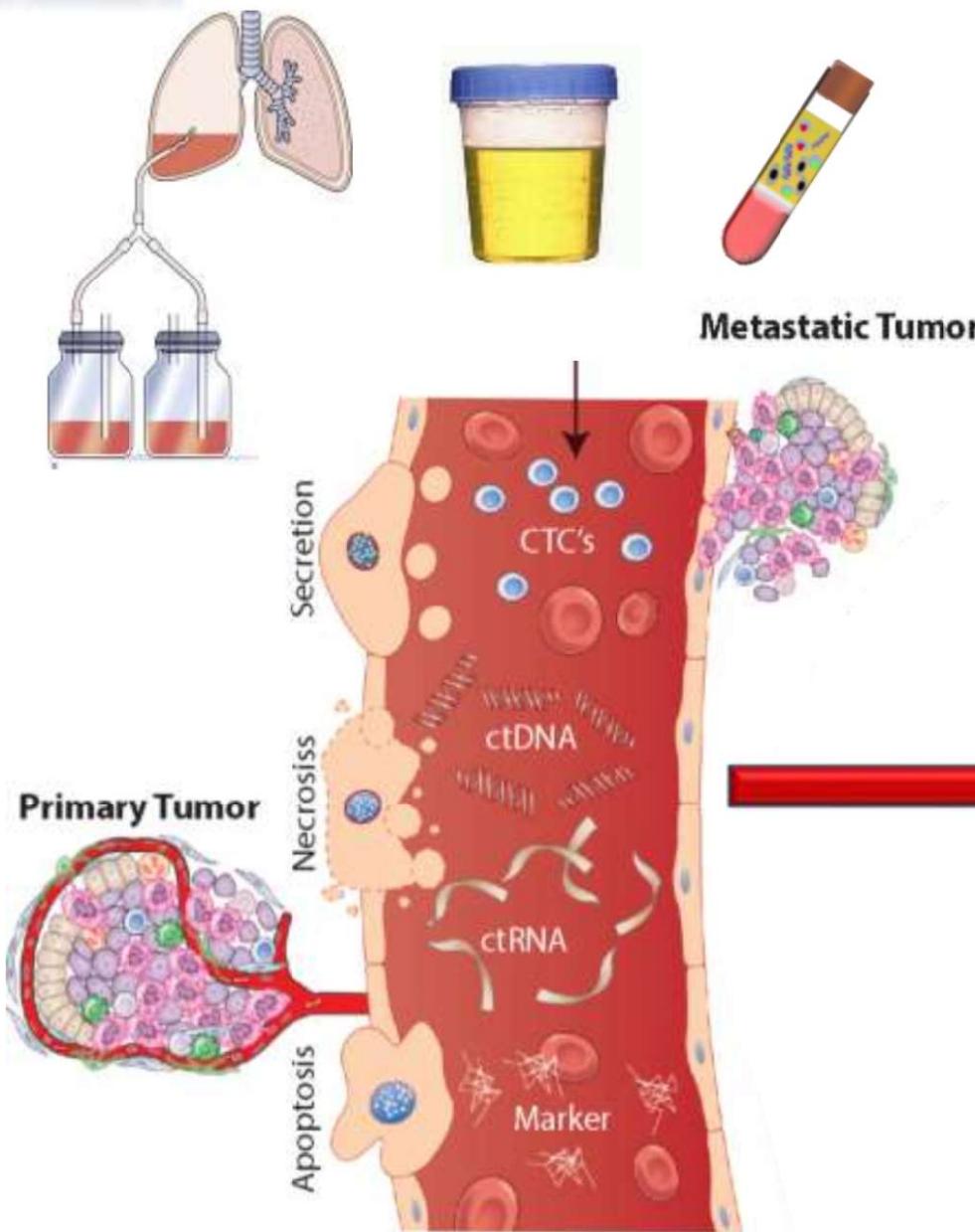
Ann Oncol. 78-83, 2006

**Detection and quantification of mammaglobin in the blood of breast cancer patients: can it be useful as a potential clinical marker? Preliminary results of a GOIM (Gruppo Oncologico dell'Italia Meridionale) prospective study**

G. Gargano<sup>1\*</sup>, V. Agnese<sup>1#</sup>, V. Calò<sup>1</sup>, S. Corsale<sup>1</sup>, C. Augello<sup>1</sup>, L. Bruno<sup>1</sup>, L. La Paglia<sup>1</sup>, A. Gulic<sup>2</sup>, L. Ottini<sup>3</sup>, A. Russo<sup>1</sup>, F. Fultaro<sup>1</sup>, G. Rinaldi<sup>1</sup>, A. Crosta<sup>1</sup>, G. Cloerc<sup>1</sup>, O. Majorana<sup>1</sup>, L. Palmeri<sup>1</sup>, C. Cicotta<sup>1</sup>, A. Agrusa<sup>1</sup>, G. Gulotta<sup>1</sup>, V. Micralto<sup>2</sup>, G. Di Fede<sup>1</sup>, V. Adamo<sup>1</sup>, G. Colucci<sup>6</sup>, R. M. Tommasi<sup>2</sup>, M. R. Valerio<sup>1</sup>, V. Bazzan<sup>1</sup> & Antonio Russo<sup>1</sup>

# Liquid Biopsy

## Definition



### Circulating Tumor Cells (CTCs)

- Protein expression
- Gene expression
- DNA abnormalities
- miRNAs
- Epigenetic alteration
- Functional studies
- Single cell analysis
- Tumor heterogeneity



### Circulating Tumor DNA (ctDNA)

- Tumor mutational burden
- Amplifications/deletions
- Translocations
- Point mutations
- Chromosomal abnormalities
- Tumor heterogeneity



### ctDNA/methylation

- Epigenetic alterations
- DNA methylation
- Tumor heterogeneity



### Circulating miRNAs



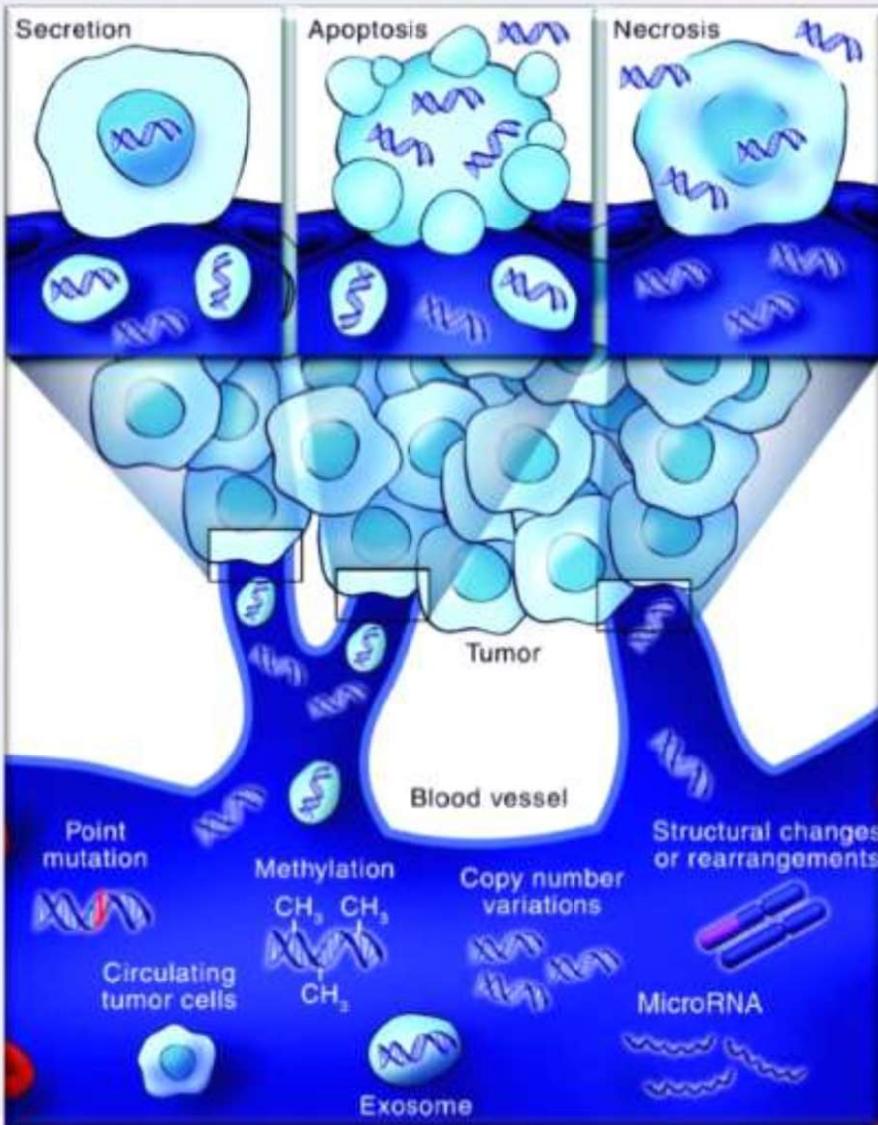
### Extracellular vesicles





# Liquid Biopsy

## Cell-free DNA (cfDNA): Mechanisms of release



cfDNA is mostly released from cells through:

- ✓ Apoptosis
- ✓ Necrosis
- ✓ Active secretion

Mechanism of cfDNA clearance from circulation:

- ✓ Nuclease action
- ✓ Renal excretion into urine
- ✓ cfDNA uptake in the liver and the spleen

# Liquid Biopsy

Where does the need for liquid biopsy come from?

## Liquid Biopsy

VS

## Tissue Biopsy

- Patient-friendly, Minimally invasive
- ↓ costs and risks of complications

Easily repeatable procedure

Rapid turnaround time

Representative of the tumor TUMOR HETEROGENEITY → **GLOBAL INTEGRATED**

SERIAL: Real-Time monitoring/Dynamic



Treatment selection and assignment to clinical trials. (larger sample sizes)

- Often invasive and expensive
- Risks of complications

Not always feasible (multiple sampling?)

Low turnaround time

Not always representative for the all tumor  
TUMOR HETEROGENEITY → **LOCALIZED**

SINGLE (snapshot of the tumor)

Difficulties in tissue sampling  
(smaller sample sizes)

# Liquid Biopsy in clinical practice

## Relevant issues to be considered

### BIOLOGICAL ISSUES

Reduced amount of ctDNA  
(compared to cfDNA)



Risk of "false negative"  
results

Tumor heterogeneity



MUST be considered for  
results from ctDNA analysis

Choice of cf/ctDNA  
source



Blood, urine, cavitary  
fluids....

Tumor load



Sampling time

### TECHNICAL AND METHODOLOGICAL ISSUES

- Sample collection, processing and storage
- cfDNA extraction and storage
- Identify the most appropriate methods for analysis:
  - Single-target analysis\* → Digital PCR, Real-time PCR
  - Multi-target analysis → NGS

\* Previously characterized on T tissue or highly recurrent mutation



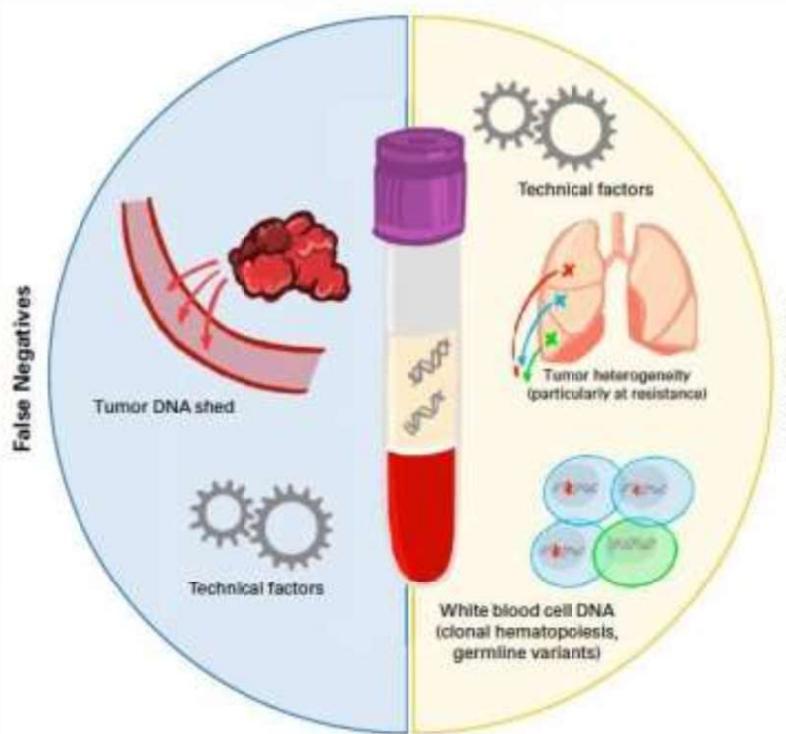
# Relevant issues to be considered... Risk of false negative and false positive results

## «False Negatives» in Liquid Biopsy

**Insufficient DNA shed  
into plasma:**  
(low tumor volume,  
eliminated by therapy)

**cfDNA/ctDNA source:**  
Plasma,  
pleuric/peritoneal  
effusion, CSF

**Technical Issues:**  
Insufficient sensitivity  
in older assays



## «False Positives» in Liquid Biopsy

**Technical Factors:**  
Sample differences  
(>6 months from tissue to  
plasma sampling)

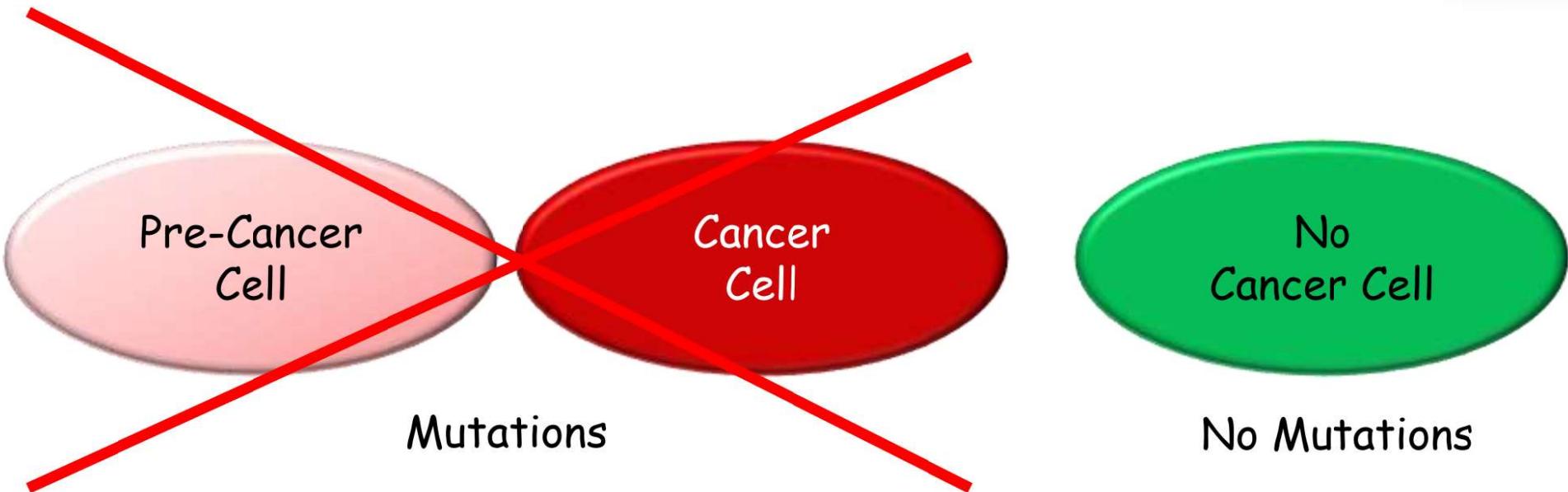
**WBC contamination:**  
Germline Variants  
Clonal Hematopoiesis

**Tumor Heterogeneity:**  
Positive Plasma & Negative  
Tissue  
(assumes tissue is  
Gold standard)



# Liquid biopsy

## Not all somatic mutations are cancer...



THE NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

### Cancer-Associated Mutations in Endometriosis without Cancer

M.S. Anglesio, N. Papadopoulos, A. Ayhan, T.M. Nazeran, M. Noë, H.M. Horlings, A. Lum, S. Jones, J. Senz, T. Seckin, J. Ho, R.-C. Wu, V. Lac, H. Ogawa, B. Tessier-Cloutier, R. Alhassan, A. Wang, Y. Wang, J.D. Cohen, F. Wong, A. Hasanovic, N. Orr, M. Zhang, M. Popoli, W. McMahon, L.D. Wood, A. Mattox, C. Allaire, J. Segars, C. Williams, C. Tornasetti, N. Boyd, K.W. Kinzler, C.B. Gilks, L. Diaz, T.-L. Wang, B. Vogelstein, P.J. Yong, D.G. Huntsman, and I.-M. Shih

THE NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

### Clonal Hematopoiesis and Blood-Cancer Risk Inferred from Blood DNA Sequence

Giulio Genovese, Ph.D., Anna K. Kähler, Ph.D., Robert E. Handsaker, B.S., Johan Lindberg, Ph.D., Samuel A. Rose, B.S., Samuel F. Bakhoum, M.D., Ph.D., Kimberly Chambert, M.S., Eran Mick, B.S., Benjamin M. Neale, Ph.D., Menachem Fromer, Ph.D., Shaun M. Purcell, Ph.D., Oscar Svartesson, M.S., Mikael Landén, Ph.D., Martin Höglund, M.D., Ph.D., Sören Lehmann, M.D., Ph.D., Stacey B. Gabriel, Ph.D., Jennifer L. Moran, Ph.D., Eric S. Lander, Ph.D., Patrick F. Sullivan, M.D., Pamela Sklar, M.D., Ph.D., Henrik Grönberg, M.D., Ph.D., Christina M. Hultman, Ph.D., and Steven A. McCarroll, Ph.D.

THE NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

### Aneurysm Syndromes Caused by Mutations in the TGF- $\beta$ Receptor

Bart L. Loeys, M.D., Ph.D., Ulrike Schwarze, M.D., Tammy Holm, M.D., Bert L. Callewaert, M.D., George H. Thomas, Ph.D., Hariyadarshi Pannu, Ph.D., Julie F. De Backer, M.D., Gretchen L. Oswald, M.S., Sofie Symoens, B.S., Sylvie Manouvrier, M.D., Ph.D., Amy E. Roberts, M.D., Francesca Faravelli, M.D., M. Alba Greco, M.D., Reed E. Pyeritz, M.D., Ph.D., Dianna M. Milewicz, M.D., Ph.D., Paul J. Coucke, Ph.D., Duke E. Cameron, M.D., Alan C. Braverman, M.D., Peter H. Byers, M.D., Anne M. De Paepe, M.D., Ph.D., and Harry C. Dietz, M.D.



# Relevant issues to be considered...

Choose the appropriate method for analysis:  
is technique sensitivity clinically relevant?



Unknown genetic  
alterations → NGS

Technique	Sensitivity (% mutated DNA)	Application	Clinical study
Sanger sequencing	> 10%		
Pyrosequencing	10%	Advanced and metastatic disease	
Next-generation sequencing	1-2%		
Quantitative PCR	1%	Diagnosis, early stage and MMR	



# Liquid biopsy

## Limitations in daily clinical practice

### Tissue Biopsy

vs

### Liquid Biopsy



Evaluation of micro-environment  
(e.g. TILs adv Melanoma or adv BC)

Drug concentration inside the tumor

Evaluation of protein expression (e.g. ER or HER2)

Reliable assessment of signatures (e.g. MSI )

Evidence for treatment selection in multiple tumors for early and adv cancers

**NO** evaluation of micro-environment

**NO** drug concentration inside the tumor

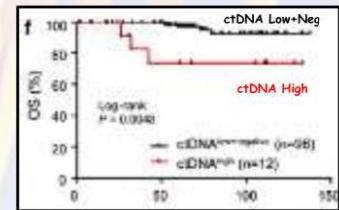
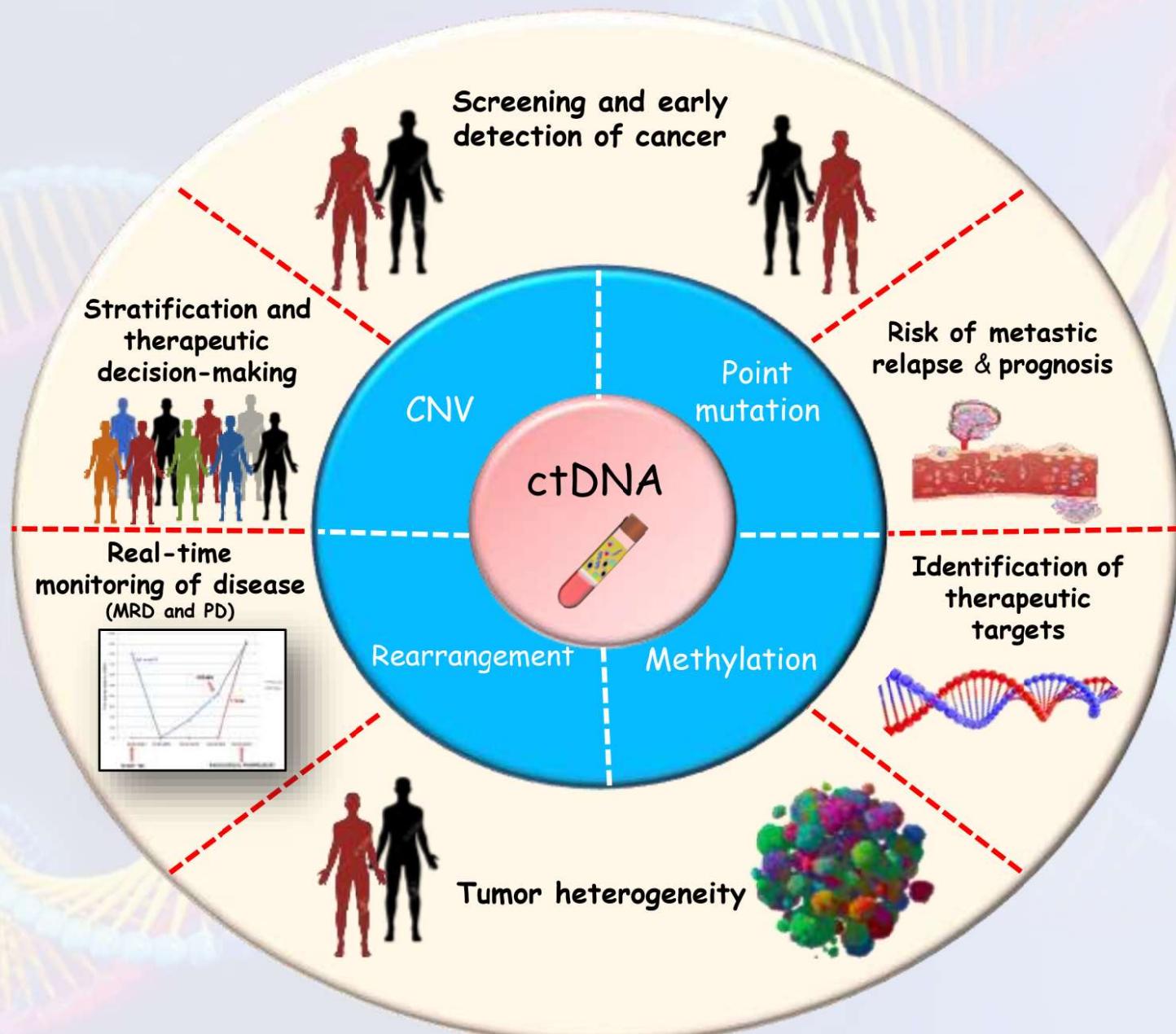
**NO** evaluation of protein expression  
(except for CTCs)

**NO** reliable assessment of signatures like MSI

Restricted number of 'actionable' genes available

# Liquid biopsy

## Potential clinical application





## Liquid biopsy

# Guidelines & Recommendations

### 2017 NCCN Practice Guidelines

- "If repeat biopsy is not feasible, plasma biopsy should be considered"
- "Testing should be conducted as part of broad molecular profiling".

### 2017 ASCO Clinical Cancer Advances

"Even for pts who are able to undergo a traditional tissue biopsy, a liquid biopsy may be safer, quicker, and more convenient and perhaps even more informative."

### 2018 AMP/CAP/IASLC: Molecular Testing Guidelines for Lung Cancer

"Key new recommendations include the inclusion of additional genes (*ERBB2*, *MET*, *BRAF*, *KRAS*, and *RET*) [...] and the use of cell-free DNA to "rule in" targetable mutations when tissue is limited or hard to obtain"

### 2018 A Statement Paper from the IASLC

"Currently, there are two important scenarios in which the liquid biopsy might confer an advantage to pts with adv NSCLC:

initial molecular diagnosis and progression during targeted therapy."

### 2018 ESMO- NSCLC: Clinical Practice Guidelines for diagnosis, treatment and follow-up

The guidelines also recommended the use of cell-free DNA for testing when tissue was unavailable...

**2018 AIOM-SIAPEC-SIBIOC-SIF:** Raccomandazioni per l'esecuzione di test molecolari su biopsia liquida in oncologia  
La biopsia liquida è al momento principalmente utilizzata per l'analisi mutazionale del gene dell'epidermal growth factor receptor (EGFR) in pazienti con NSCLC in stadio avanzato.

# Liquid biopsy

## OUTLINE

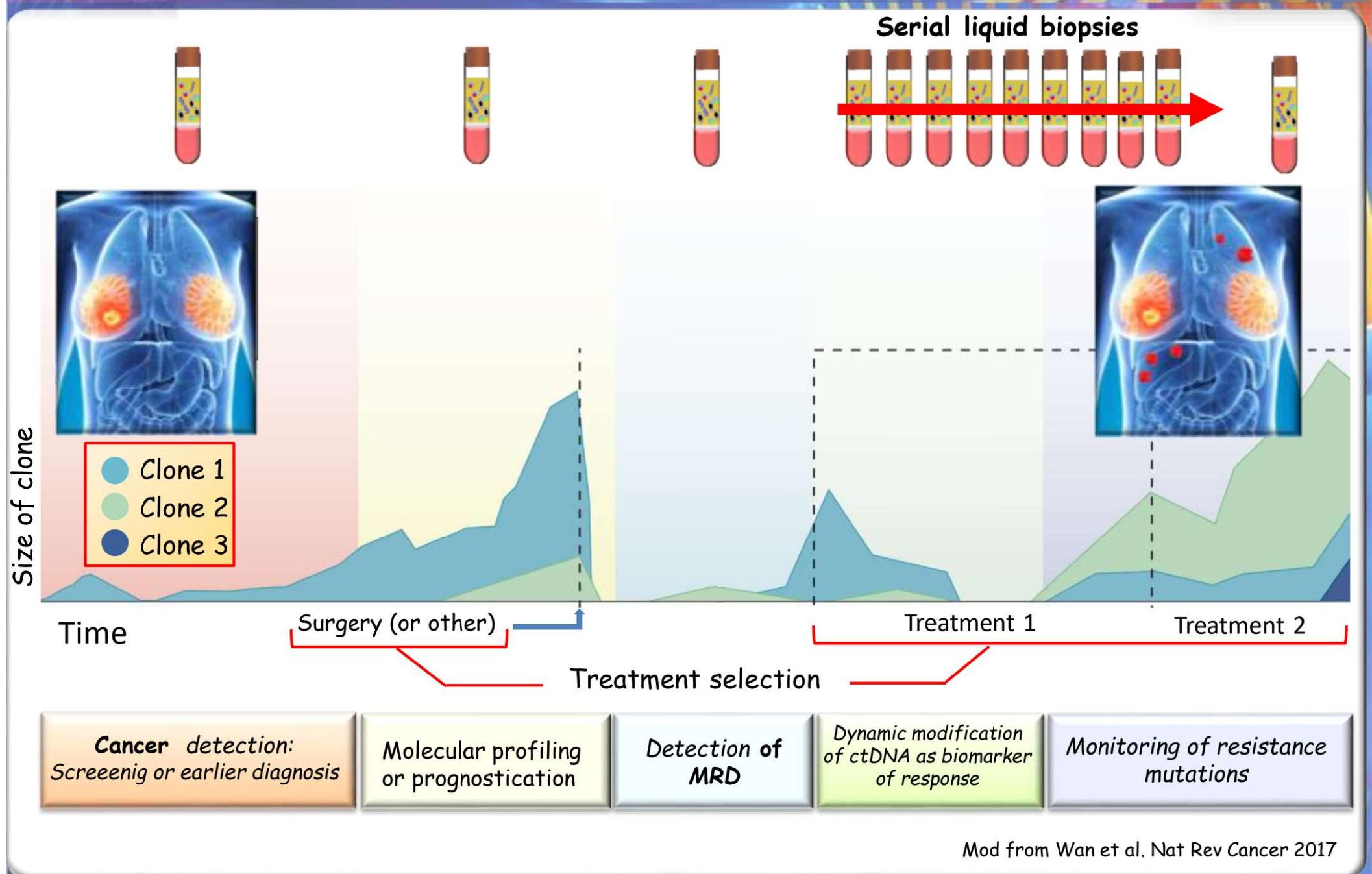


**Introduction to  
liquid biopsy**

**Potential clinical  
applications in Breast  
Cancer**

# Liquid Biopsy

## Potential clinical application in Breast Cancer



# Liquid biopsy OUTLINE

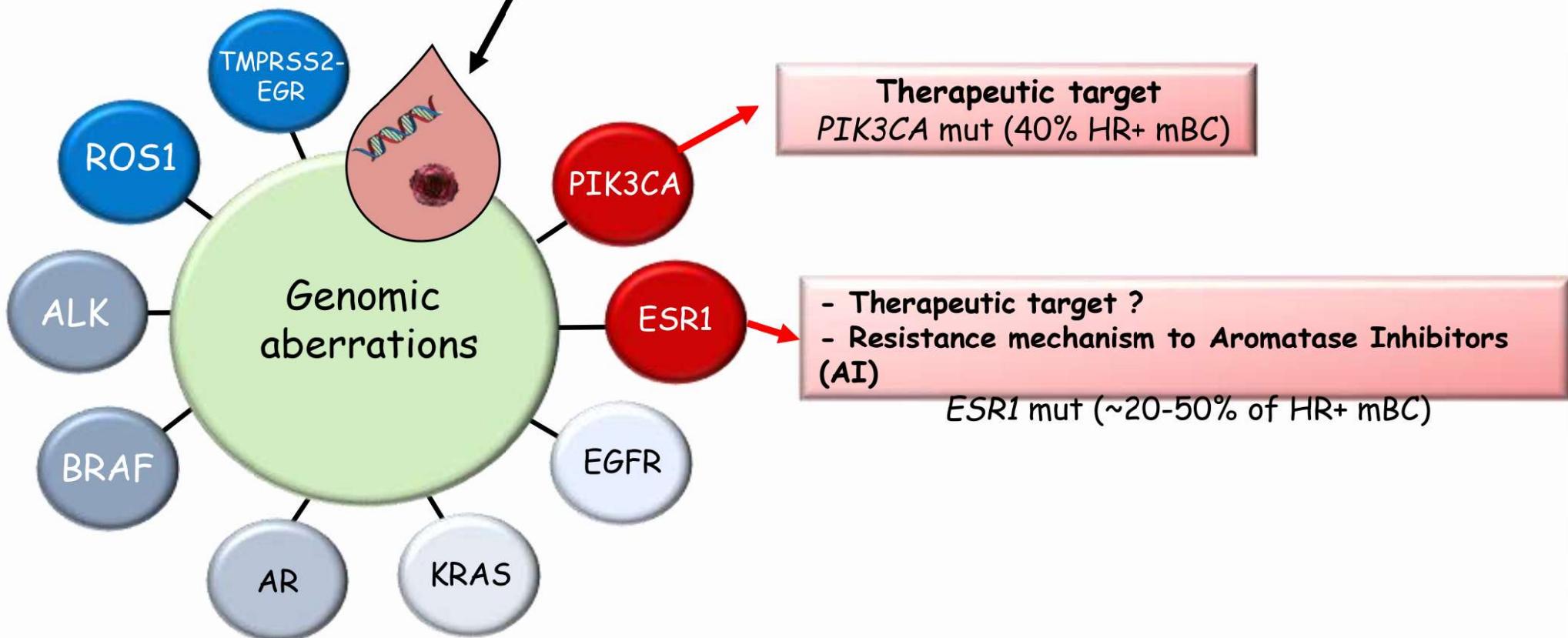


Potential clinical applications in  
Breast Cancer

Treatment  
selection

# Liquid Biopsy in clinical practice: future perspectives

## CtDNA in Metastatic Breast cancer (mBC)

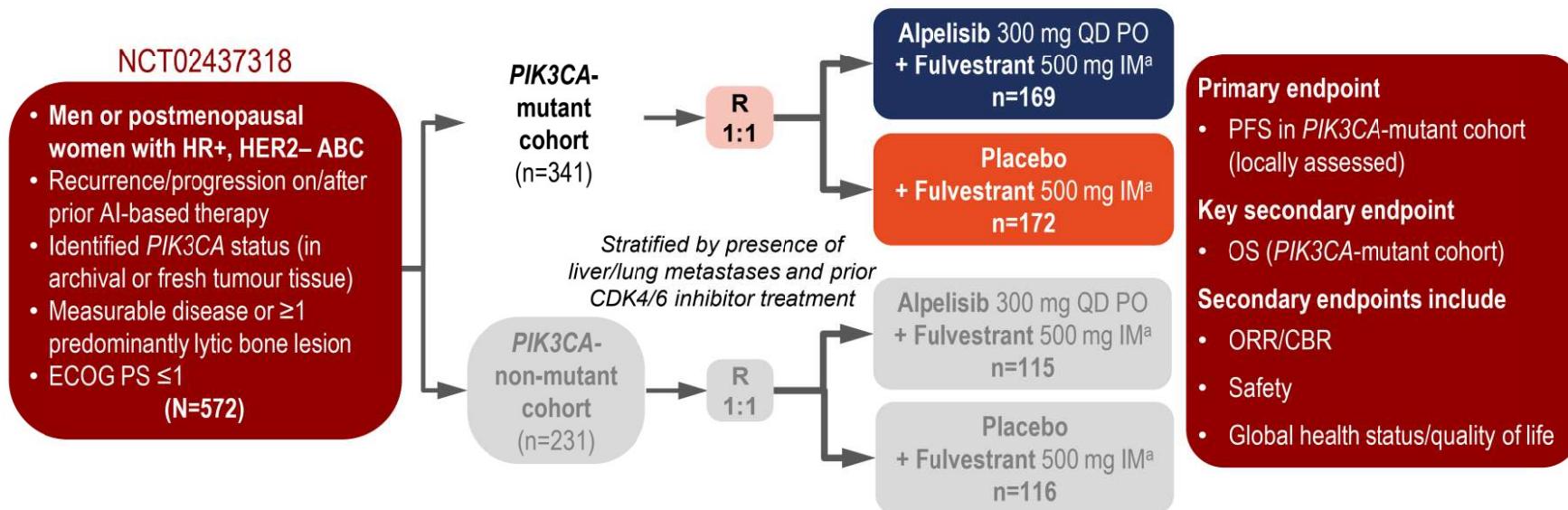


Andre et al. NEJM 2019; Juric et al. SABCS 2018; Pantel & Alix-Panabieres Nat Rev Clin Oncol 2019



## Liquid Biopsy in Breast Cancer: PI3K Treatment selection: stratifying metastatic BC pts

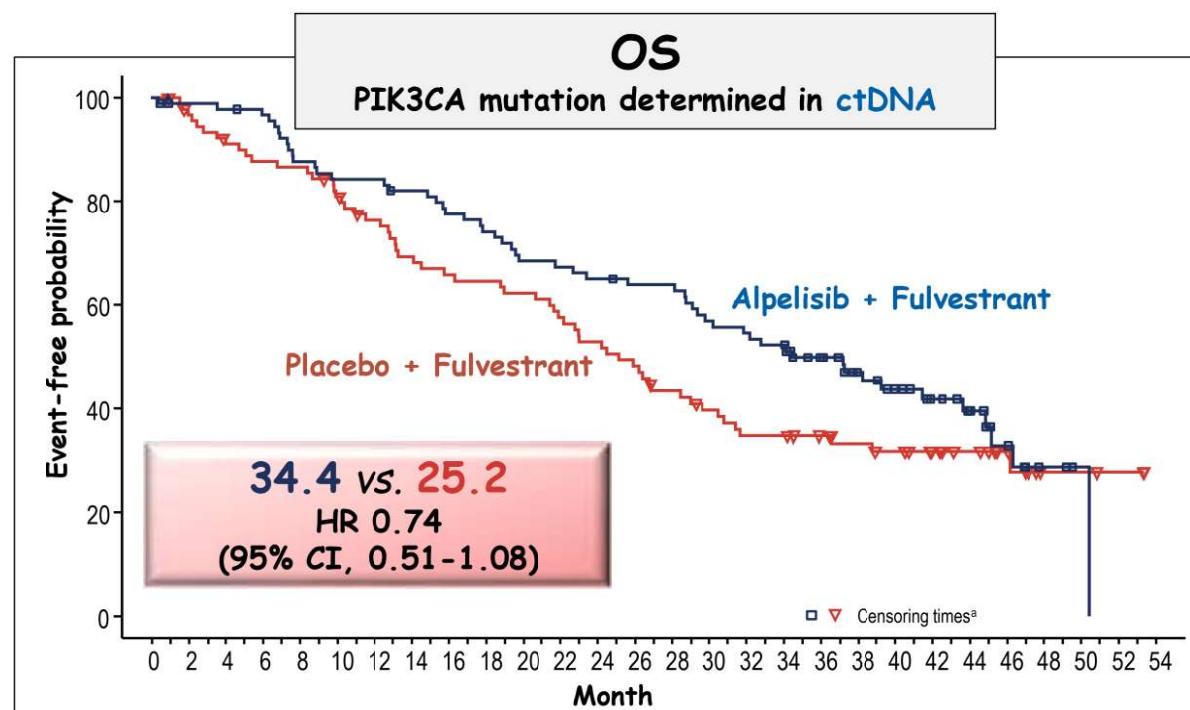
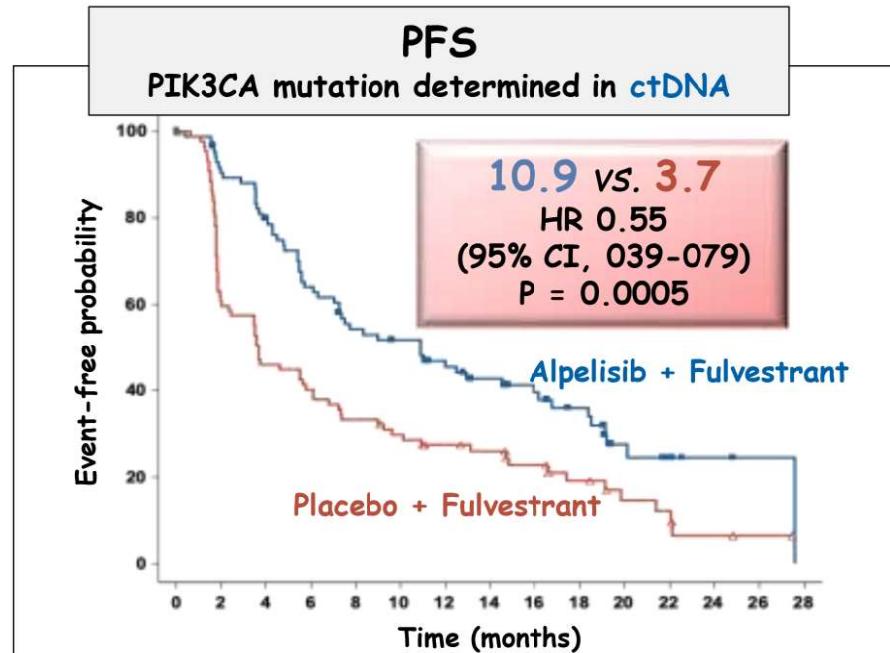
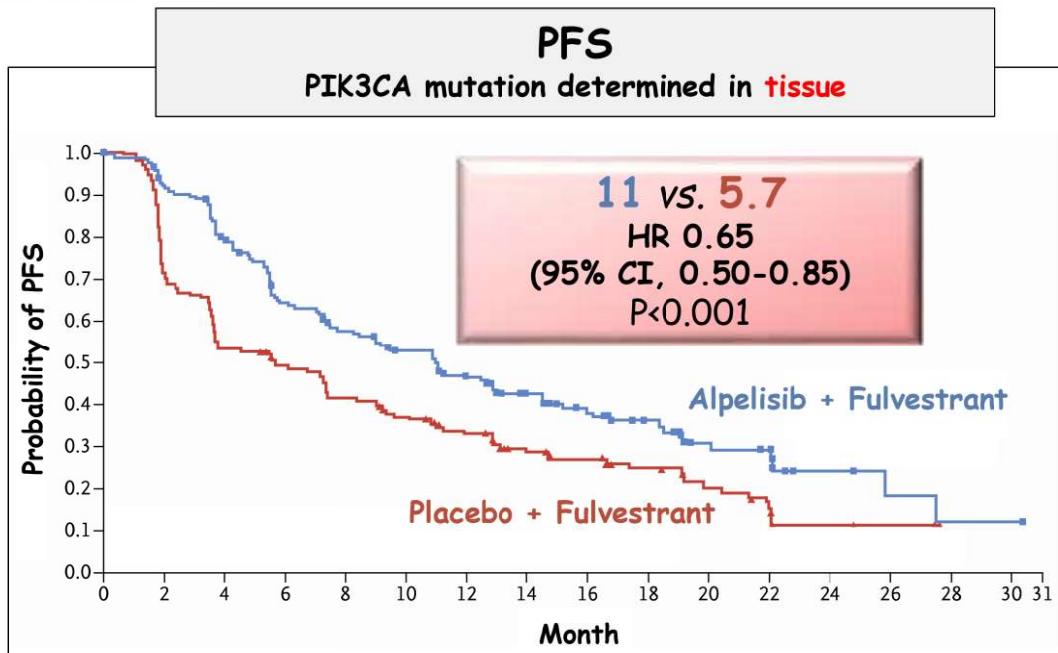
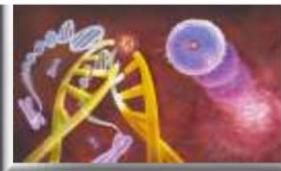
# SOLAR-1 STUDY DESIGN



- ✓ n=572 HR-positive and HER2-negative advanced breast cancer, previously treated with endocrine therapy
  - ✓ 341/572 had confirmed tissue PIK3CA mutation
  - ✓ Primary endpoint: **PFS** in PI3KCA-mutant cohort
  - ✓ Secondary endpoint: **OS** in PI3KCA-mutant cohort

# Liquid Biopsy in Breast Cancer

## Results from SOLAR-1 study

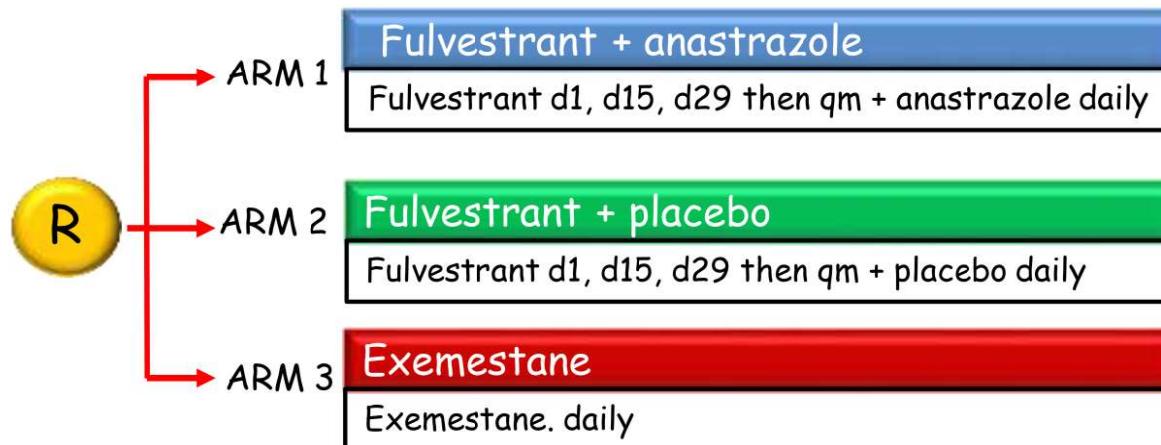


Andre F. et al. NEJM 2019  
Juric et al. SABCS 2018  
De Mattos-Arruda. ESMO Open 2020

# Liquid Biopsy in Breast Cancer: ESR1

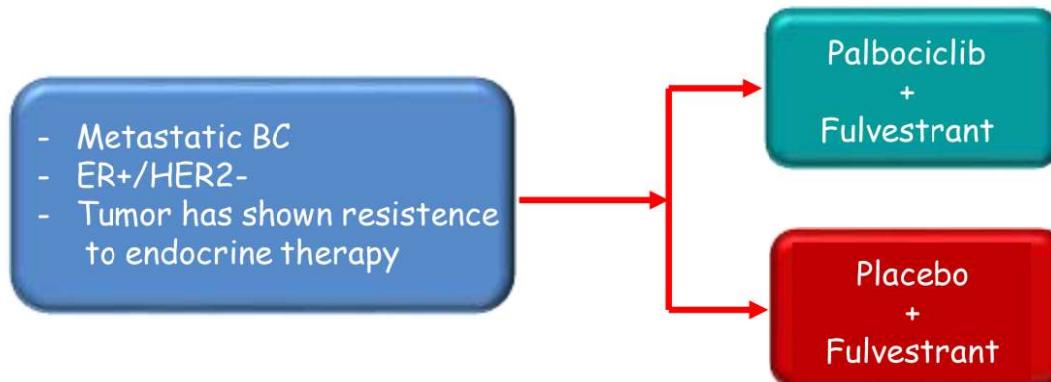
## Treatment selection: stratifying metastatic BC pts

- ✓ SOFEA trial: exemestane vs fulvestrant-containing regimens
- ✓ n= 723 post-menopausal patients with prior sensitivity to nonsteroidal aromatase inhibitors



Johnston. et al. Lancet 2013

- ✓ PALOMA-3 trial: Fulvestrant with or without Palbociclib
- ✓ n= 521 pre- and post-menopausal patients who had progressed during prior endocrine therapy

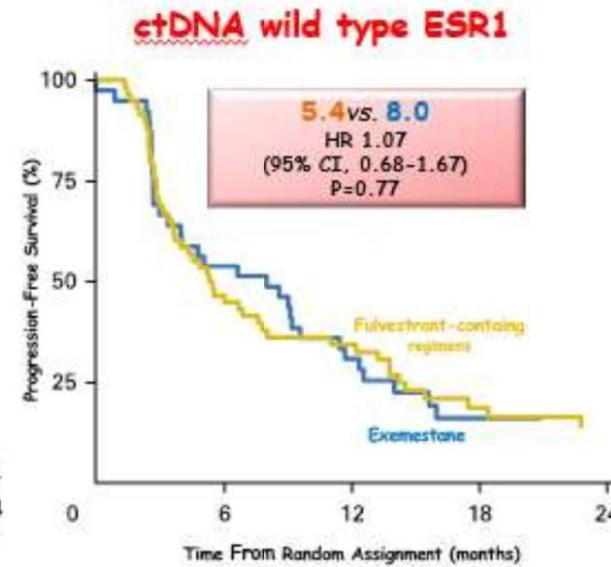
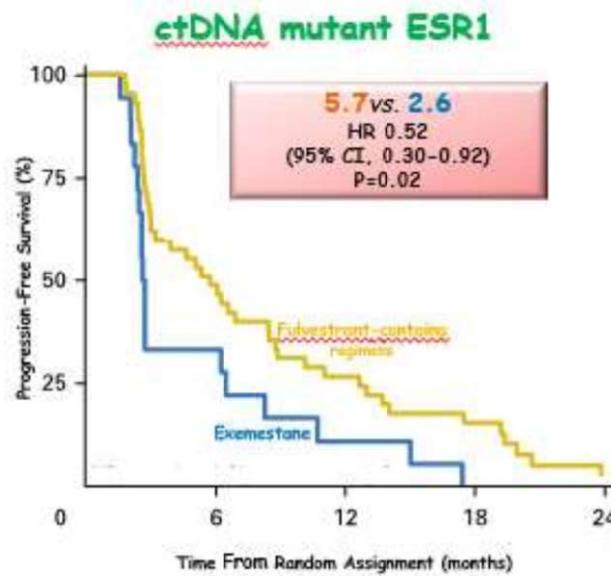


Cristofanilli et al. Lancet Onc 2016

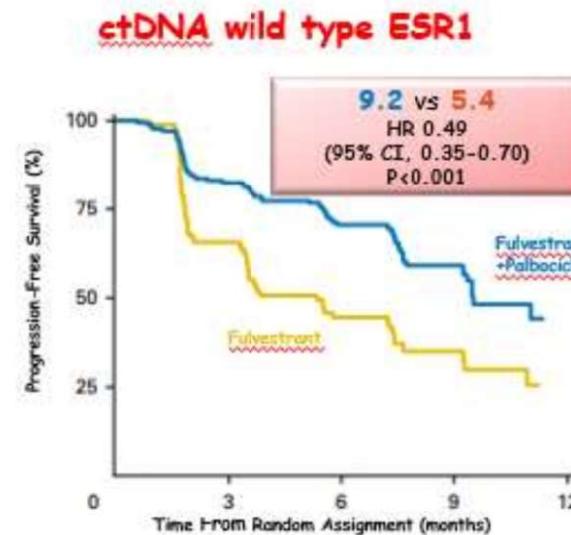
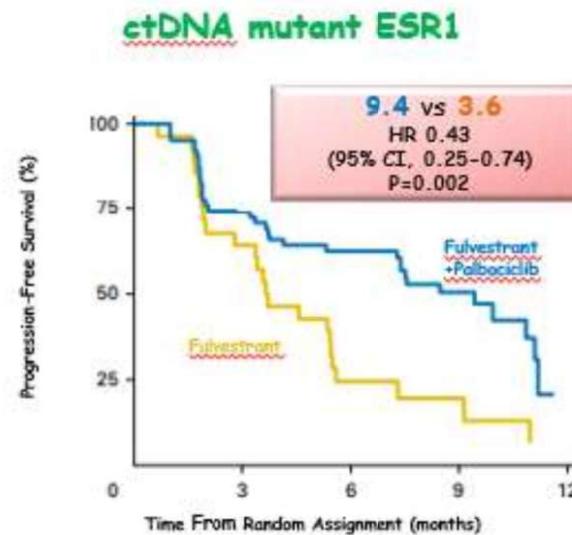


# Liquid Biopsy in Breast Cancer

## Treatment selection: stratifying metastatic cancer pts



**SOFEA**



**PALOMA-3**

**ESR1 mutation in ctDNA predicts benefit for fulvestrant combination regimens**



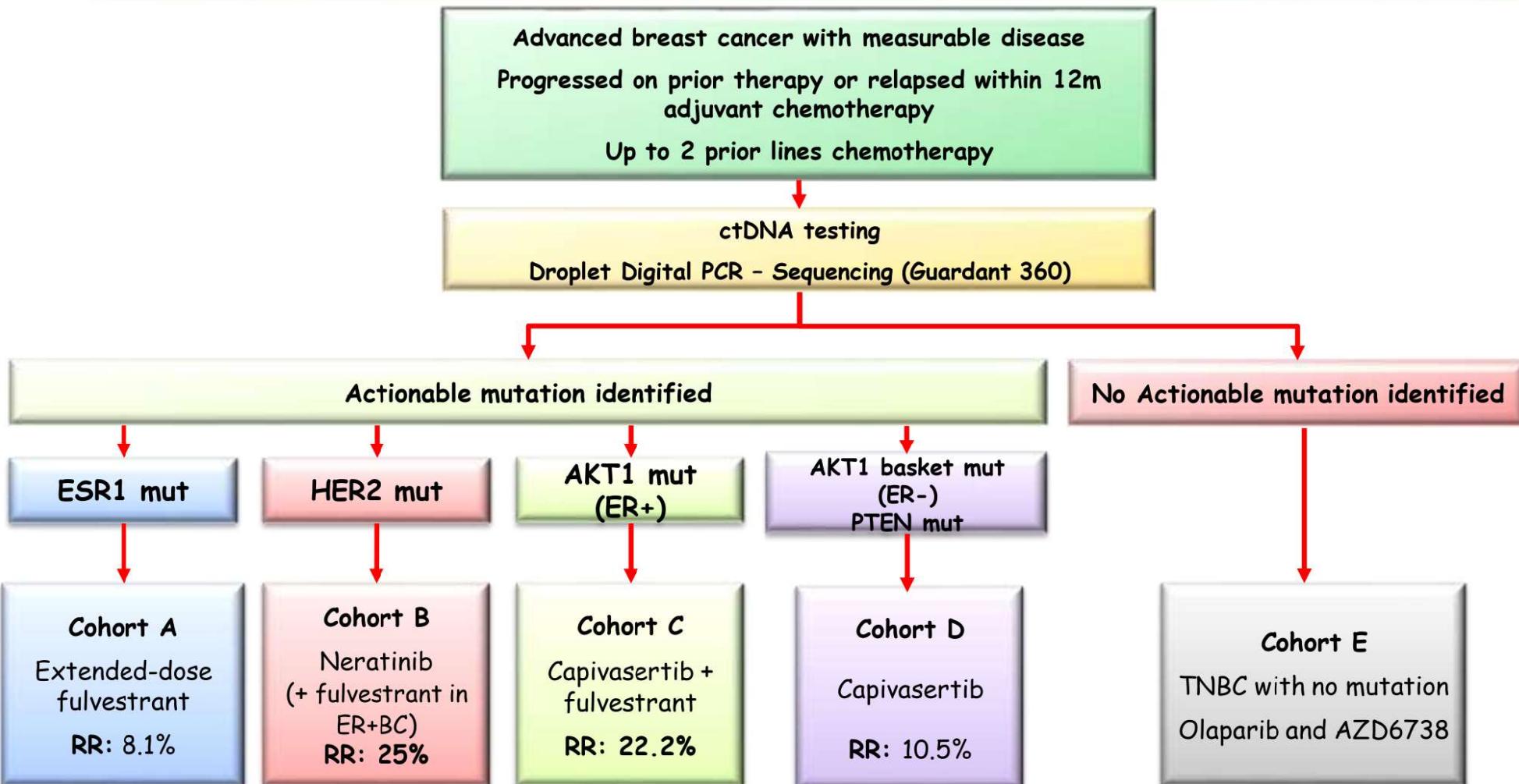
# Liquid Biopsy in Breast Cancer

Treatment selection: enrichment strategy for clinical trial



## PLASMA MATCH STUDY DESIGN

- ✓ Open-label, multicenter, multicohort trial, ctDNA testing in 1051 advanced BC
- ✓ Primary objective: RR of therapies matched to ctDNA mutations



Mod from Turner N. et al, SABCS 2019

# Liquid biopsy OUTLINE

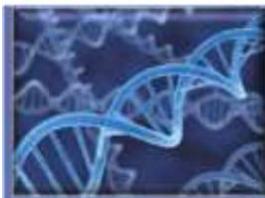


Potential clinical  
applications in Breast  
Cancer

Treatment  
selection

MRD detection





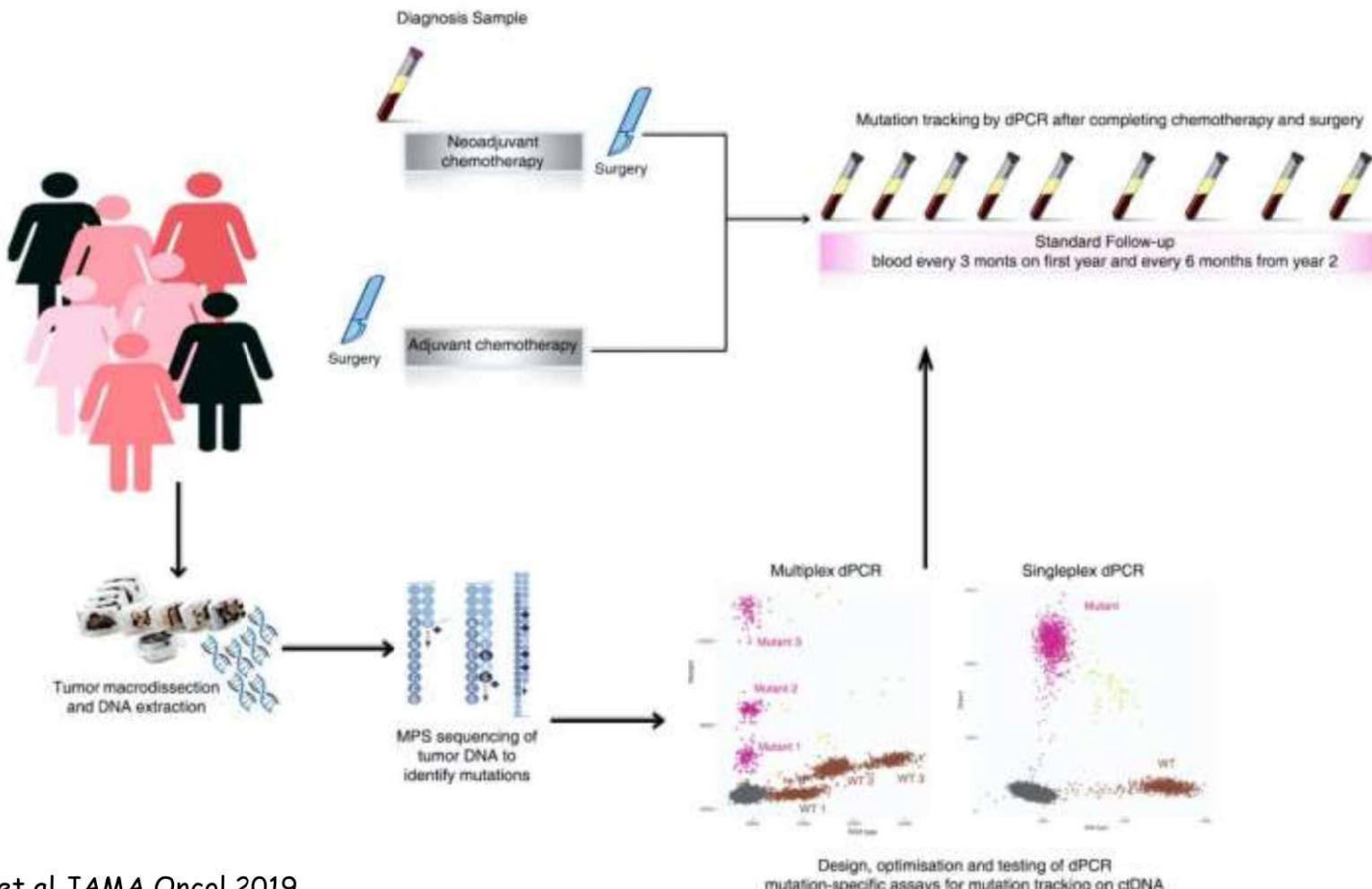
# Liquid Biopsy in Breast Cancer

## Early BC: Minimal Residual Disease detection



### ChemoNEAR and Plasma DNA study designs

- ✓ Prospective, multicentric, validation study
- ✓ N=170 early BC patients, irrespective of HR and HER2 status receiving neoadjuvant chemotherapy followed by surgery or surgery before adjuvant chemotherapy

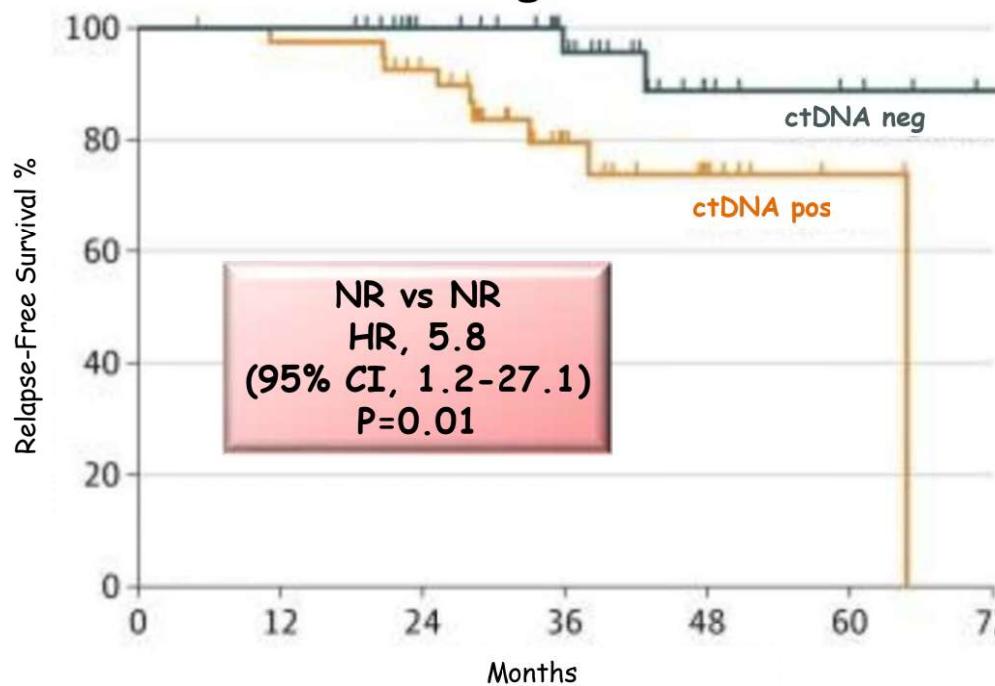


# Liquid Biopsy in Breast Cancer

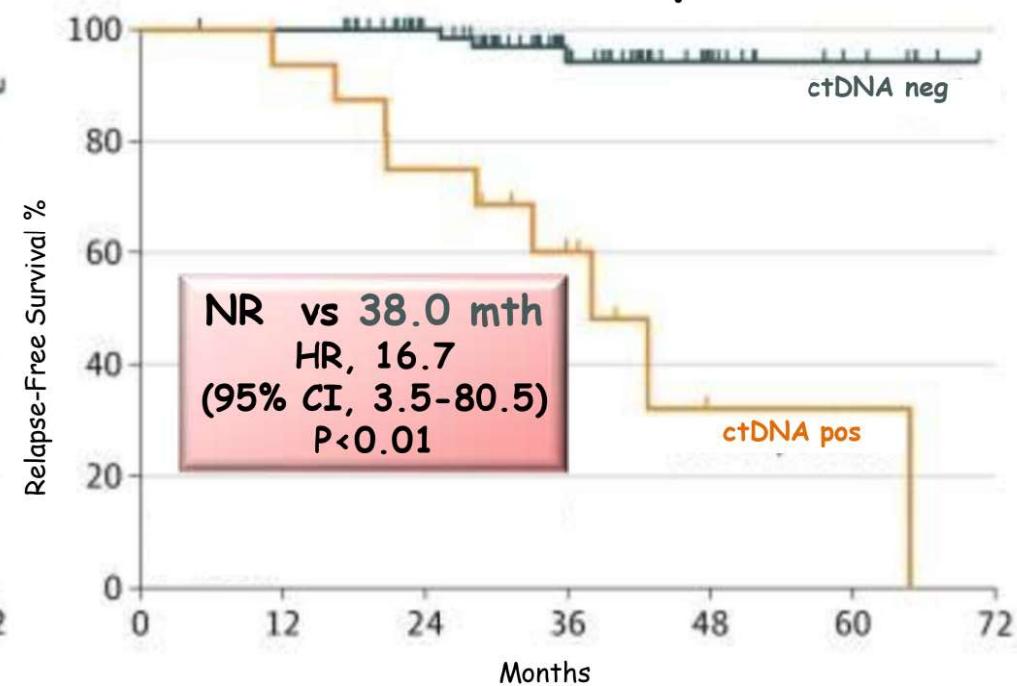
MRD detection: results from ChemoNEAR and Plasma DNA studies



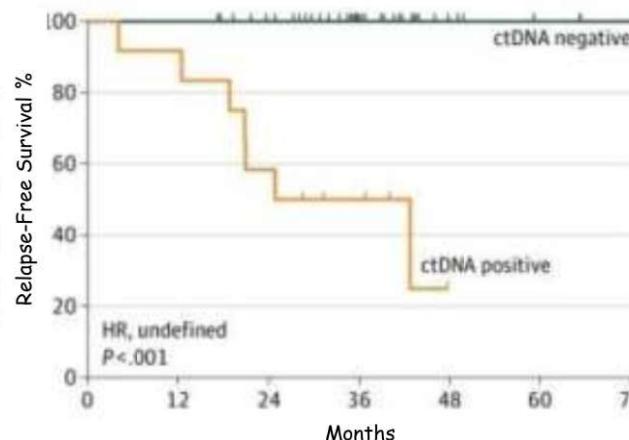
At diagnosis



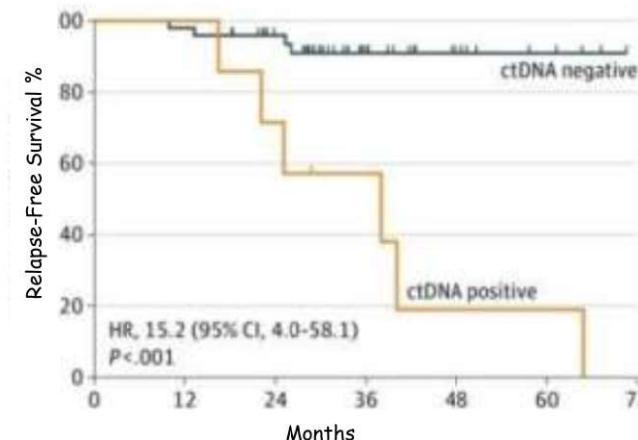
At follow-up



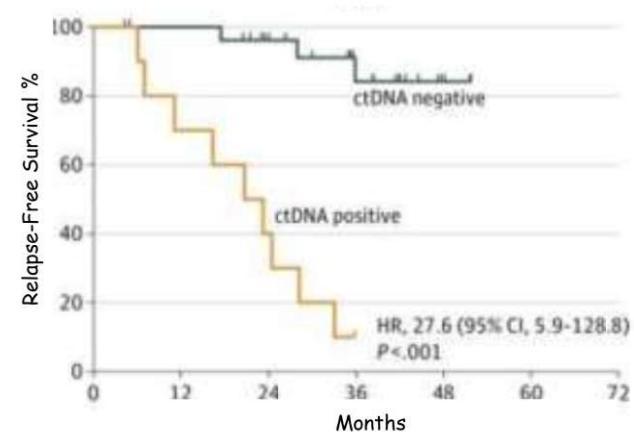
ER+



HER2+



TNBC



# Liquid biopsy OUTLINE



## Potential clinical applications in Breast Cancer

Treatment selection

MRD detection

ctDNA dynamic changes during treatment





# Liquid Biopsy in Breast Cancer

## ctDNA dynamic changes during treatment

### PALOMA-3 study design

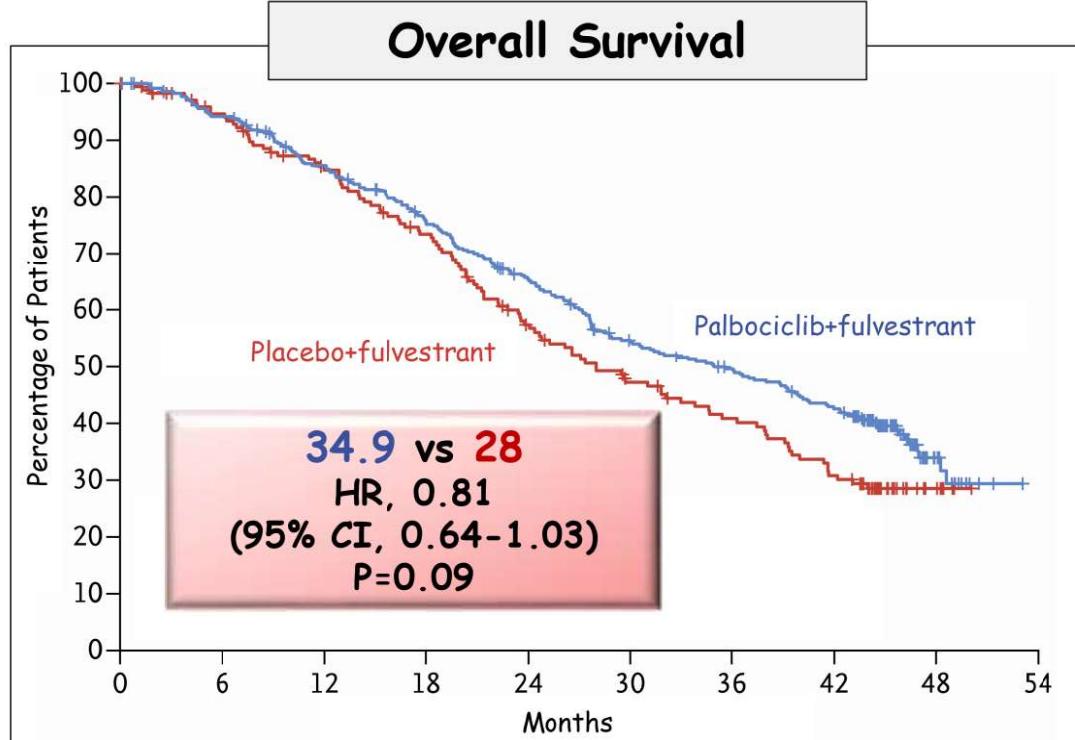
#### Study population

- Pre/perimenopausal and postmenopausal HR+ HER2- metastatic/advanced BC
- Progressed on prior endocrine therapy (on or within 12m of adjuvant therapy; while on therapy for advanced disease)
- Less or 1 prior chemotherapy regimen for advanced disease

RANDOMIZATION

Palbociclib (125mg QD, 3/1 schedule) + Fulvestrant (500mg IM Q4W)

Placebo (3/1 schedule) + Fulvestrant (500mg IM Q4W)



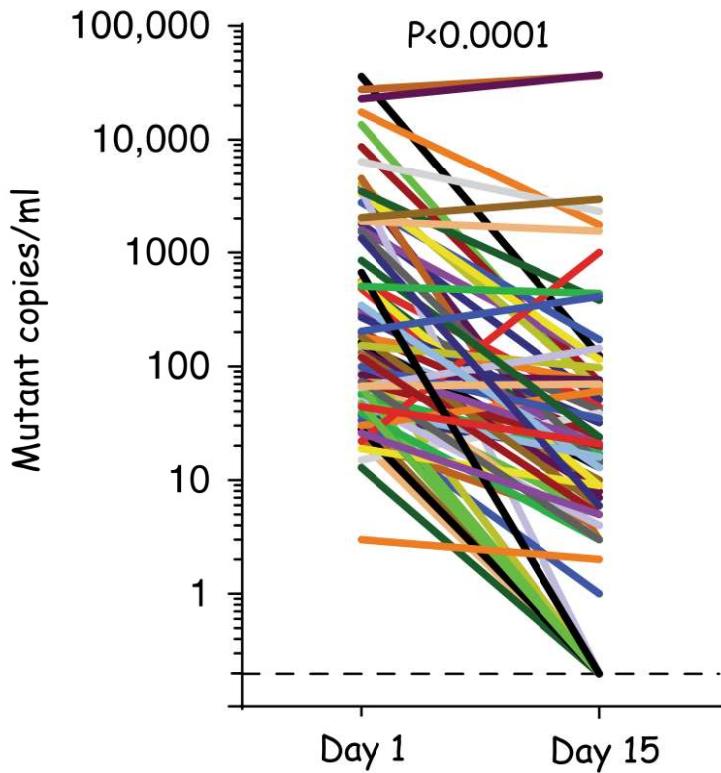


# Liquid Biopsy in Breast Cancer

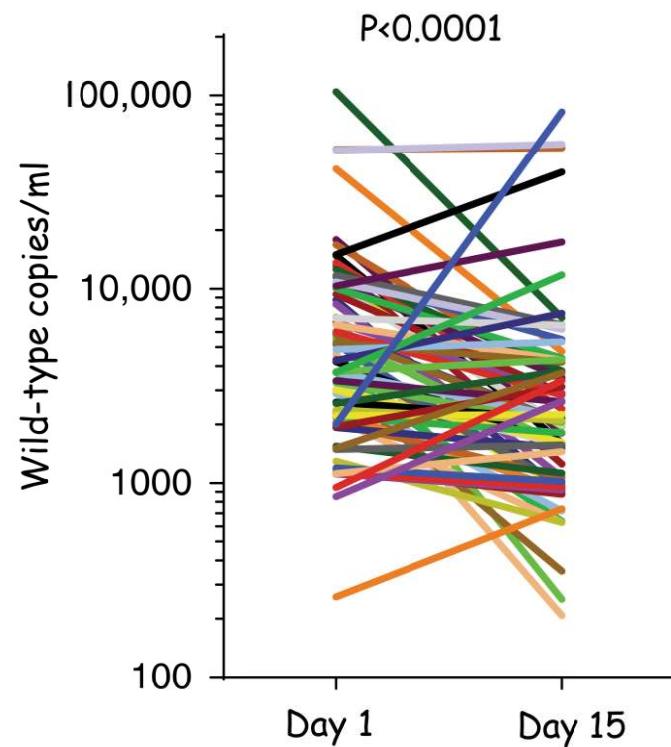
Analysis from PALOMA-3: early ctDNA changes to predict outcome on palbociclib



*PIK3CA* mutant copies/ml



*PIK3CA* wild-type copies/ml

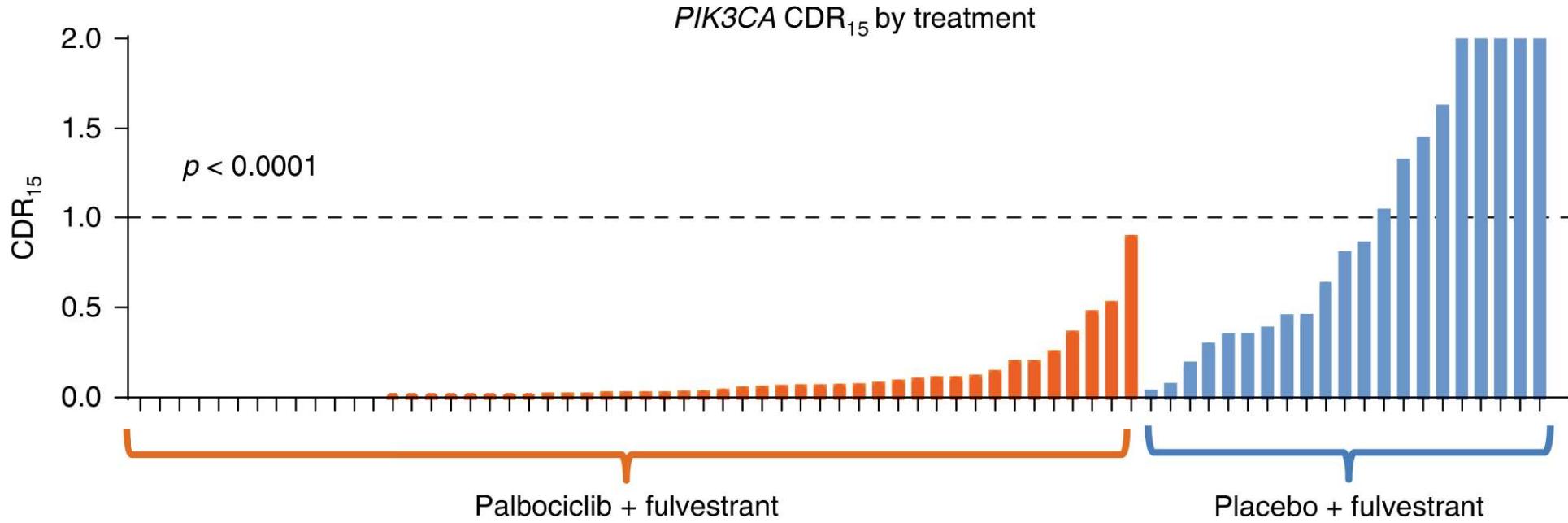


- ✓ Statistically significant decline of both *PIK3CA* mutant and wild-type alleles from day 1 to day 15 of Palbociclib
- ✓ Wild-type allele change reflected changes in total cell-free DNA, with a substantially more marked fall for mutant *PIK3CA* as a marker of ctDNA



# Liquid Biopsy in Breast Cancer

## Analysis from PALOMA-3: early ctDNA changes to predict outcome on palbociclib



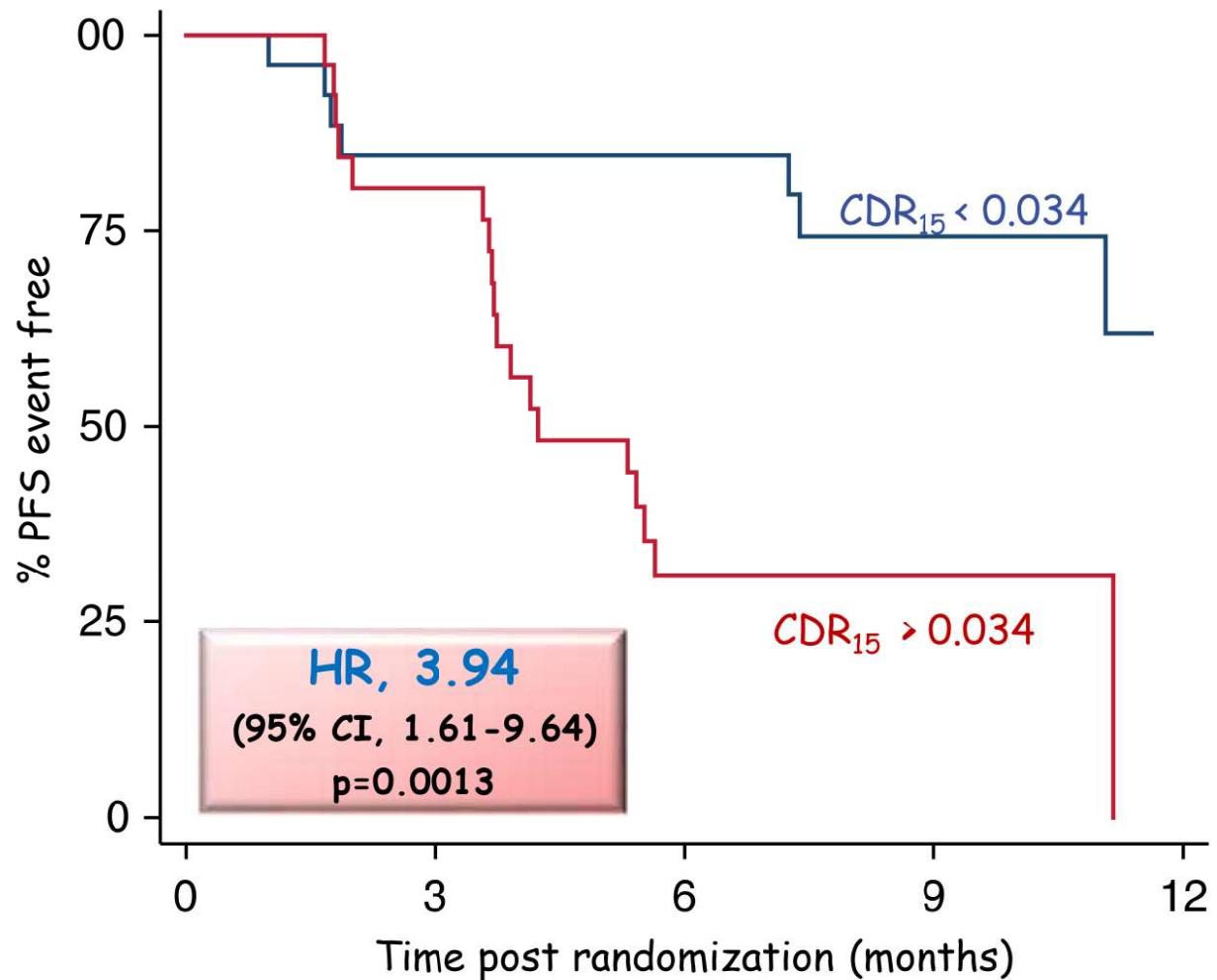
- ✓ "circulating DNA ratio" (CDR): ratio of mutant copies/ml on treatment relative to baseline
- ✓ CDR<sub>15</sub>: ratio of cycle 1 day 15 to baseline
- ✓ Patients receiving Palbociclib+fulvestrant had lower CDR<sub>15</sub> (<1) compared to fulvestrant plus placebo
- ✓ Anti-proliferative effects of palbociclib result in a rapid fall in ctDNA levels by day 15
- ✓ Evaluation of CDR<sub>15</sub> is indicative of treatment response



# Liquid Biopsy in Breast Cancer

Analysis from PALOMA-3: early ctDNA changes to predict outcome on palbociclib

PIK3CA-CDR<sub>15</sub> assessment could predict long-term outcome in patients treated with palbociclib



# Liquid biopsy OUTLINE



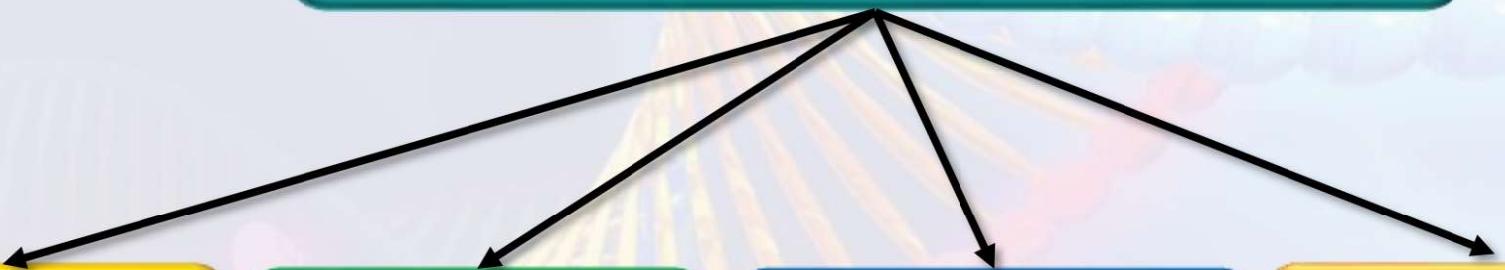
## Potential clinical applications in Breast Cancer

Treatment selection

MRD detection

ctDNA dynamic changes during treatment

Monitoring resistance mutation onset





# Liquid Biopsy in Breast Cancer

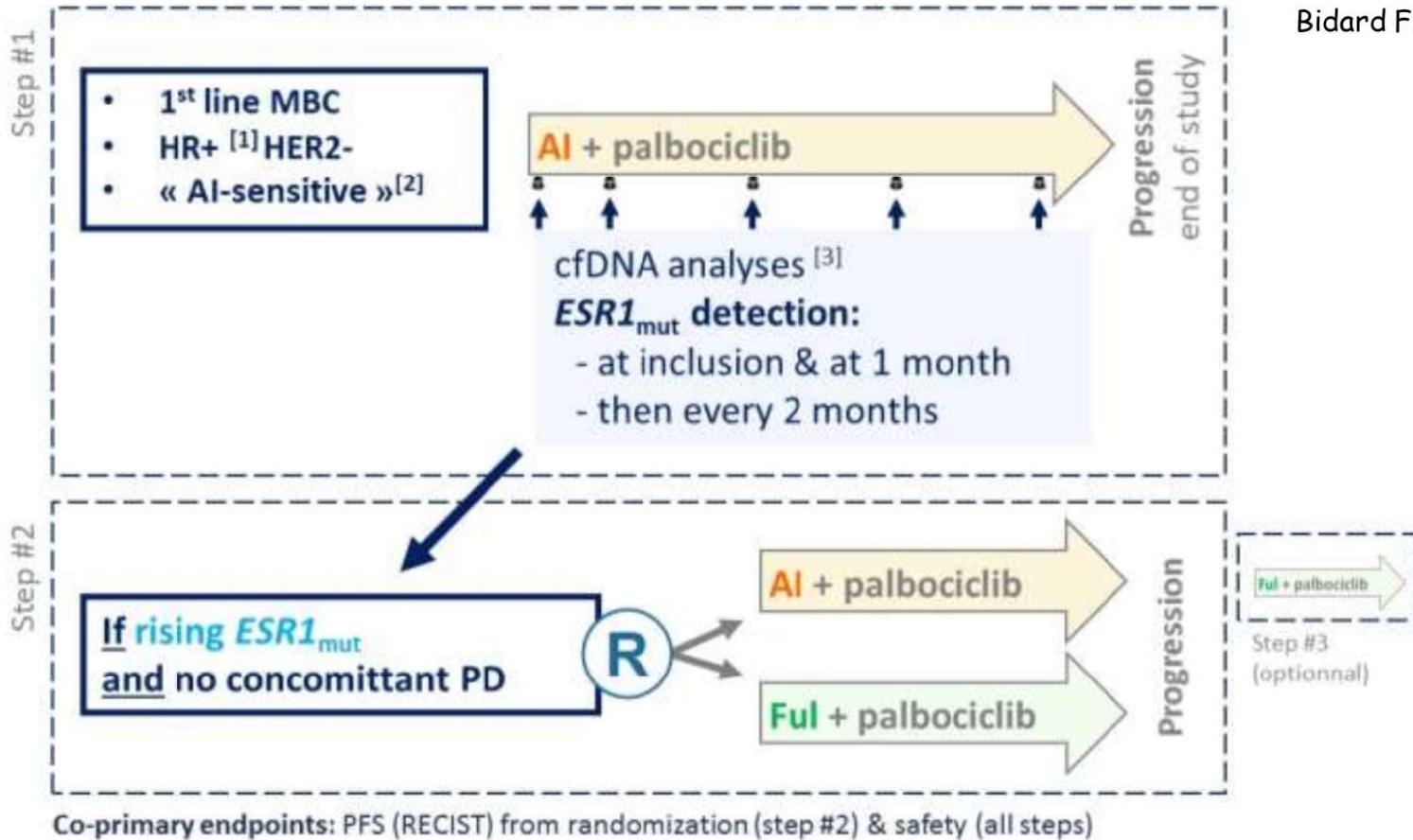
## Monitoring resistance mutation onset



PRESENTED AT: 2020 ASCO  
ANNUAL MEETING

Bidard F.C. ASCO 2020

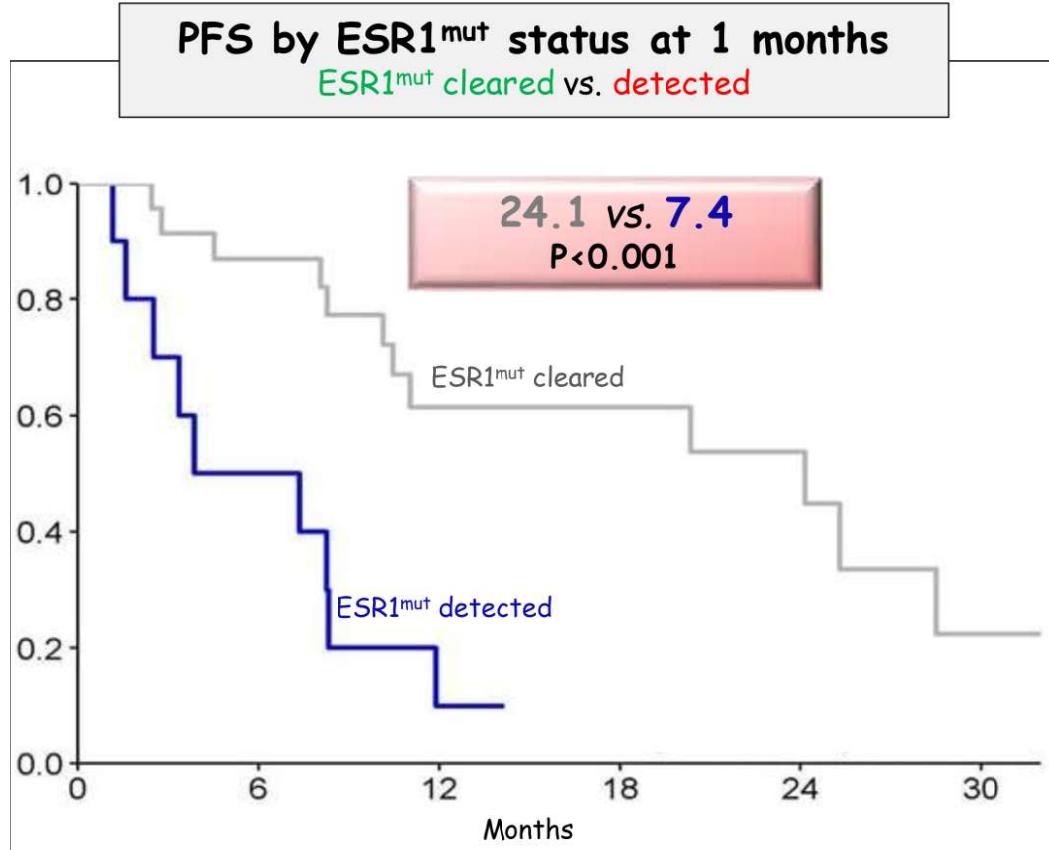
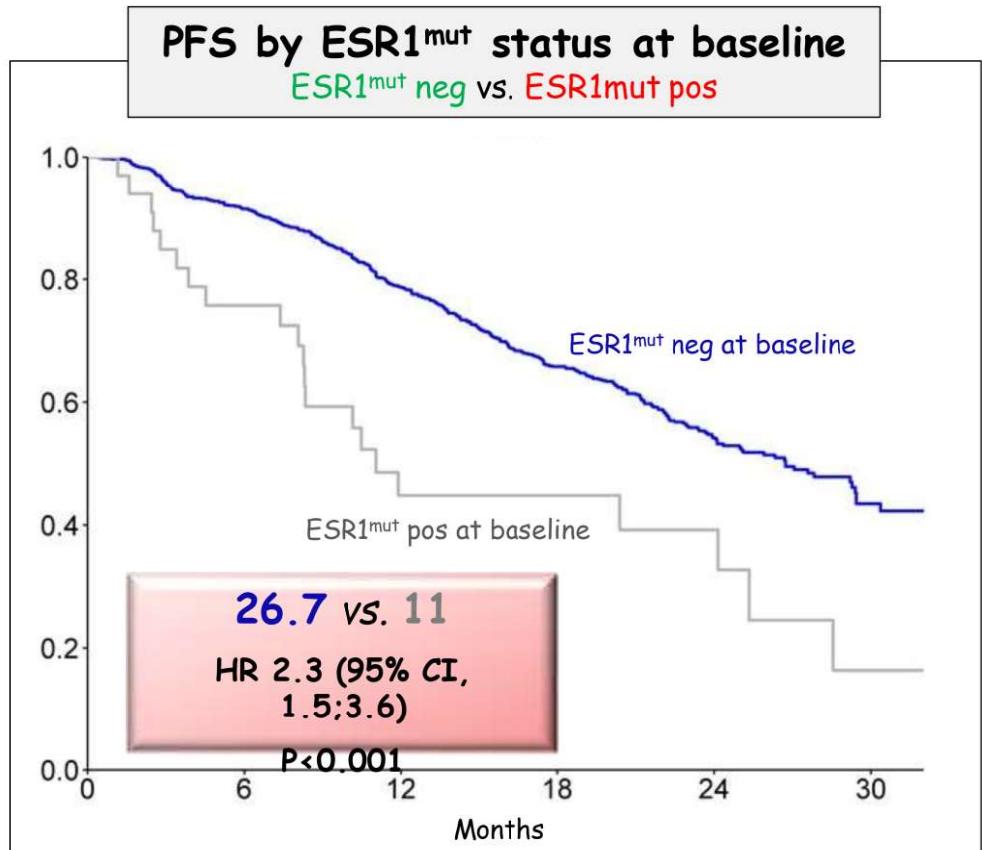
## PADA-1 STUDY DESIGN



- ✓ Randomized, open-label, multicentric, phase III trial, n=1015 pts
- ✓ Patients receiving aromatase inhibitor and palbociclib as first line therapy for ER+ MBC
- ✓ Evaluation of clinical utility of real-time detection of ESR1 mutation in ctDNA

# Liquid Biopsy in Breast Cancer

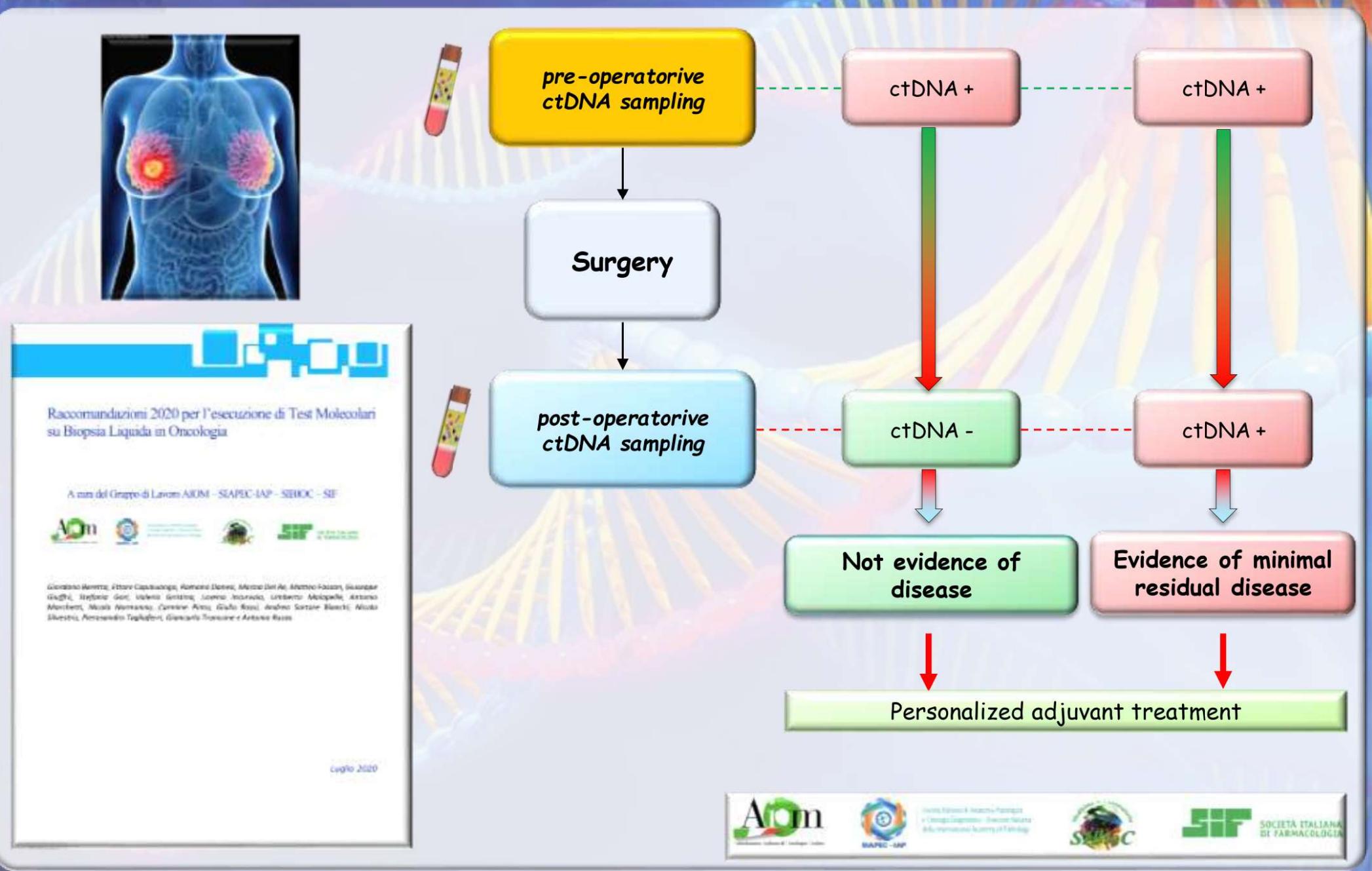
## Exploratory PFS analysis from PADA-1 study



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# Liquid Biopsy in clinical practice: future perspectives

## Early BC: AIOM RECOMMENDATIONS ON LB

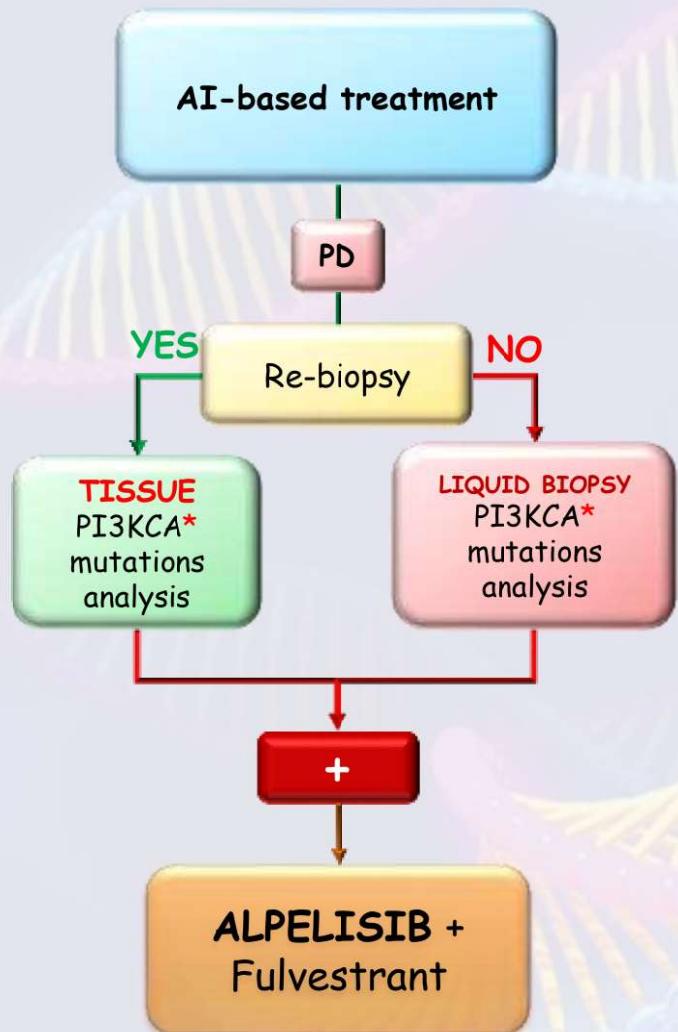


# Liquid Biopsy in clinical practice: future perspectives

## Metastatic BC: AIOM RECOMMENDATIONS ON LB



### I scenario



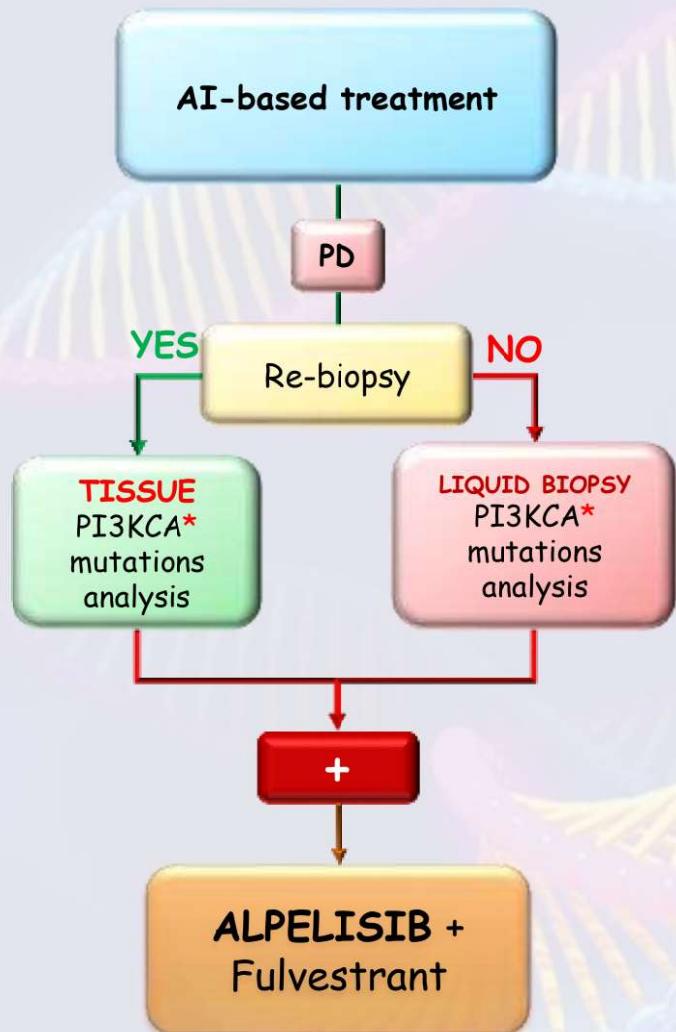
\*Analysis of the following muts: p.C420R, p.E542K, p.E545A, p.E545D, p.E545G, p.E545K, p.Q546E, p.Q546R, p.H1047L, p.H1047R, p.H1047Y.



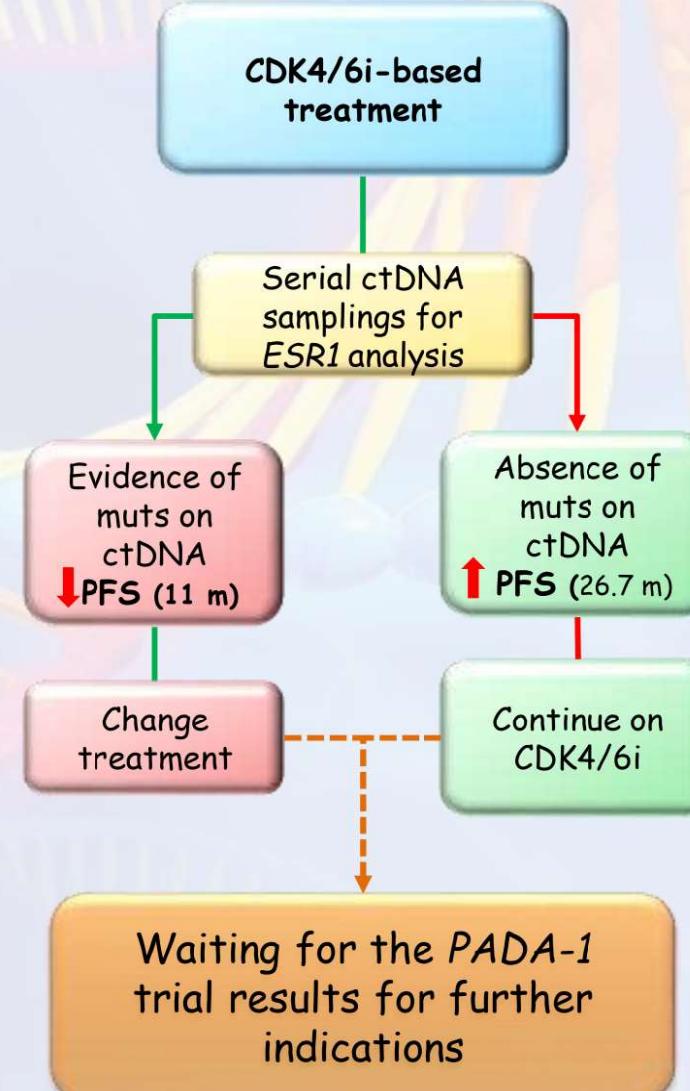
# Liquid Biopsy in clinical practice: future perspectives

## Metastatic BC: AIOM RECOMMENDATIONS ON LB

### I scenario



### II scenario



\*Analysis of the following muts: p.C420R, p.E542K, p.E545A, p.E545D, p.E545G, p.E545K, p.Q546E, p.Q546R, p.H1047L, p.H1047R, p.H1047Y.



## Liquid Biopsy in Breast Cancer

### Final remarks

- LB could be an useful tool complementary to radiological assessment and tissue analysis in patient tumor profiling.
- Among its potential applications, ctDNA analysis could serve as biomarker to monitor disease treatment or to early discover treatment resistance;
- In particular, in early stage BC ctDNA could discover recurrence/relapse or select high risk pts to suggest adjuvant treatment;
- Finally, in metastatic setting, plasma ctDNA analyses could identify tumor heterogeneity allowing patient stratification in clinical trials;
- Future/ongoing clinical studies investigating ctDNA in BC will assess its role and its utility in clinical practice.