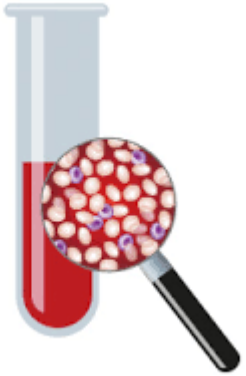
Clinical Utility of Liquid Biopsy

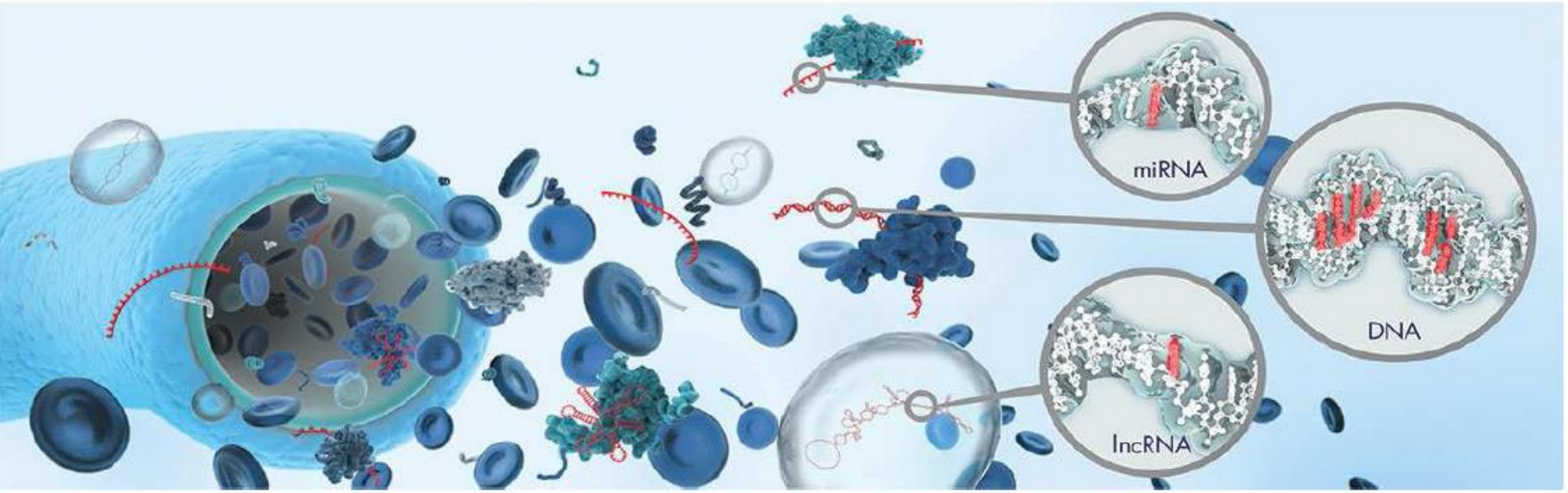


Byung Joo Chae MD., PhD., FACS

Department of Surgery

Seoul St. Mary's Hospital, The Catholic University of Korea

What is liquid biopsy?



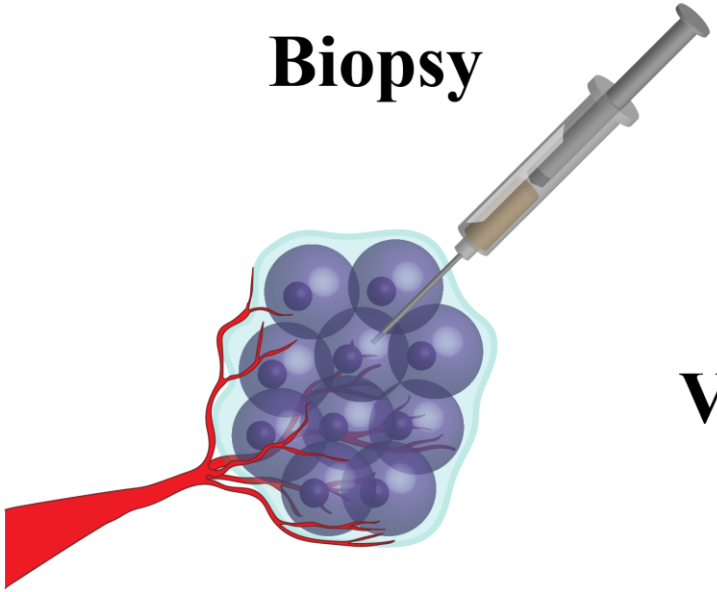
A liquid biopsy is a liquid biomarker that can be isolated from body fluids, such as blood, saliva, urine, ascites, or pleural effusion. Like a tissue biopsy, it is a representative of the tissue from which it has spread.

Liquid biopsies have become more clinically useful in recent years due to the ability to pair tests on circulating tumor cells with genomic tests.

Diaz, Jr., L.A. and Bardelli, A. (2014) "Liquid biopsies: genotyping circulating tumor DNA." Am. Soc. Clin. Oncol. **32**, 579.

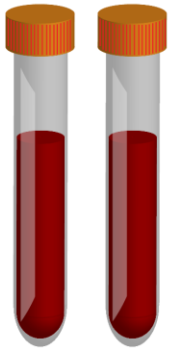
Why liquid biopsy?

Standard Biopsy



VS.

Liquid Biopsy



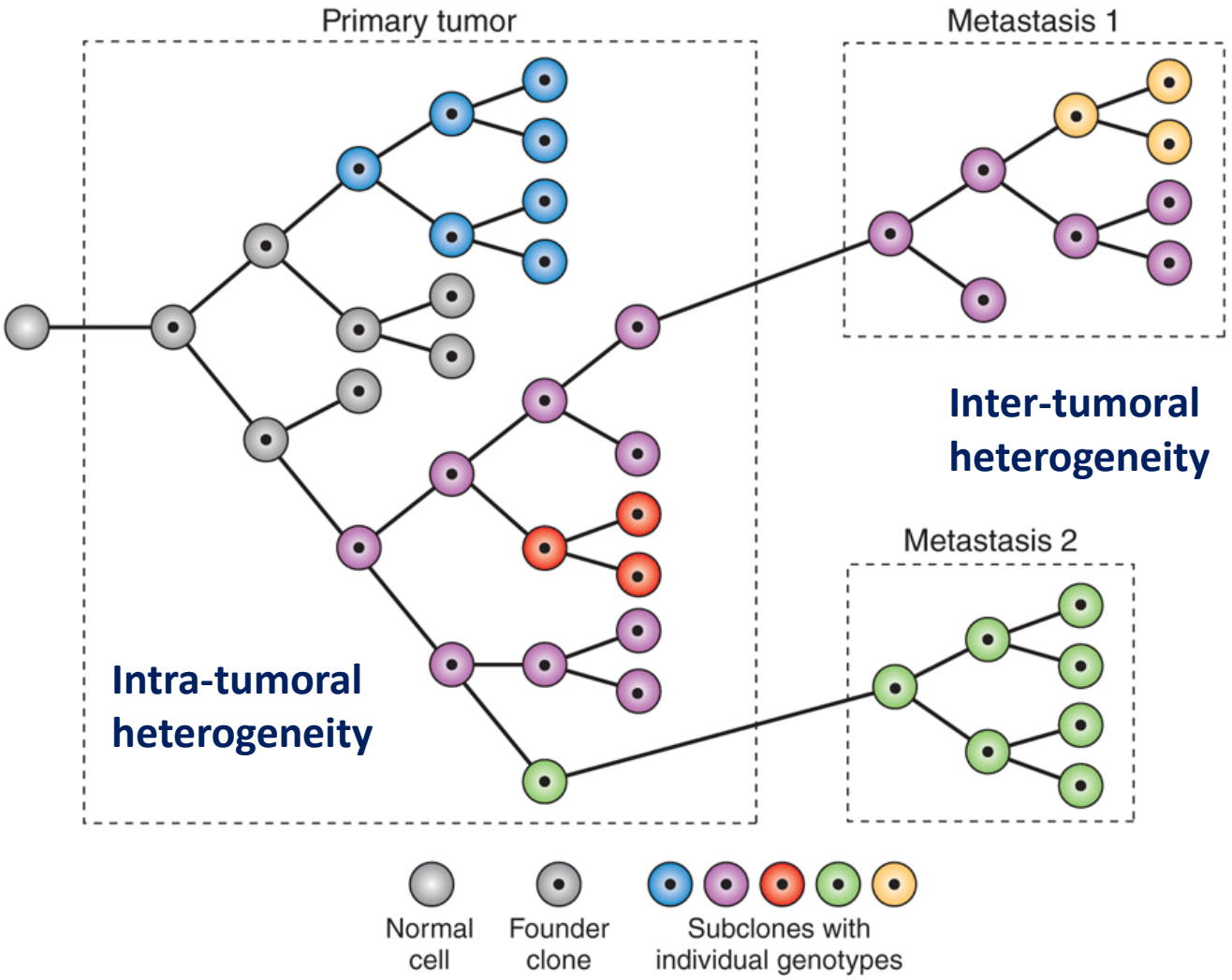
CHALLENGE !!
Isolation of high quantities of markers in a PURE fraction

Time-Intensive Procedure
Localized Sampling of Tissue
Not Easily Obtained
Some Pain/Risk
Invasive

Quick
Comprehensive Tissue Profile
Easily Obtained
Minimal Pain/Risk
Minimally Invasive

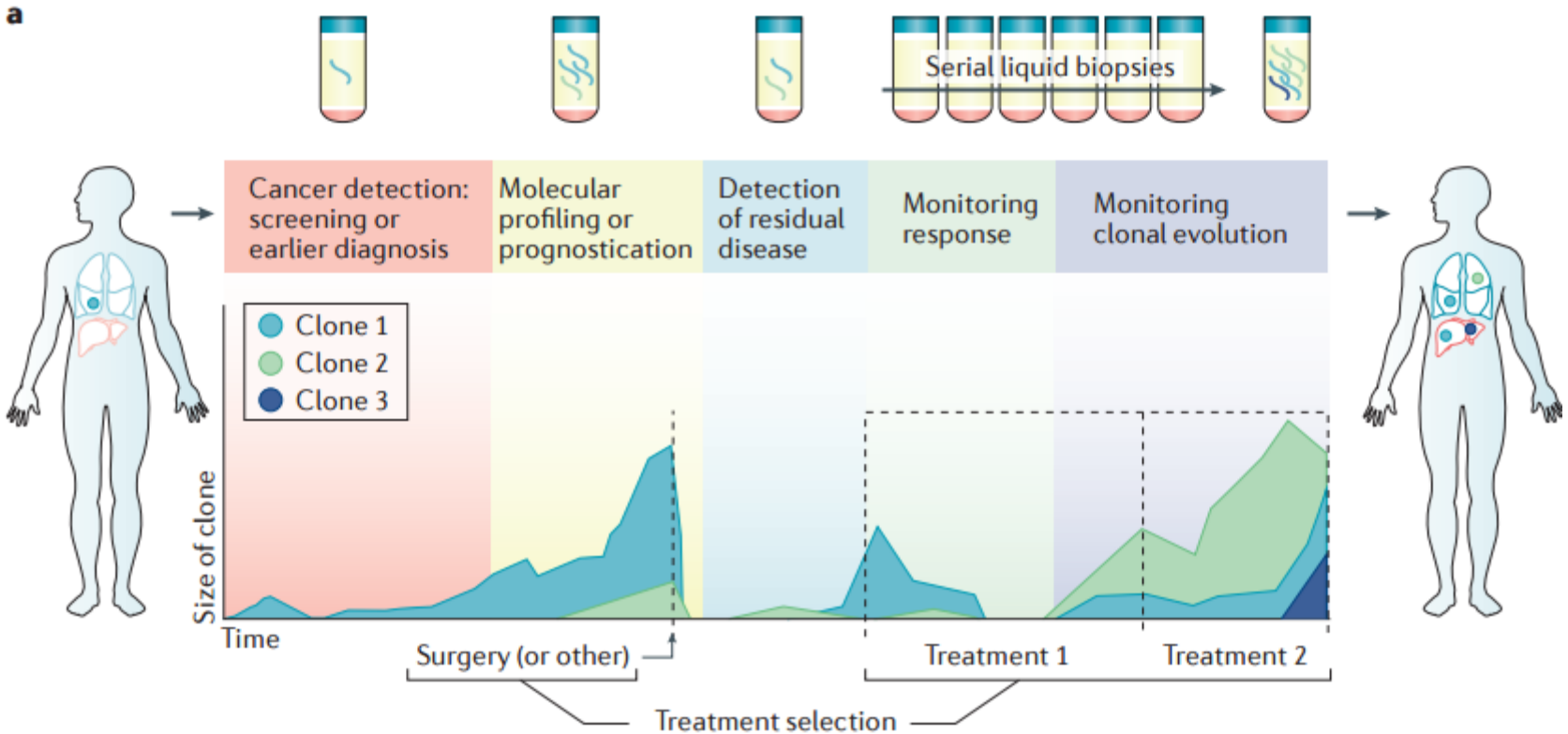
https://www.mycancergenome.org/content/molecular-medicine/circulating-tumor-dna/

Intra/Inter-tumoral heterogeneity



➔ **Characterization by metastatic tumor biopsy may not be enough to select the treatment!**

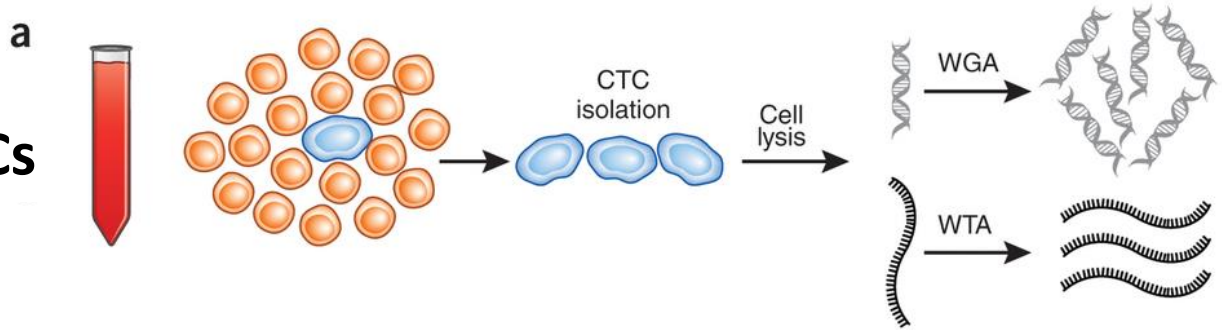
Caldas C et al, Nat Biotechnol, 2012.



Jonathan C. M. Wan *et al, Nat Review ,Apr 2017.*

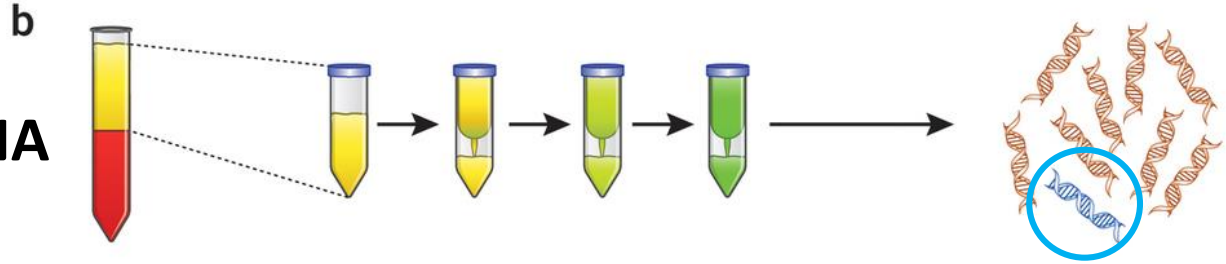
Types of Liquid Biopsies

CTCs



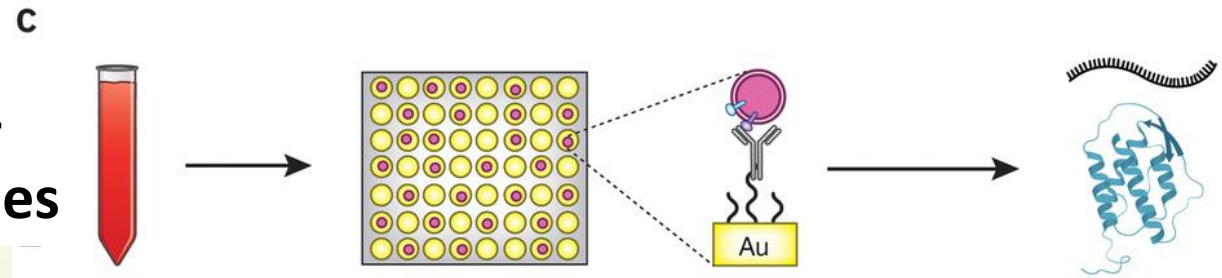
- ✓ DNA
- ✓ RNA
- ✓ Protein analysis by IHC
- ✓ Functional analysis by viable CTCs

cfDNA



DNA/RNA from tumor cells
→ NGS
→ SNV, CNV, indels, rearrangements

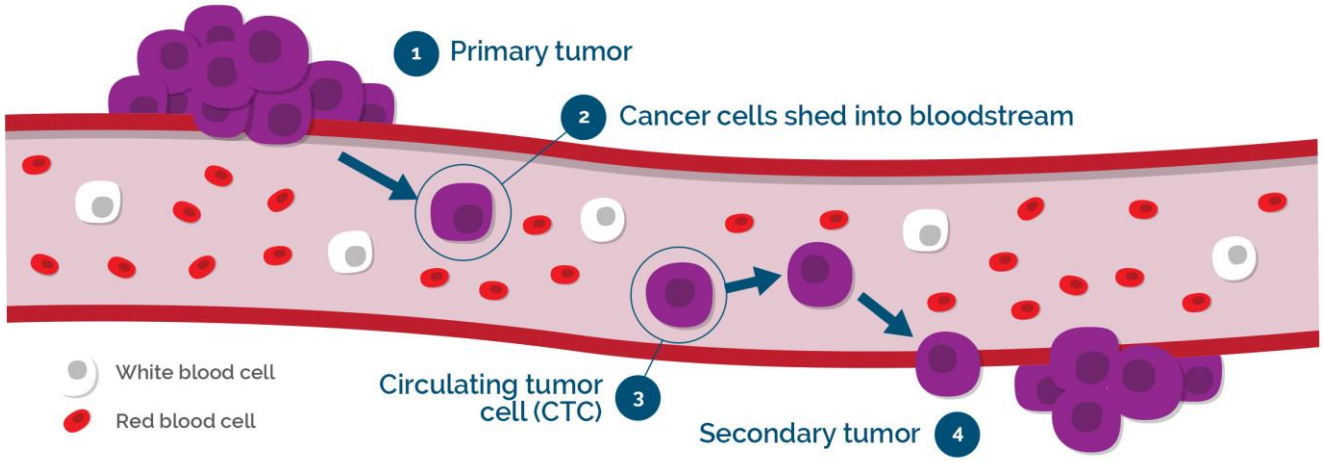
Exo-somes

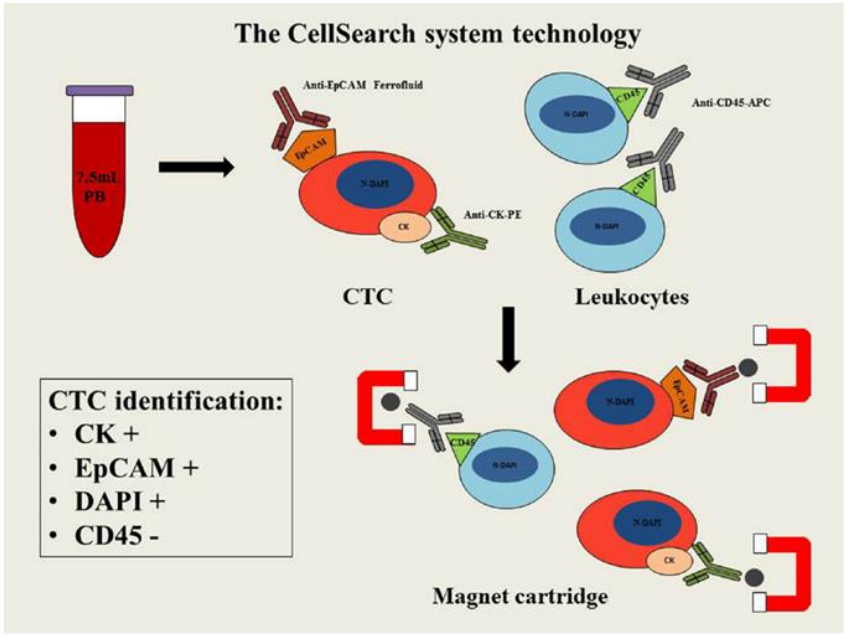


- ✓ Protein quantification
- ✓ RNA quantification, sequencing

Types of Liquid Biopsies

Circulating Tumor Cells (CTCs)



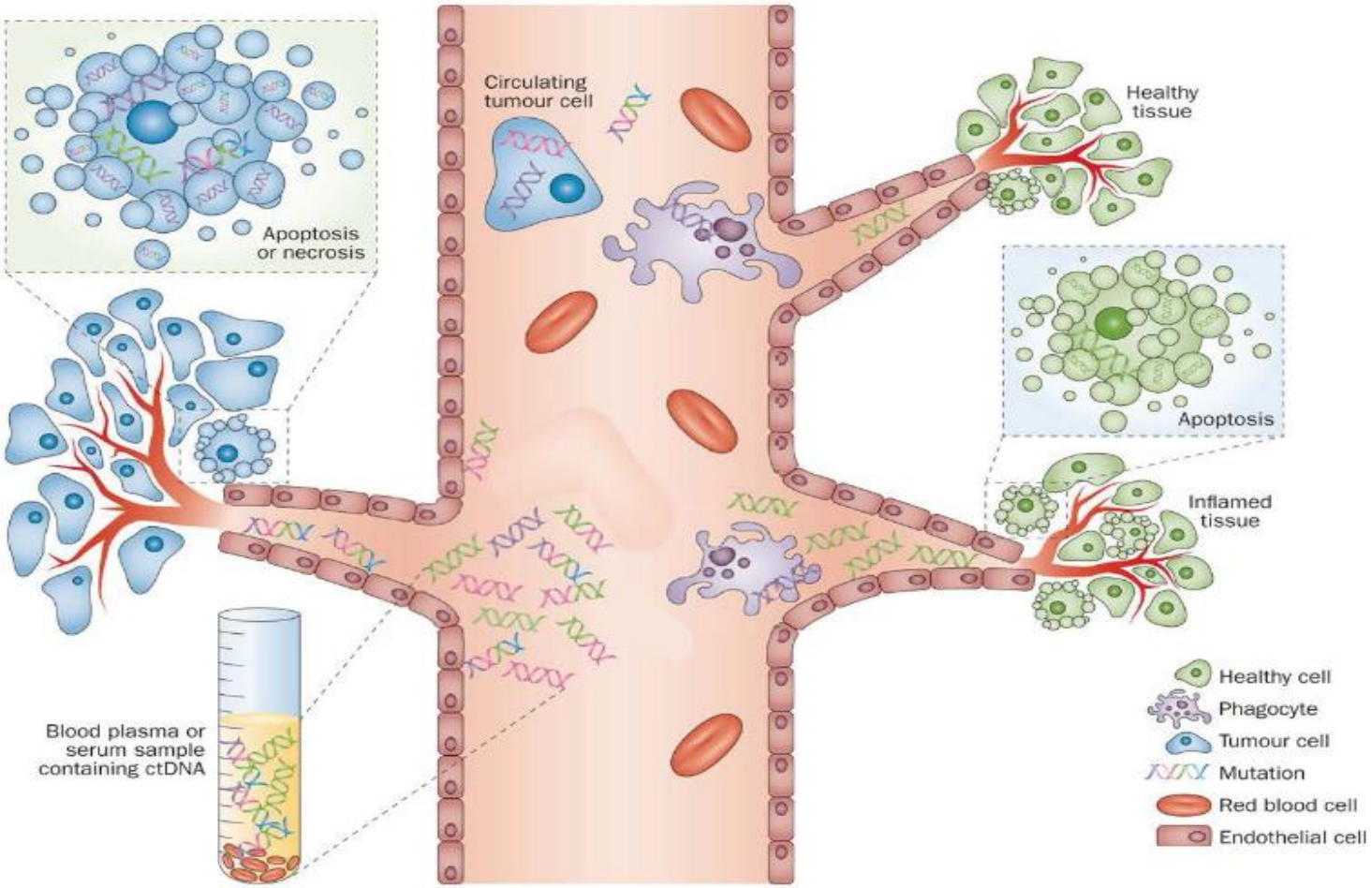


CellSearch® System

: 1st and only clinically validated, FDA-cleared system

In breast cancer, colorectal cancer, prostate cancer

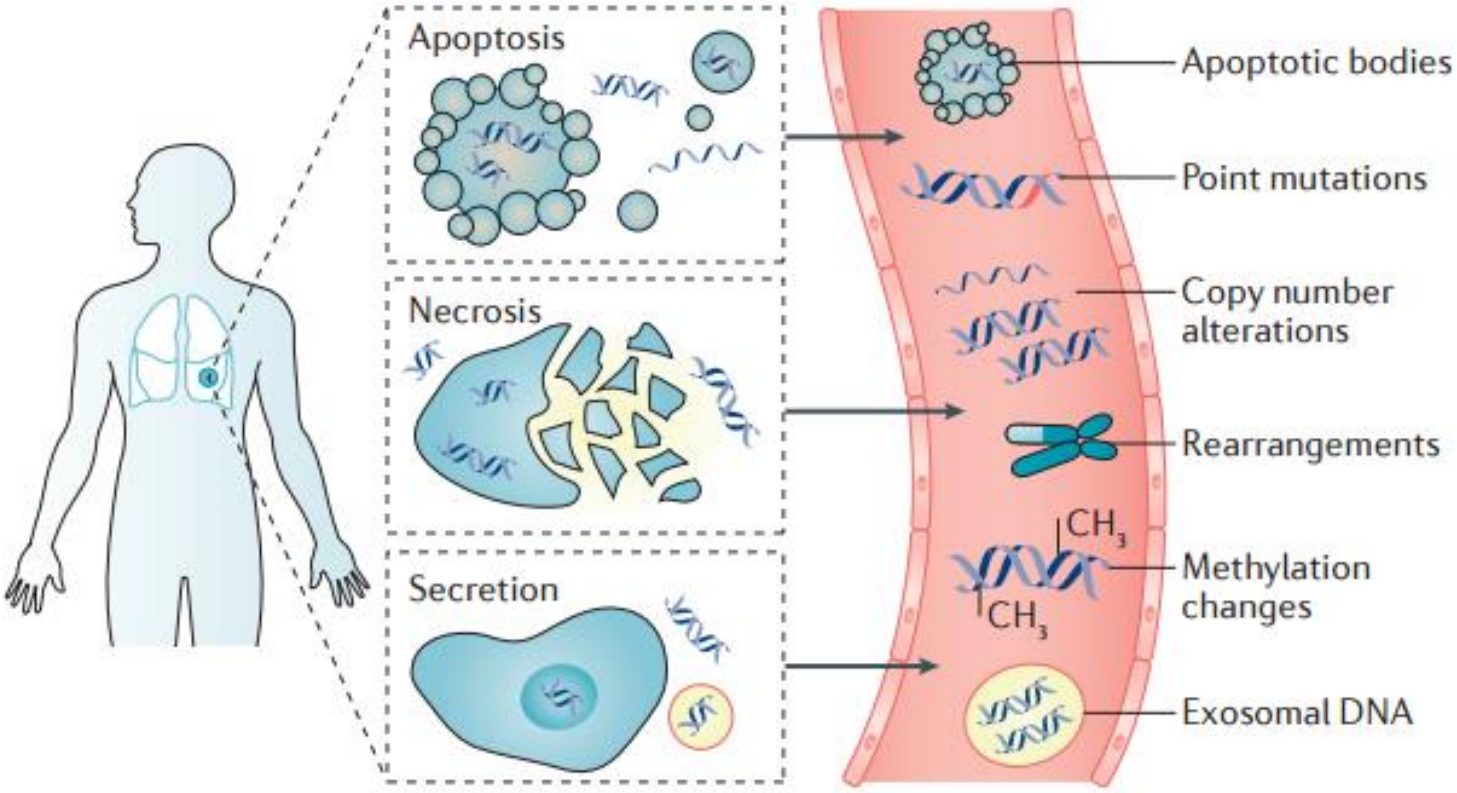
Cell-Free DNA (cfDNA)



Crowley, E. *et al.* (2013) Liquid biopsy: monitoring cancer-genetics in the blood *Nat. Rev. Clin. Oncol.* doi:10.1038/nrclinonc.2013.110

Types of Liquid Biopsies

Cell-free DNA (cfDNAs)



Cell-free DNAs from tumor tissues are released through secretion, necrosis and apoptosis, but mainly through apoptosis

Jonathan C. M. Wan *et al, Nat Review ,Apr 2017.*

Types of Liquid Biopsies

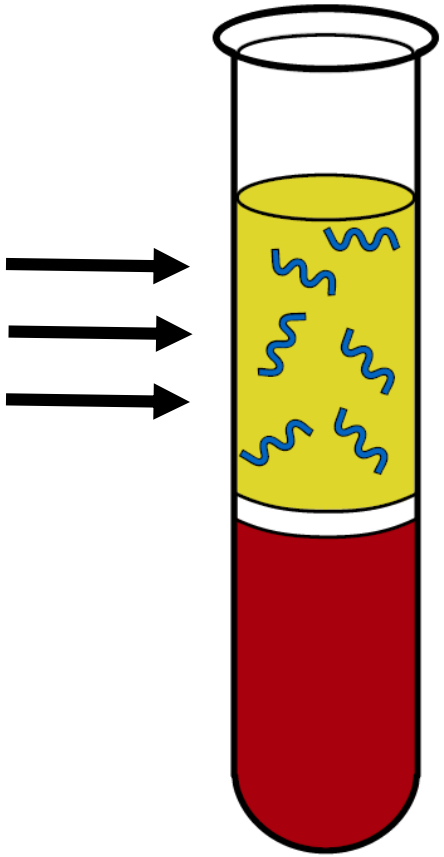
Cell-free DNA (cfDNAs)

Advantages

- single tube of blood
- non-invasive
- standard lab procedure
- no fasting required

Major challenges

- scant 1 cell has ~6pg of DNA
- short lived 15 minute half-life
- short 150bp fragments
- damaged and degraded

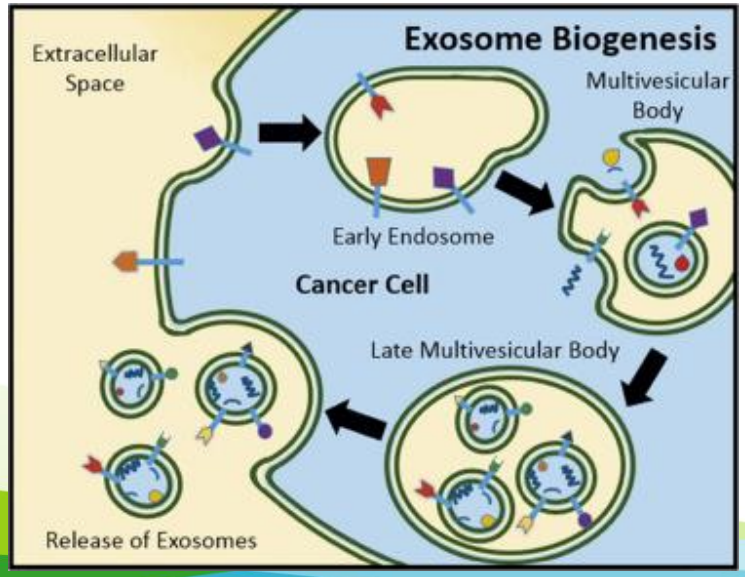


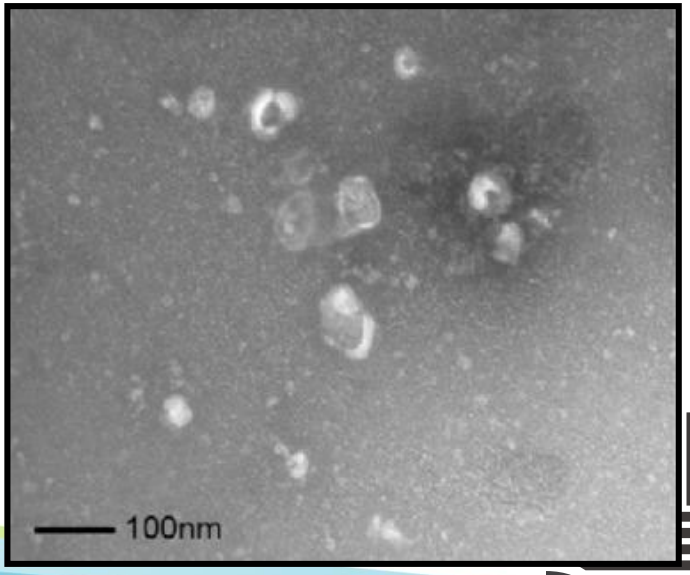
The Liquid Biopsy Summit, 2016
Andrew Hill

Exosome

- Subset of extracellular vesicles
- 30~200nm in size
- Originate from the intracellular endosome compartment
- Contain miRNA, mRNA and proteins in the lumina
- Cellular communication

Cancer progression through interaction with target cells and cargo deposition into the target cell intracellular space

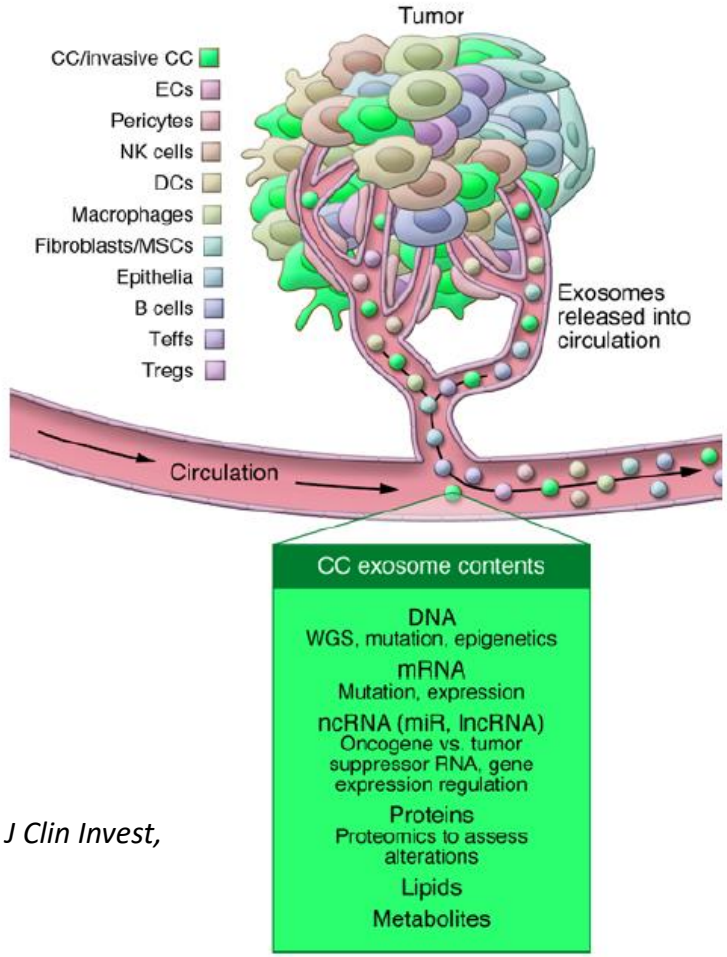


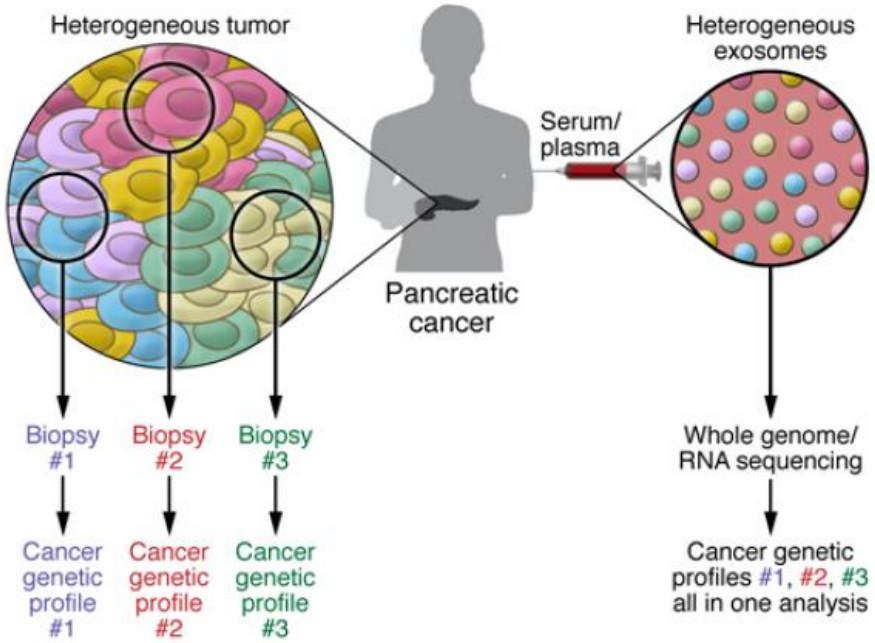


Franzen CA et al. *J Urol* 2016

Types of Liquid Biopsies

Exosomes





Kalluri, J Clin Invest, 2016.

➔ Offering genetic information reflecting the status of all the cancer cells in the tumor accounting for tumor heterogeneity?

- Heterogeneous population that generates a unique tumor nanoenvironment
- Participates in cell-to-cell communications within the microenvironment
- Contributes to the heterogeneity of circulating exosomes

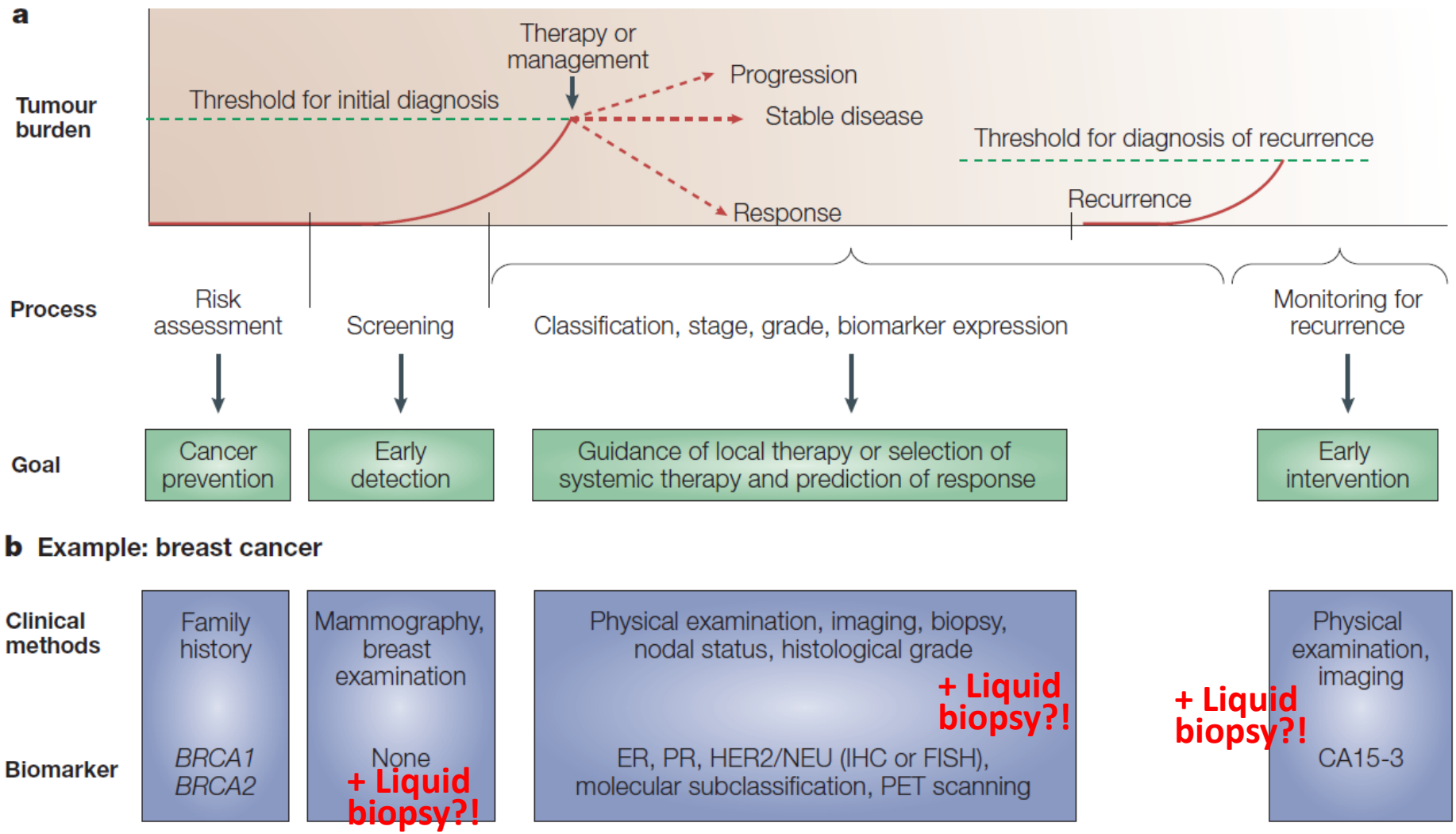
Table 1 Comparison of the analysis capability of CTC's, cfDNA and exosomes

Analysis capability	Examples	CTCs	cfDNA	Exosomes
Mutations	Point mutations, InDels, amplifications, deletions, translocations	Yes	Yes	Yes
Epigenetic modifications	Methylation patterns	Yes	Yes	Yes
RNA transcription profiles	Levels/activity of mRNA, microRNA, long non codingRNA, RNA splice variants	Yes	No	Yes
Phenotypic studies of cells from the tumor	Cell morphology, protein localization, *in vivo* studies	Yes	No	No
Inflammatory response, stromal and other systemic changes	Inflammatory RNA and protein markers	No	No	Yes
Analysis of RNA as well as DNA and protein profiles from tumor cells	Separate or in combination	Yes	No	Yes
Can utilize biobanked samples	Frozen plasma, urine and other biofluids	No	Yes	Yes

CTCs, circulating tumor cells; cfDNA, cell free DNA; InDels, insertions/deletions.

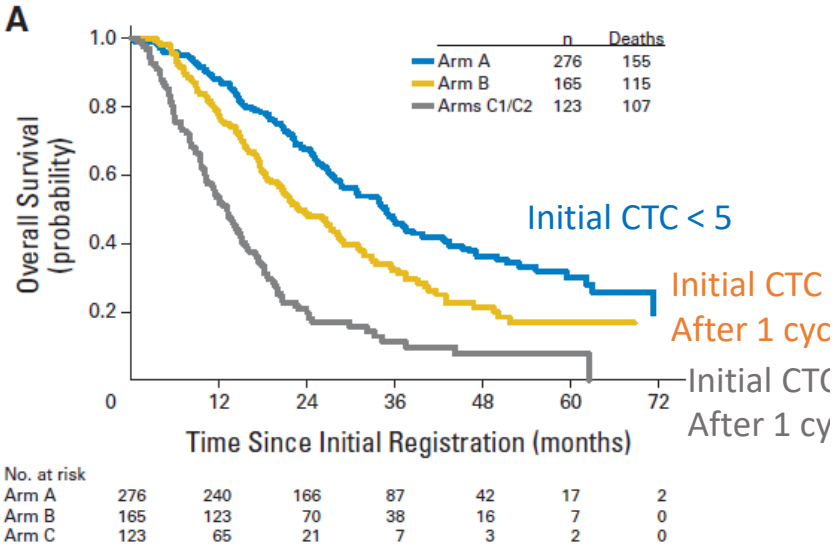
Brock G et al, Transl Cancer Res, 2015.

Biomarkers for precision medicine



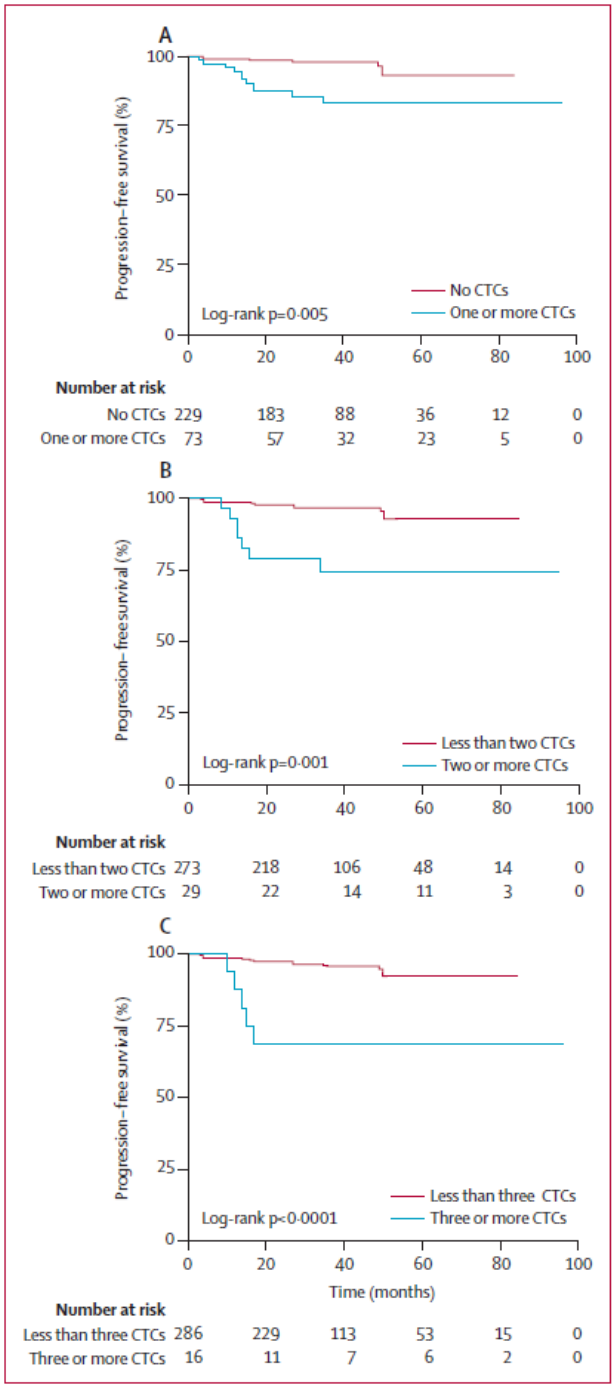
Ludwig and Weinstein, Nat Rev Cancer, 2005.

Liquid biopsies in bre s



Metastatic breast cancer

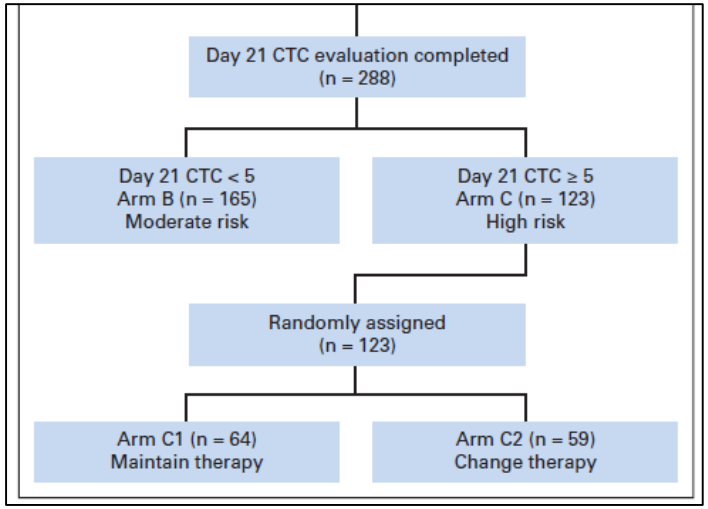
→ **CellSearch® approved by FDA for MBC**



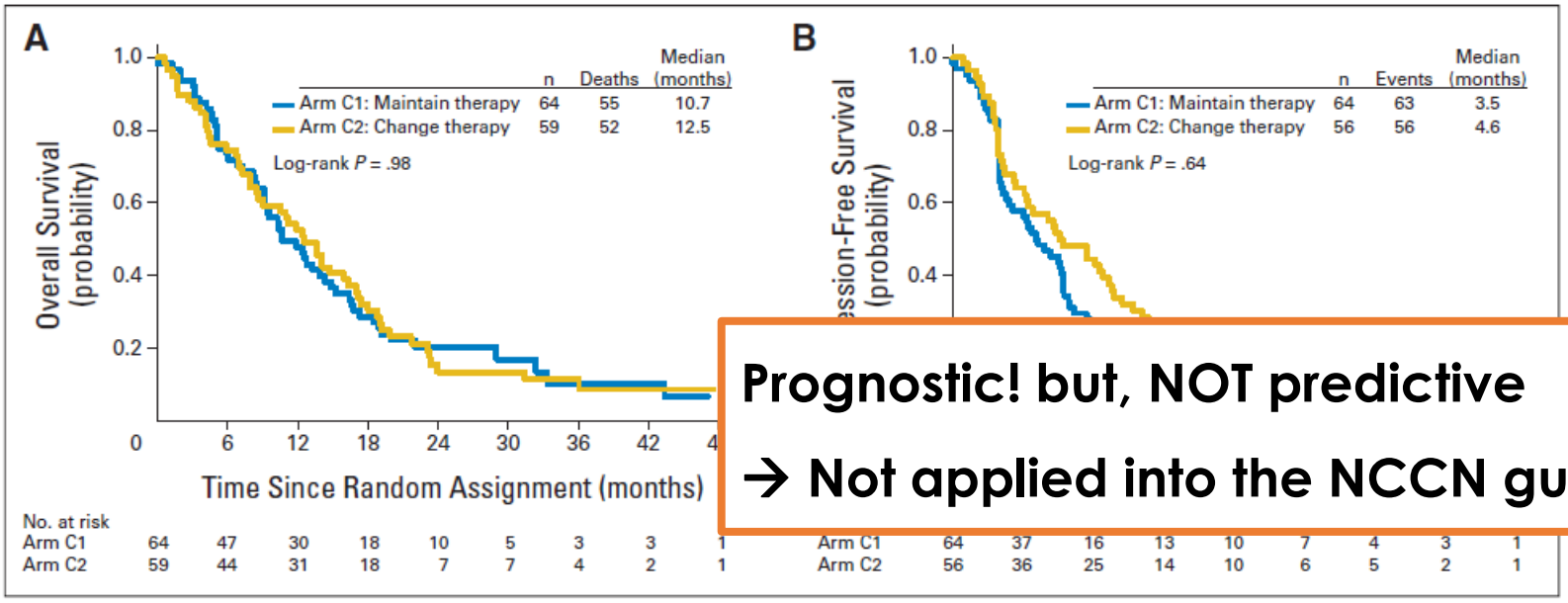
Stage I-III breast cancer

Smerage JB et al, JCO, 2014.
Lucci A et al, Lancet Oncol, 2012

Liquid biopsies in breast cancer: CTCs



RCT for **therapy change** when CTC persists after 1 cycle of treatment

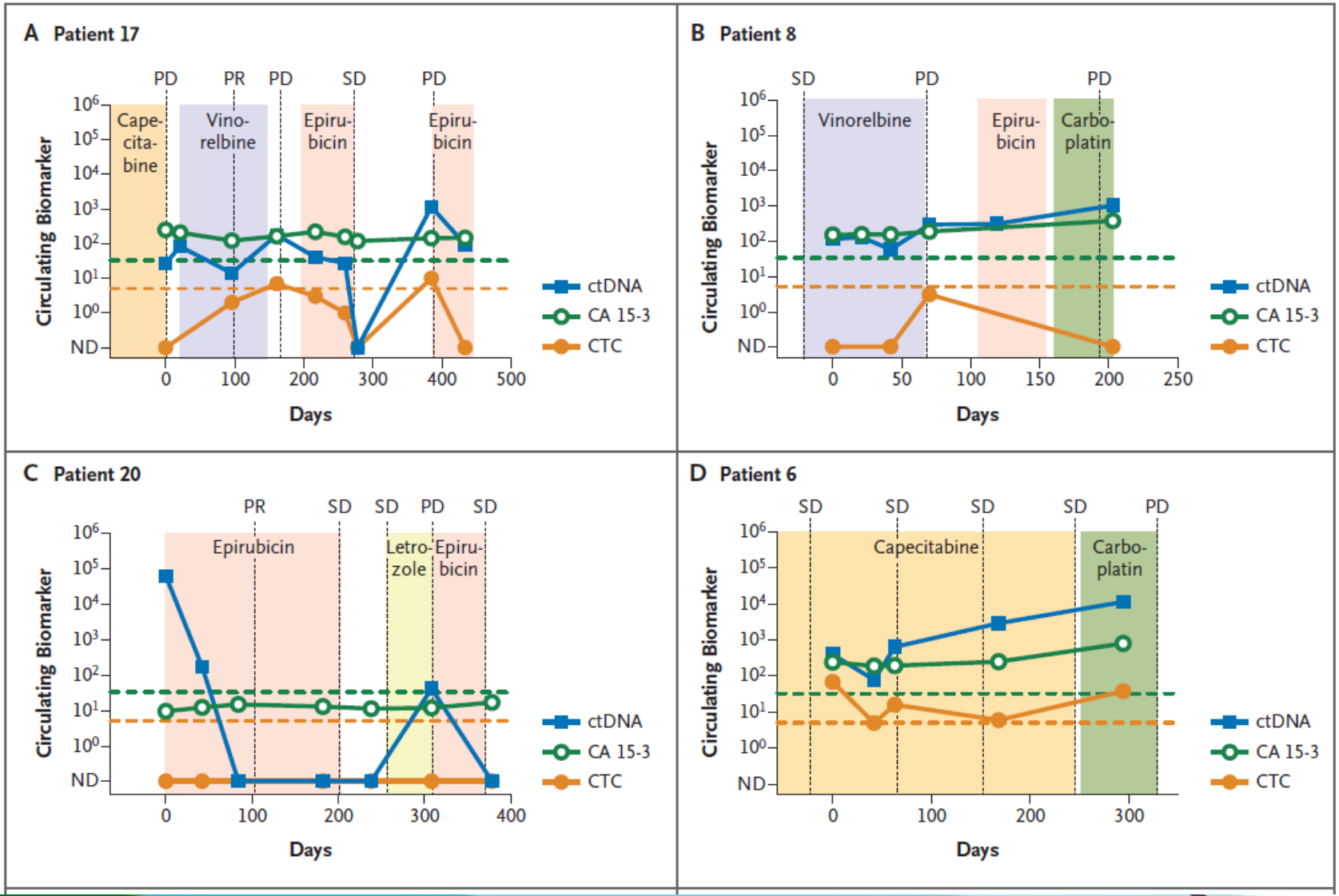


Prognostic! but, NOT predictive

→ Not applied into the NCCN guidelines

cfDNAs in breast cancer

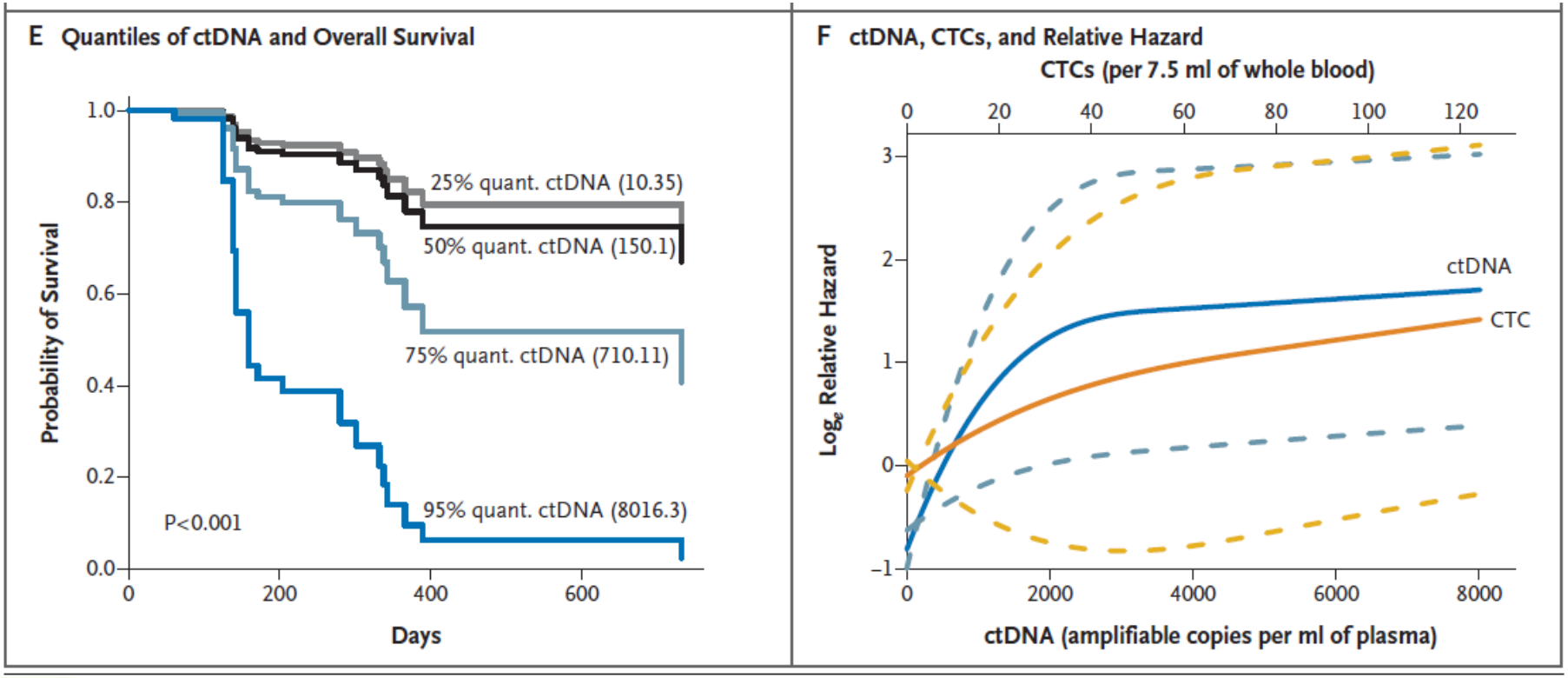
Longitudinal Monitoring



Dawson SJ et al, NEJM, 2013.

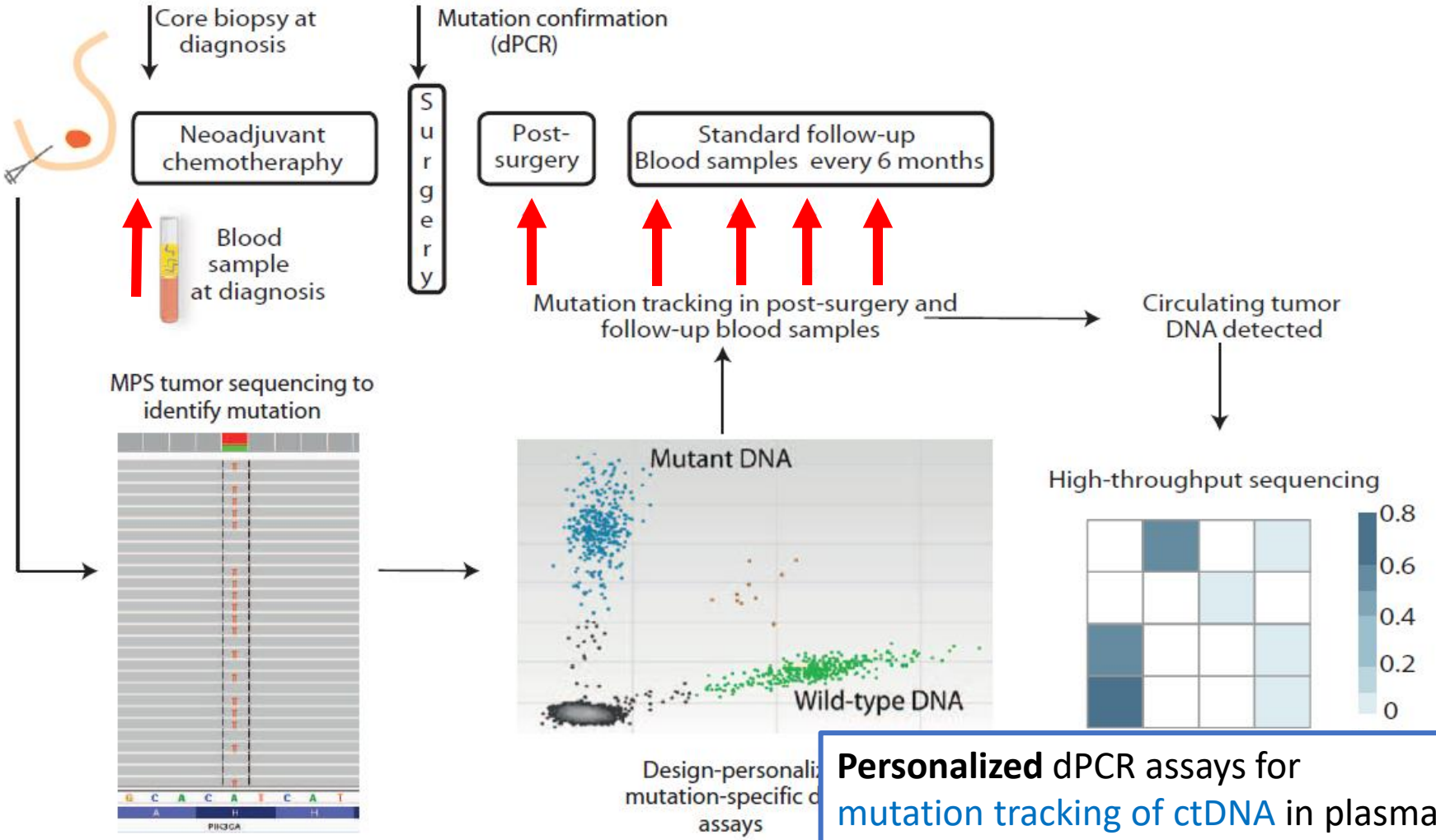
cfDNAs in breast cancer

Prognostication



cfDNAs in breast cancer

Longitudinal Monitoring & Early Detection

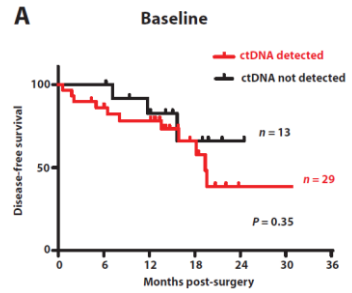


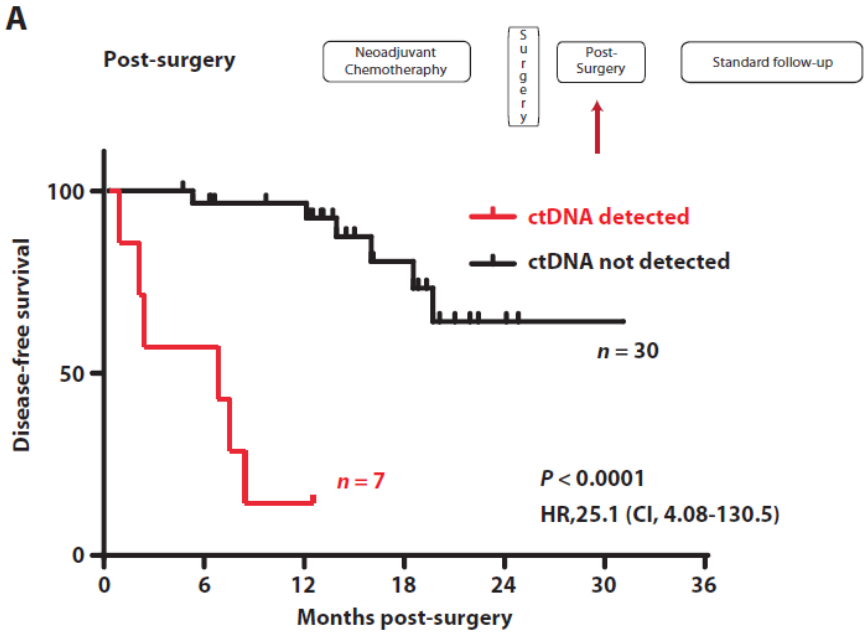
Personalized dPCR assays for mutation tracking of ctDNA in plasma of patients with early breast cancer

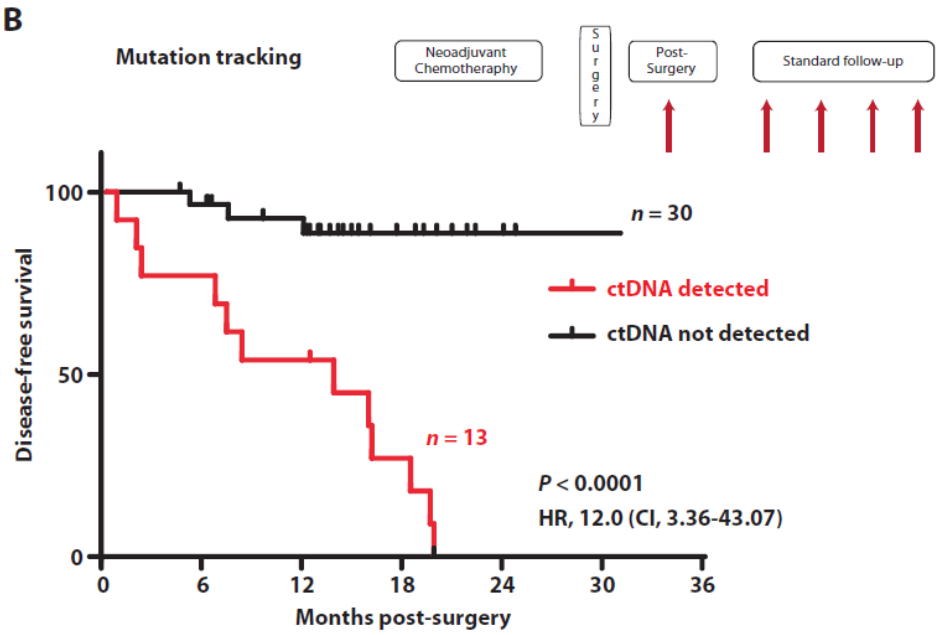
Garcia-Murillas et al, Sci Trans Med, 2015.

cfDNAs in breast cancer

Prognostication



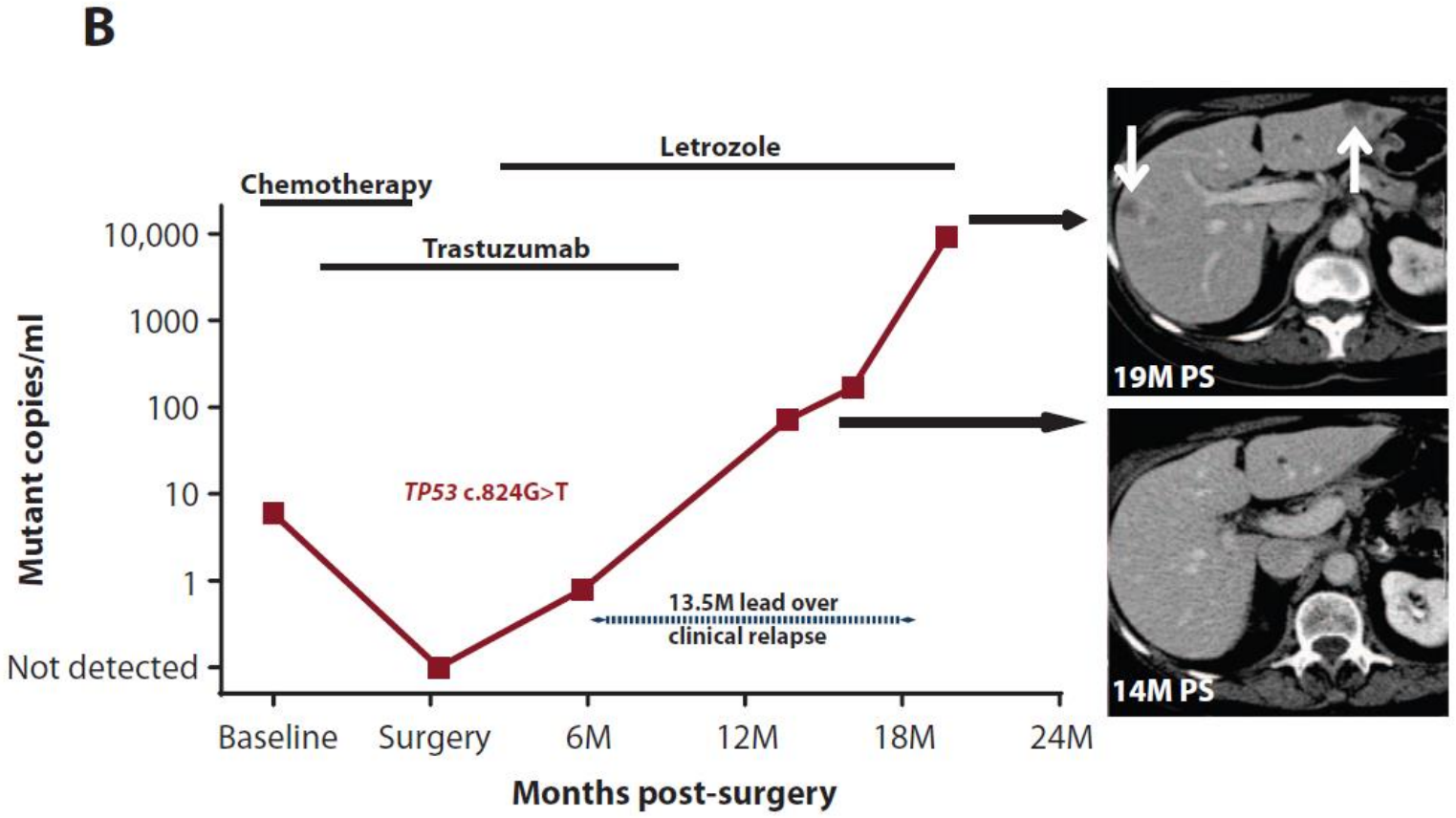




Garcia-Murillas et al, Sci Trans Med, 2015.

cfDNAs in breast cancer

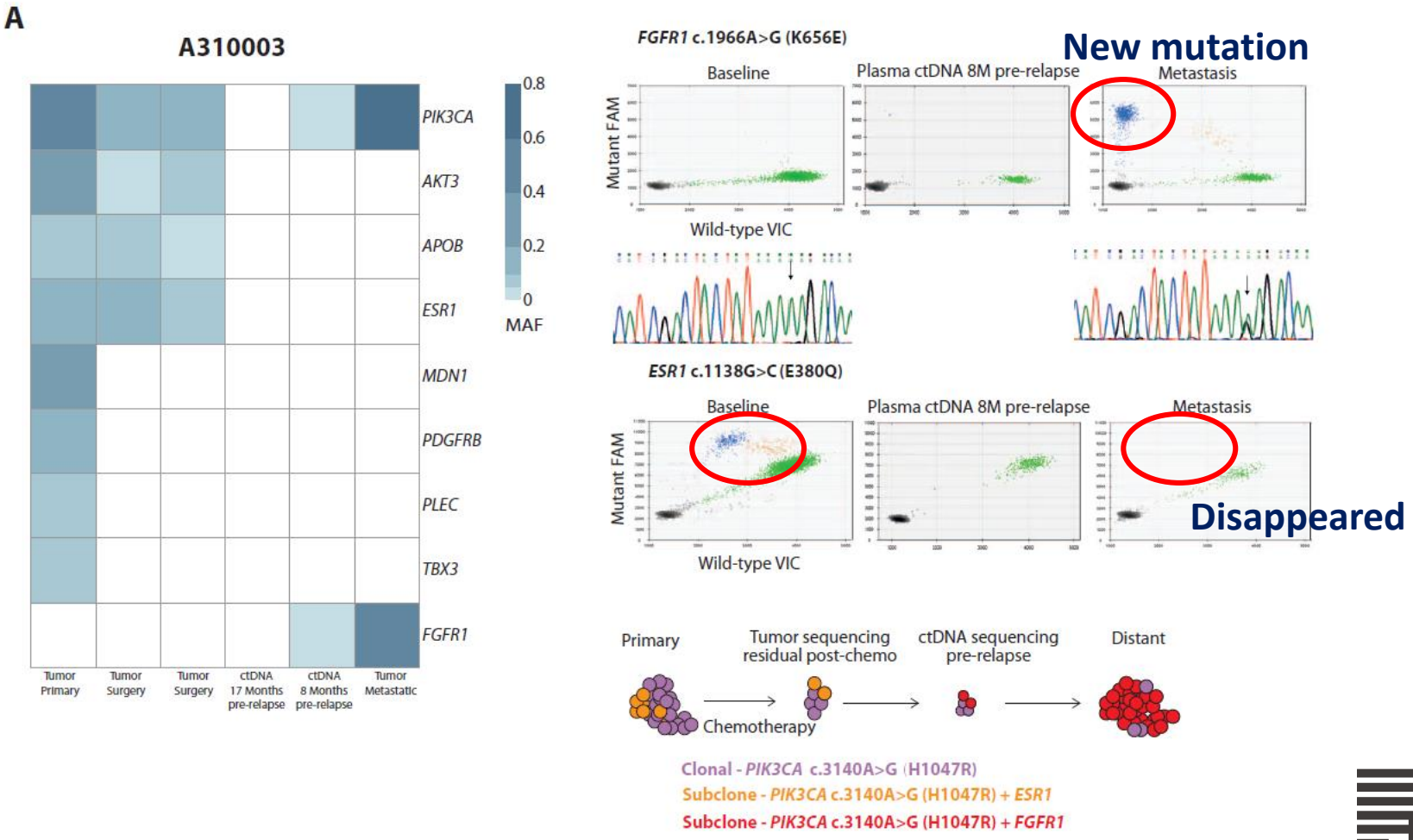
Early Detection



Garcia-Murillas et al, Sci Trans Med, 2015.

cfDNAs in breast cancer

Intra-/Inter-tumoral heterogeneity



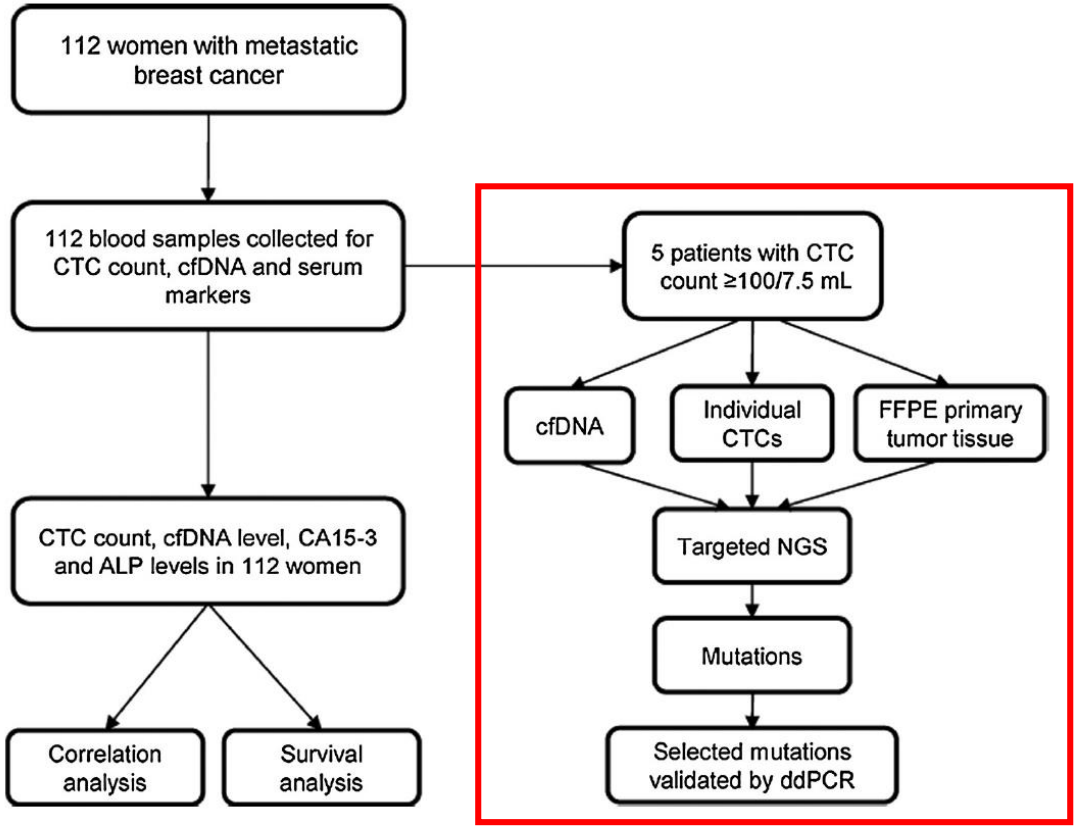
Garcia-Murillas et al, Sci Trans Med, 2015.

cfDNAs in breast cancer

Intra-/Inter-tumoral heterogeneity

- Compare CTC and ctDNA mutation profiles in metastatic breast cancer

→Does ctDNA reflect tumor heterogeneity?



Shaw JA et al, Clin Canc Res, 2017.

Table 2. Mutations identified in matched FFPE, cfDNA,

Mutations matched!

Sample ID	Receptor status		CTC	cfDNA (AF)	Primary tumor region 1 (AF)	Primary tumor region 2 (AF)
CTCM155	ER+/PR+/HER2−	C1	PIK3CA p.H1047R; ESR1 p.E380Q	PIK3CA p.H1047R (23.7%); ESR1 p.E380Q (3.9%)	PIK3CA p.H1047R (67.8%)	PIK3CA p.H1047R (72.4%)
		C2	PIK3CA p.H1047R; ESR1 p.E380Q			
		C3	PIK3CA p.H1047R; ESR1 p.E380Q			
		C4	PIK3CA p.H1047R; ESR1 p.E380Q			
		C5	PIK3CA p.H1047R			
		Cpool	PIK3CA p.H1047R; ESR1 p.E380Q			
CTCM138	ER+/PR+/HER2−	C1	—/NC	PIK3CA p.H1047R (31%); ESR1 p.E380Q (23.8%); TP53 p.R175H (0.3%); ESR1 Y537C (0.43%)	PIK3CA p.H1047R (14.2%); TP53 p.R175H (1.9%)	PIK3CA p.H1047R (36%); TP53 p.R175H (0.99%)
		C2	PIK3CA p.H1047R; ESR1 p.E380Q			
		C3	PIK3CA p.H1047R; ESR1 p.E380Q			
		C4	—			
		C5	—			
		Cpool	PIK3CA p.H1047R; ESR1 p.E380Q			
CTCM105	ER−/PR−/HER2−	C1	KRAS p.G12D	KRAS p.G12D (3.8%); TP53 p.P278R (2.3%)	—	—
		C2	KRAS p.G12D			
		C3	KRAS p.G12D			
		C4	—			
		C5	KRAS p.G12D			
		C6	KRAS p.G12D			
		C7	NC			
		C8	KRAS p.G12D			
		C9	—			
		C10	KRAS p.G12D			
		C11	NC			
		Cpool	KRAS p.G12D			
CTCM292	ER+/PR+/HER2−	C1	PIK3CA p.E545K; ESR1 p.D538G	PIK3CA p.E545K (0.17%); ESR1 p.D538G (0.27%)	None available	None available
		C2	PIK3CA p.E545K; ESR1 p.D538G			

Mutations unique to cfDNA and CTCs

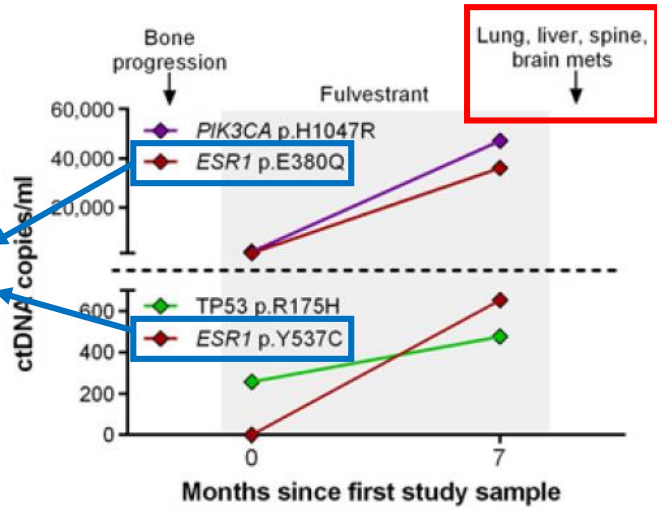
Shaw JA et al, Clin Canc Res, 2017.

Sample ID	Receptor status	CTC		cfDNA (AF)	Primary tumor region 1 (AF)	Primary tumor region 2 (AF)
CTCM138	ER$^+$/PR$^+$/HER2$^-$	C1	—/NC	PIK3CA p.H1047R (31%); ESR1 p.E380Q (23.8%); TP53 p.R175H (0.3%); ESR1 Y537C (0.43%)	PIK3CA p.H1047R (14.2%); TP53 p.R175H (1.9%)	PIK3CA p.H1047R (36%); TP53 p.R175H (0.99%)
		C2	PIK3CA p.H1047R; ESR1 p.E380Q			
		C3	PIK3CA p.H1047R; ESR1 p.E380Q			
		C4	—			
		C5	—			
		Cpool	PIK3CA p.H1047R; ESR1 p.E380Q			

Low frequency
- ESR1 Y527C
- TP53 p.R175H

Not detected

Acquired new mutations



cfDNA copies/ml		6970	159091
PIK3CA p.H1047R	AF (%)	32	31
	mutant copies/ml	2230	49318
ESR1 p.E380Q	AF (%)	24.4	23.8
	mutant copies/ml	1701	37864
TP53 p.R175H	AF (%)	3.7	0.3
	mutant copies/ml	258	477
ESR1 p.Y537C	AF (%)	0	0.43
	mutant copies/ml	0	684
CTC count		12	171

Shaw JA et al, Clin Canc Res, 2017.

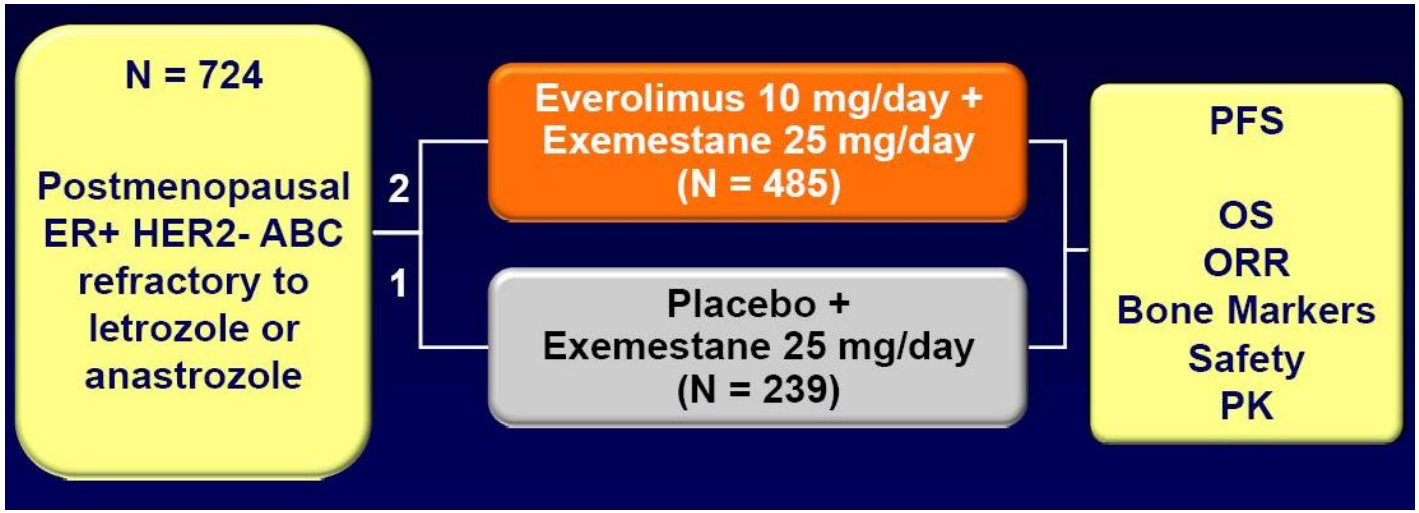
Mini-Summary

- Baseline ctDNA status has not been consistently shown to affect breast cancer prognosis.
- However, changes in ctDNA during treatment or follow-up have been associated with subsequent progression
- Moving forward, we predict that ctDNA analysis will largely overtake genomic analysis from tissue biopsy.
- A key advantage of ctDNA analysis is the ability to repeat the test at each new progression event to detect resistance mutations.
- In addition, serial sampling rather than a one-off analysis may result in more accurate results

cfDNAs in breast cancer

Stratification

BOLERO-2 Trial Design (mTOR inhibitor: Everolimus)



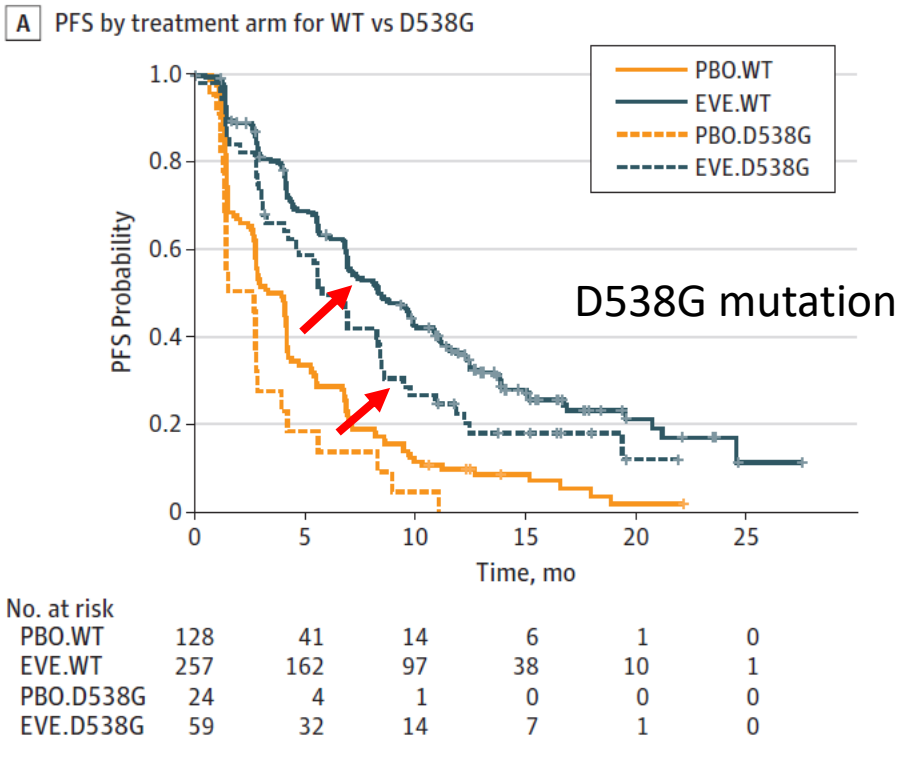
ctDNA analysis for ESR1 D538G and Y537S mutation by ddPCR

	D538G and/or Y537S mutation	D538G mutation	Y537S mutation	Double mutation
Overall, N = 541 (74.7% of ITT)	156 (28.8%)	83 (15.3%)	42 (7.8%)	30 (5.5 %)

Chandarlapaty S et al, JAMA Oncol, 2016.

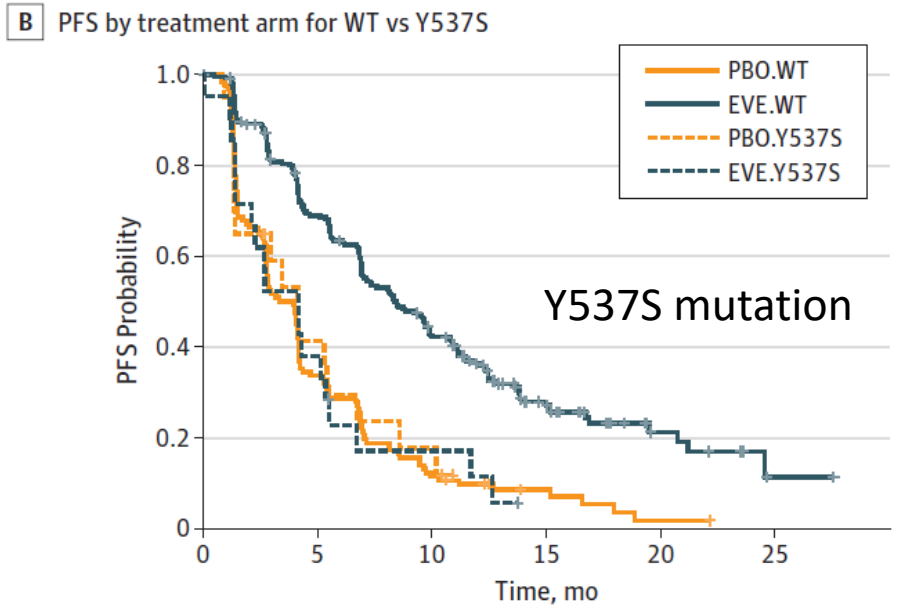
cfDNAs in breast cancer

Stratification



No. at risk

PBO.WT	128	41	14	6	1	0
EVE.WT	257	162	97	38	10	1
PBO.D538G	24	4	1	0	0	0
EVE.D538G	59	32	14	7	1	0



No. at risk

PBO.WT	128	41	14	6	1	0
EVE.WT	257	162	97	38	10	1
PBO.Y537S	21	7	3	0	0	0
EVE.Y537S	21	8	3	0	0	0

Everolimus improved PFS in patients with **wild type** & **D538G mutation**

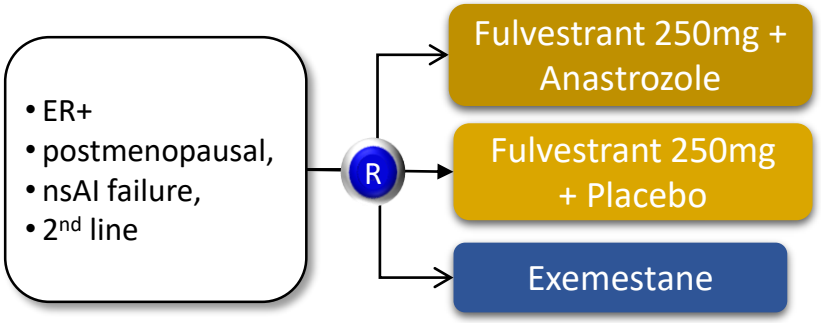
No benefit of everolimus in patients with **Y537S mutation**

Chandarlapaty S et al, JAMA Oncol, 2016.

cfDNAs in breast cancer

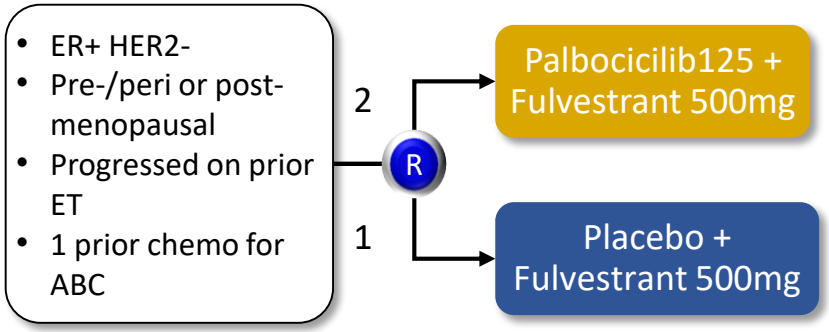
Stratification

SoFEA



➔ No significant difference in PFS

PALOMA3



➔ Improved PFS with palbociclib (CDK4/6 inhibitor)

Table 2. Frequency of Specific *ESR1* Mutations in the SoFEA and PALOMA3 Cohorts

	SoFEA (n = 161)				PALOMA3 (n = 360)			
ESR1 Mutation	No. of Mutations Observed in Cohort		% of SoFEA *ESR1* Mutant (n = 63)	% of All SoFEA Cohort (n = 161)	No. of Mutations		% of PALOMA *ESR1* Mutant (n = 91)	% of All PALOMA Cohort (n = 360)
D538G	29		46.0	18.0	51		56.0	14.2
Y537N	23		36.5	14.3	14		15.4	3.9
Y537S	16		25.4	9.9	23		25.3	6.4
E380Q	6	**39%**	9.5	3.7	22	**27%**	24.2	6.1
S463P	6		9.5	3.7	4		4.4	1.1
Y537C	3		4.8	1.9	5		5.5	1.4
L536R	2		3.2	1.2	1		1.1	0.3

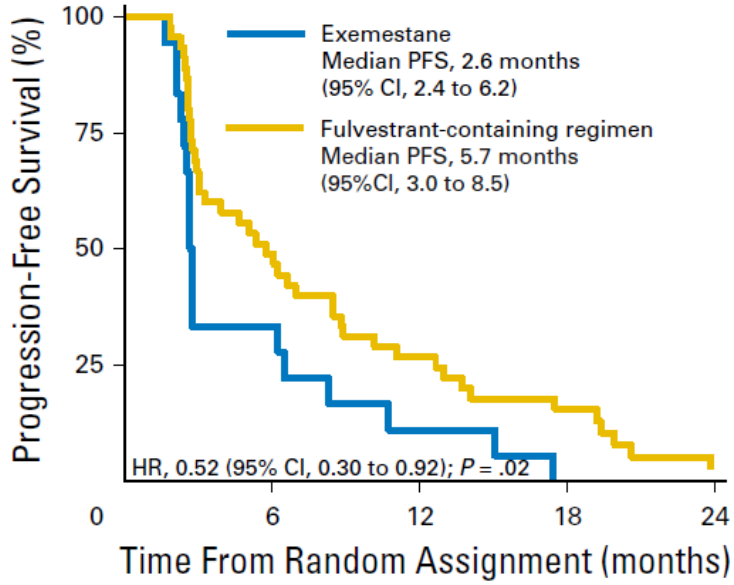
Fribbens C et al, J Clin Oncol, 2016.

cfDNAs in breast cancer

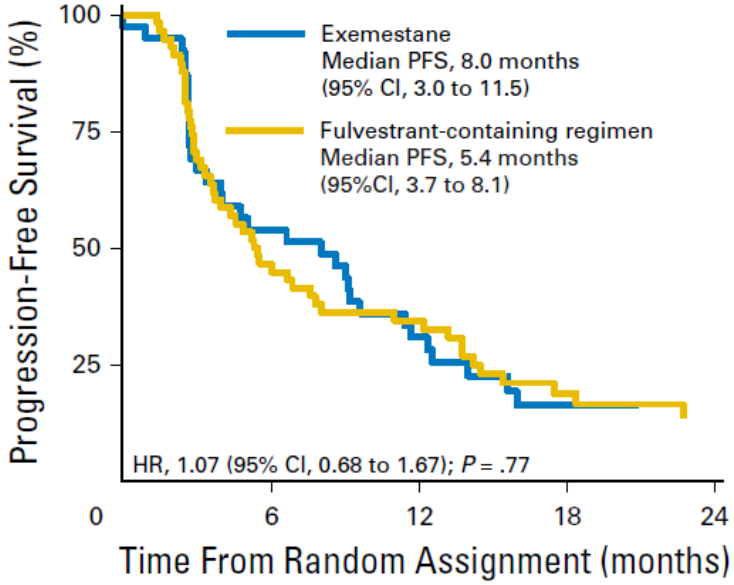
Stratification

SoFEA Trial

A Patients with *ESR1* mutant cancers



B Patients without *ESR1* mutant cancers



- Considering *ESR1* mutation status within the **EXE group**, patients with an *ESR1* mutation had worse PFS than ESR1 wild type (HR, 2.12; 95% CI, 1.18 to 3.81; P = 0.01).
- This provides the **first evidence of potential clinical utility** for the use of ESR1 plasma DNA analysis in **selecting the most appropriate endocrine therapy**.

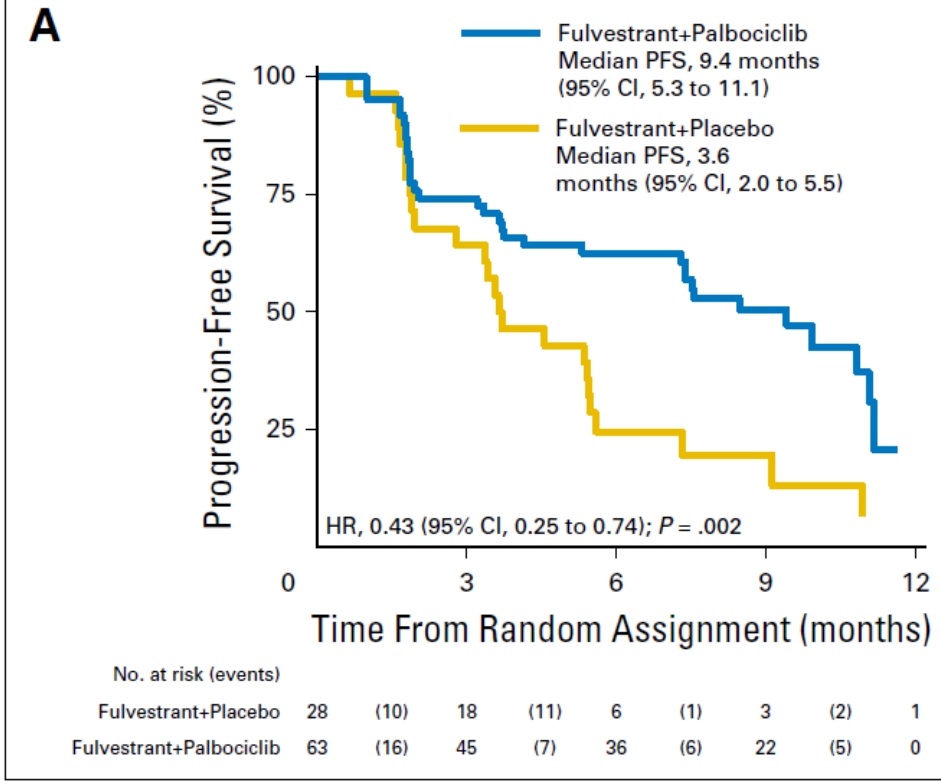
Fribbens C et al, J Clin Oncol, 2016.

cfDNAs in breast cancer

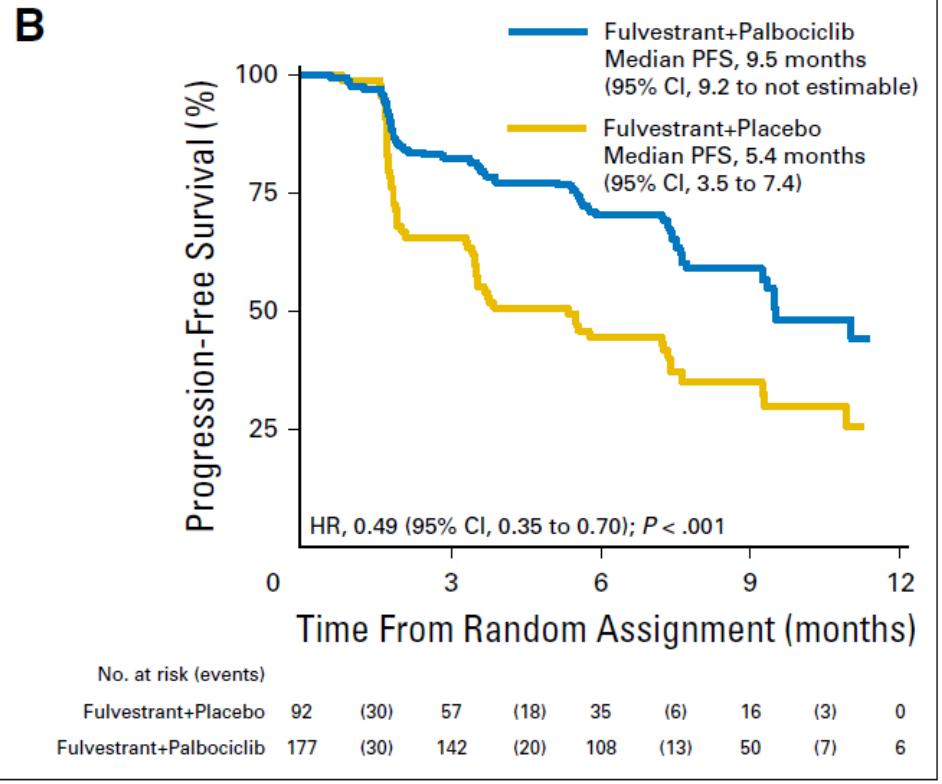
Stratification

PALOMA-3 Trial

Patients with *ESR1* mutant cancers



Patients without *ESR1* mutant cancers



- Palbociclib offers high efficacy regardless of ESR1 mutation status.

Fribbens C et al, J Clin Oncol, 2016.

cfDNAs in cancer

Detection of Acquired Resistance

Table 2 | Mutations responsible for acquired resistance to targeted therapies

Gene	Genetic aberration	Tumour type	Acquired drug resistance	Reference*
EGFR	T790M	Advanced NSCLC	Gefitinib Erlotinib	Yun *et al.* (2008)[113] Murtaza *et al.* (2013)[105]
KRAS	Codon 12, 13 and 61	Colorectal cancer	Cetuximab	Diaz *et al.* (2012)[7] Misale *et al.* (2012)[8]
KIT	T670I	GIST	Imatinib	Tamborini *et al.* (2006)[155]
PIK3CA	NS	NSCLC	Erlotinib Gefitinib	Sequist *et al.* (2011)[134] Murtaza *et al.* (2013)[105]
ALK	C1156Y L1196M	NSCLC	Crizotinib	Choi *et al.* (2010)[156]
MEK1	C121S	Melanoma	Vemurafenib	Wagle *et al.* (2011)[128]
BRAF	Amplification	Melanoma	Vemurafenib	Shi *et al.* (2012)[123] Gevensleben *et al.* (2013)[98]
NRAS	Q61K	Melanoma	Vemurafenib	Nazarian *et al.* (2010)[124]

*References include a selection of studies in which detection of the genetic alteration has been technically achieved in circulating DNA of patients with cancer or proof-of-principle demonstrated.
Abbreviations: GIST, gastrointestinal stromal tumour; NS, not specified; NSCLC, non-small-cell lung cancer.

Crowley, E. *et al. Nat. Rev. Clin. Oncol.* 10, 472–484 (2013)

Liquid biopsies in other cancers

Lung Cancer

FDA Approves First Liquid Biopsy Test for Lung Cancer Patients

Posted on June 6, 2016 by Srivani Ravoori, PhD

On June 1, the U.S. Food and Drug Administration (FDA) approved a liquid biopsy test, a companion diagnostic test called cobas EGFR Mutation Test v2. The test uses plasma samples to identify patients with metastatic non-small cell lung cancer (NSCLC) eligible for treatment with the EGFR-targeted therapeutic erlotinib (Tarceva).

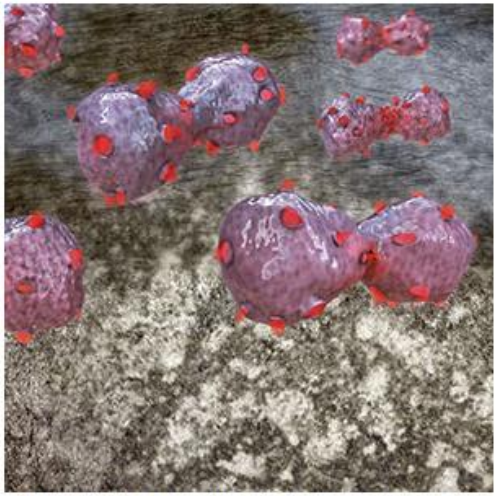
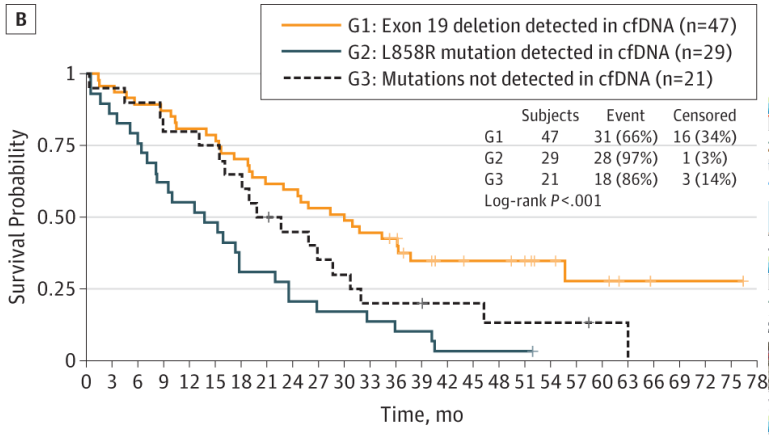


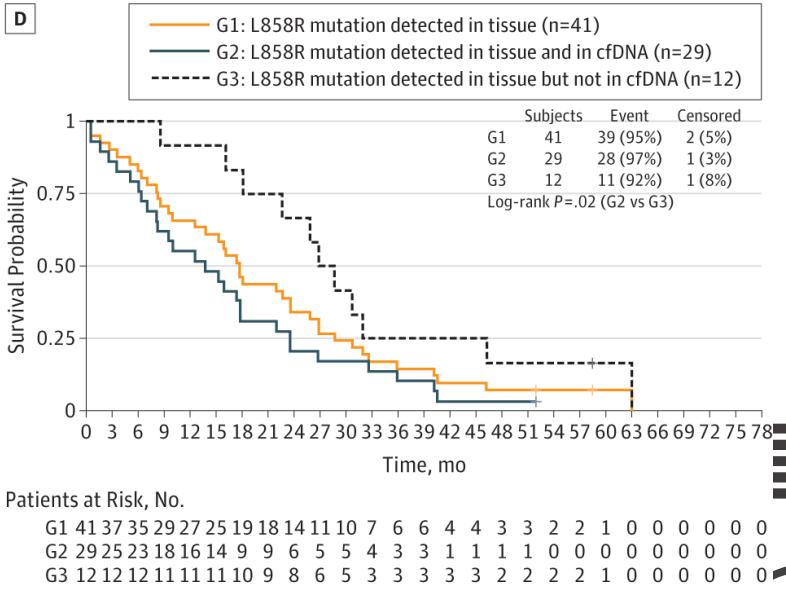
Illustration of lung cancer cell during cell division.

The test detects specific alterations in the gene epidermal growth factor receptor (EGFR): exon 19 deletions or exon 21 (L858R) substitution mutations. These mutations are present in about 10 to 20 percent of NSCLCs, the most common type of lung cancer.

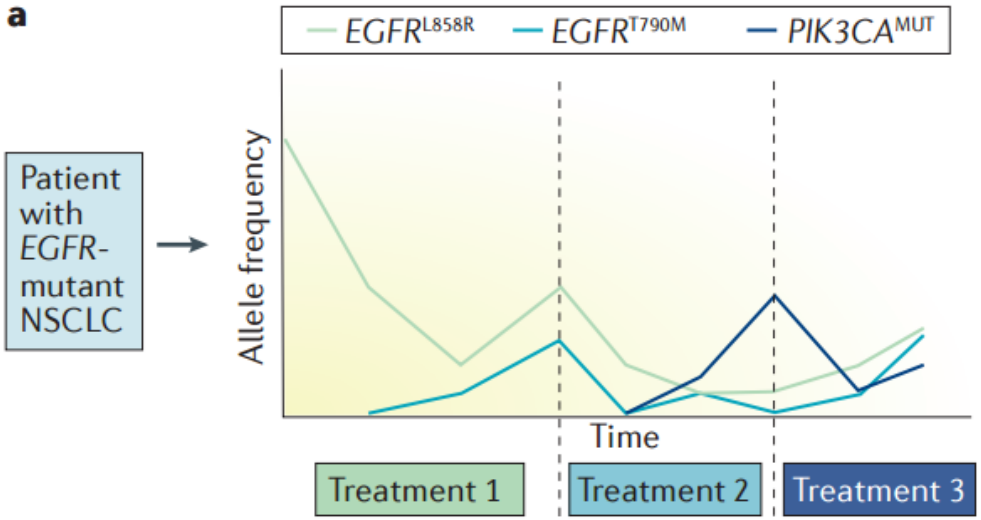
This is the first liquid biopsy test approved for use by the FDA.

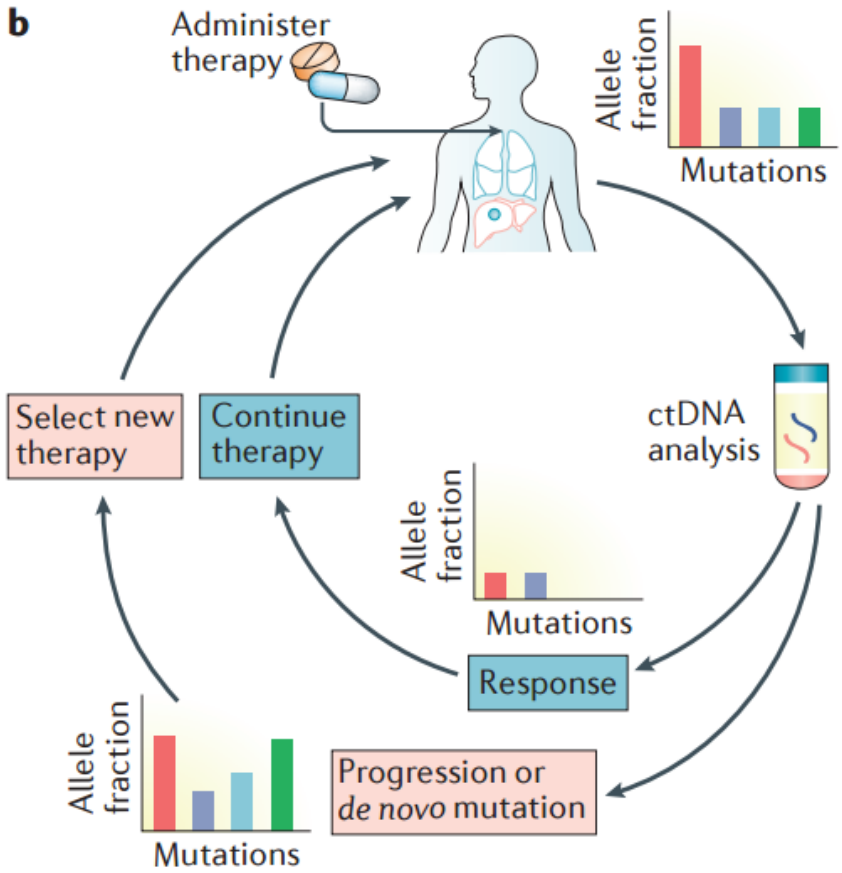
EGFR mutations in cfDNA predict patient survival!



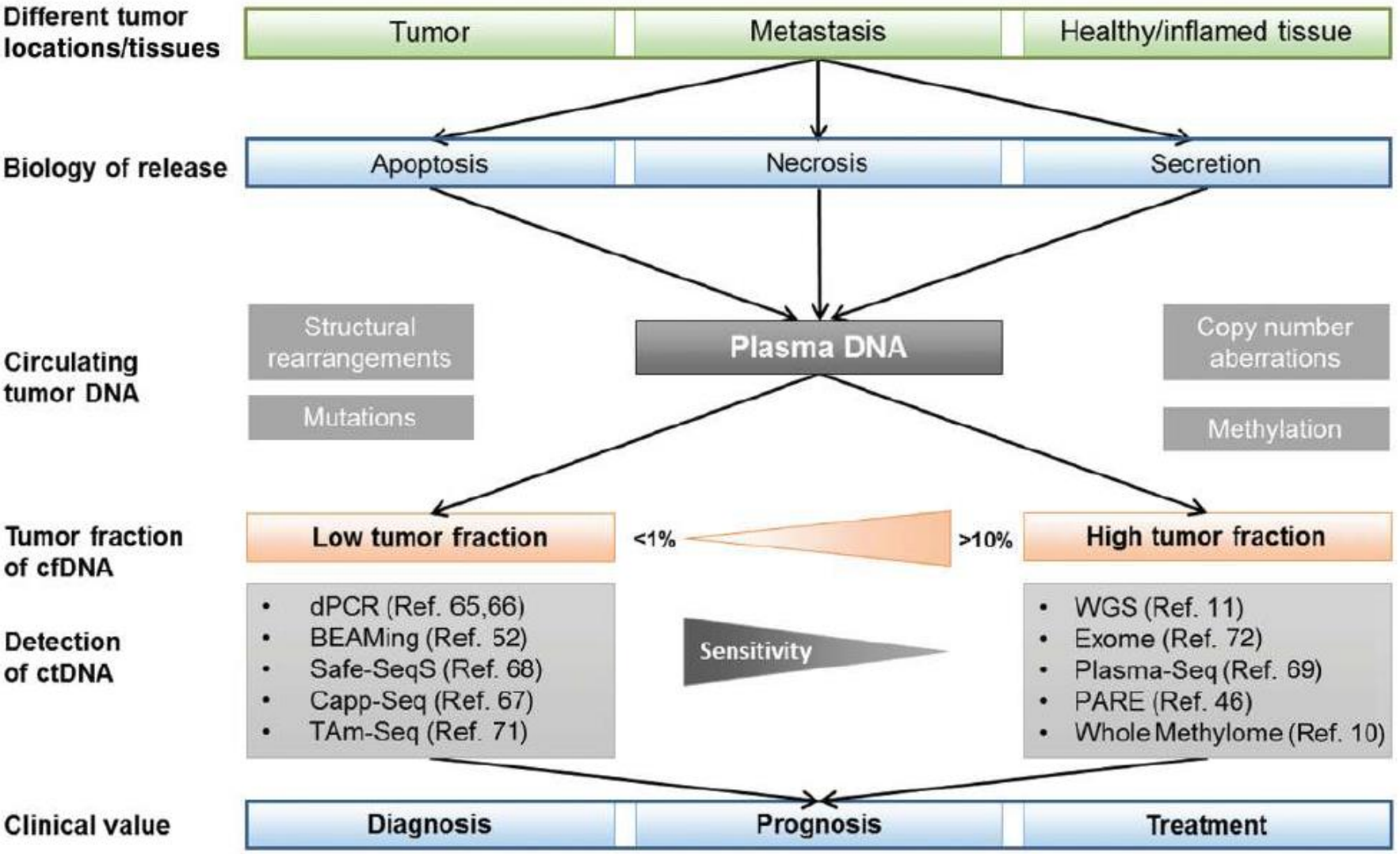


Karachaliou N et al, JAMA Oncol, 2015



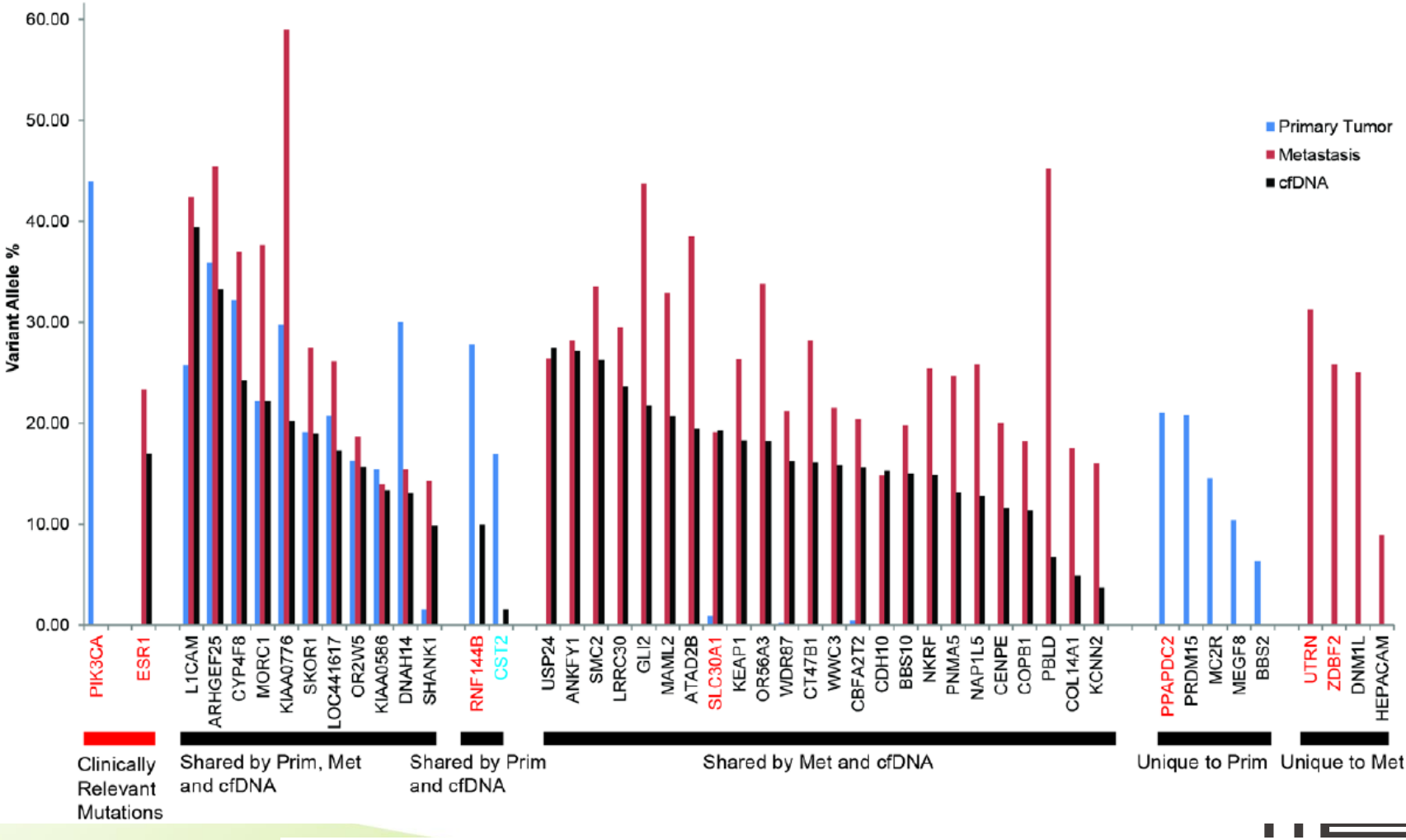


Schematic representation of the liquid biopsy as a tool for cancer monitoring



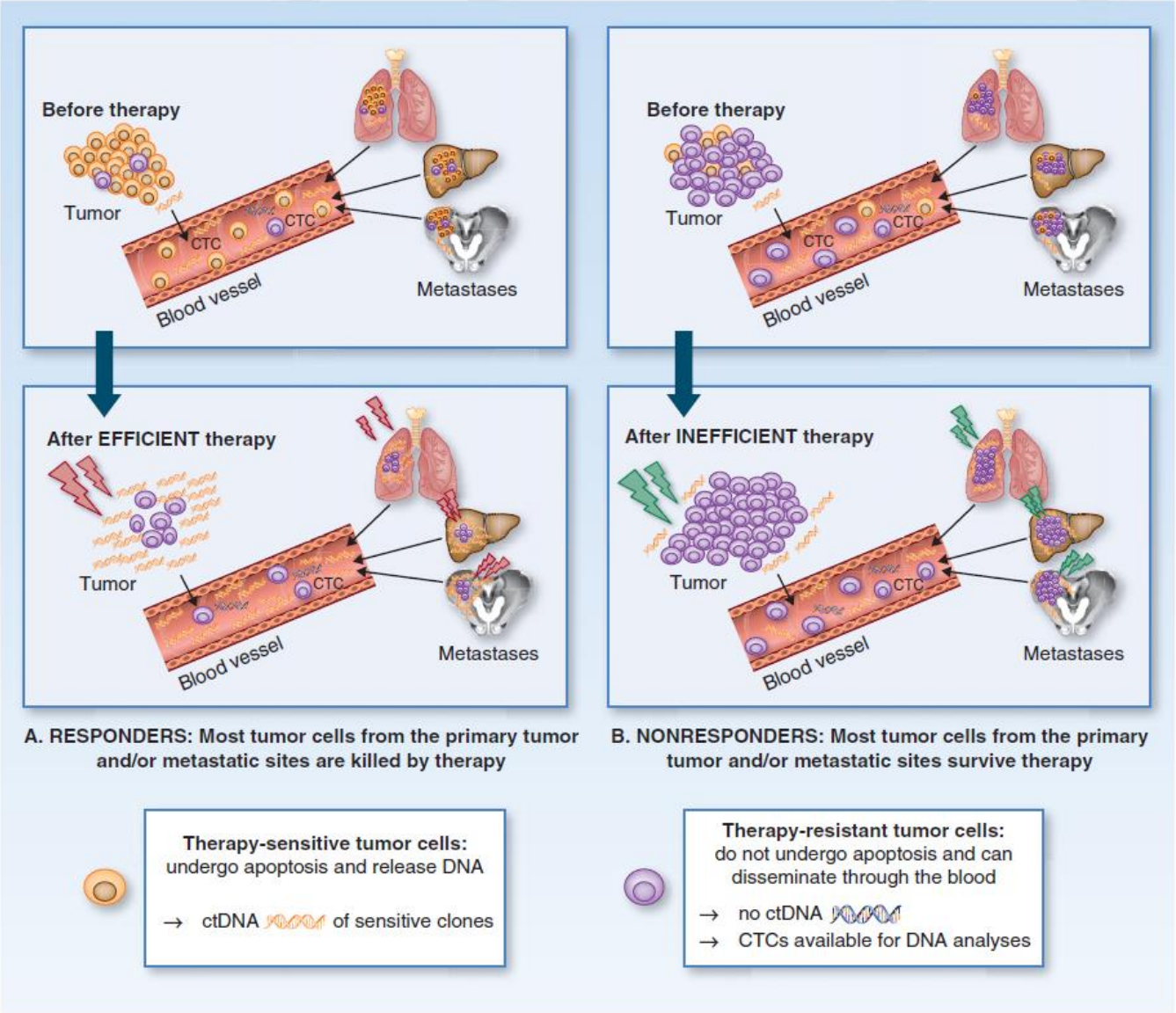
Moving Forward and Considerations

ctDNA as a Liquid Biopsy



Butler, T.M., Johnson-Camacho, K., Peto, M., et. al. Exome sequencing of cell-free DNA from metastatic cancer patients identifies clinically actionable mutations distinct from primary disease (2015) PLoS ONE, 10 (8)

CTC vs. ctDNA? **Complementary Roles!!**



Alix-Panabieres and Pantel, Canc Disc, 2016.

Limitations of Liquid biopsy

- Cost

-improvements in technology and reductions in the cost of sequencing mean the cost is unlikely to remain a limiting problem.

- how tumor markers should be selected, that is, whether multiple mutational panels should be adopted or personalized panels based on the sequence of the cancer of an individual .

- the lack of standardization of techniques

Moving Forward

1. CTC and cfDNA analysis should be incorporated into ongoing clinical trials where blood collection is mandatory, thus allowing for greater generalizability and more impactful results.

2. Need to develop SOPs for cfDNA and CTC and exoxome sample archiving, and make this routine practice for ongoing clinical trials, thus allowing reassessment or further assessment of archived samples following technological advances.

3. Need to design trials that incorporate both CTCs and cfDNA to allow for direct comparison and determination of each biomarker's role and value in various disease settings.

4. Need rationally designed prospective trials from which to draw meaningful conclusions.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Thank you for your attention.