Liquid Biopsies and Their Application in Clinical Trials

David Cescon MD PhD FRCPC
Medical Oncologist and Clinician Scientist
Princess Margaret Cancer Centre
University of Toronto

Canadian Cancer Trials Group
A national program of the Canadian Cancer Society

New Investigator Course
August, 2019

UNIVERSITY OF TORONTO

UHN Princess Margaret Cancer Centre

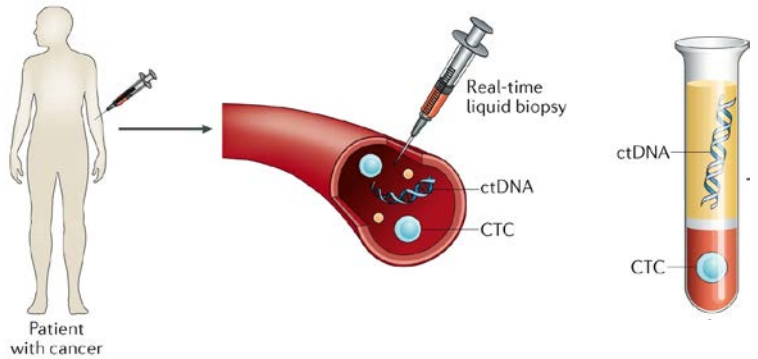
Disclosures

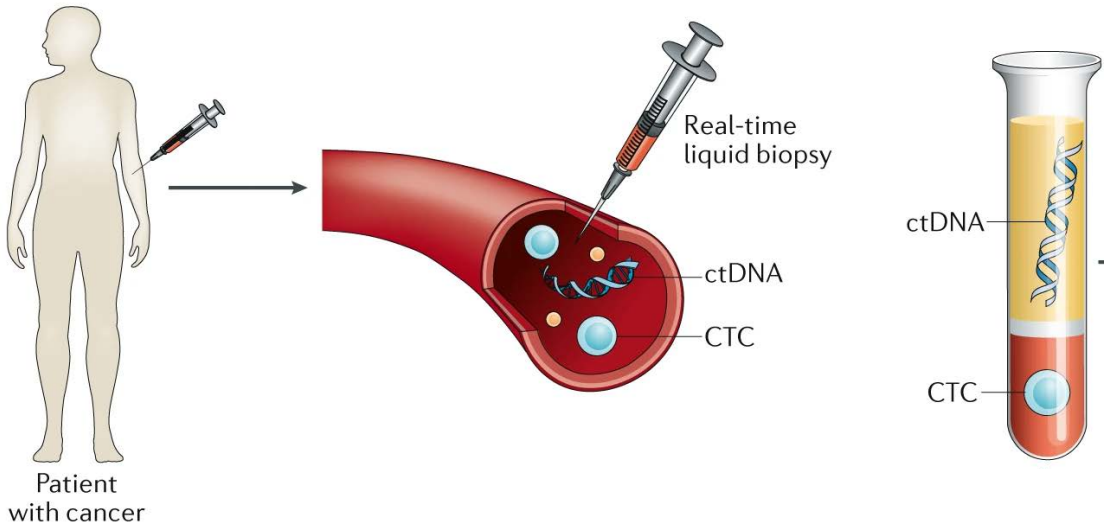
- Research Funding (Institution): Merck, Pfizer, GSK
- Consulting/Advisory: Merck, Pfizer, Novartis, Puma, GSK, AstraZeneca, Agendia, Roche, Dynamo Therapeutics

Educational Objectives

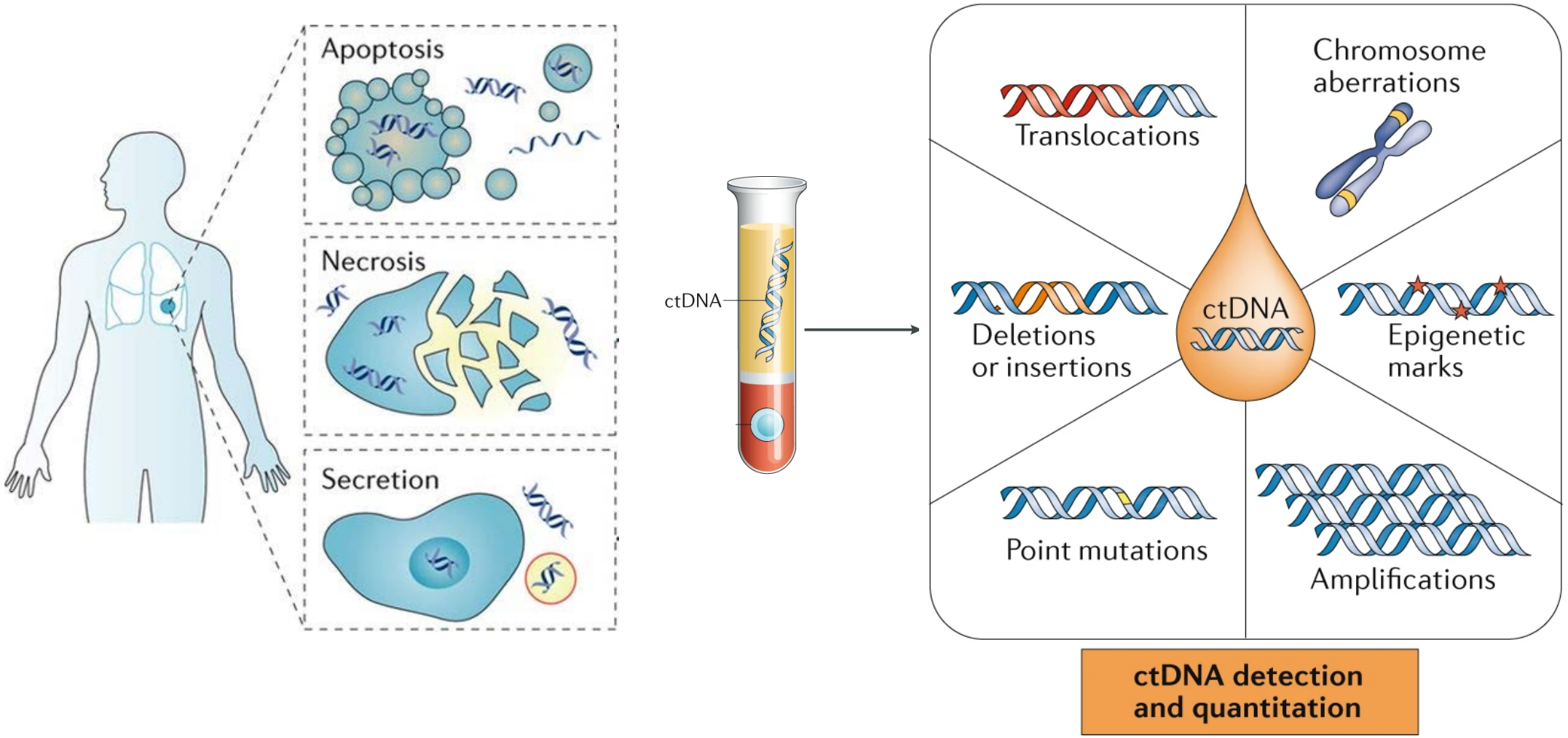
- Appreciate the principles of liquid biopsy analysis
- Identify potential applications of liquid biopsies in clinical trials
- Understand advantages and disadvantages of liquid biopsy compared to tissue-based assays

Liquid Biopsy – Assays and Analytes



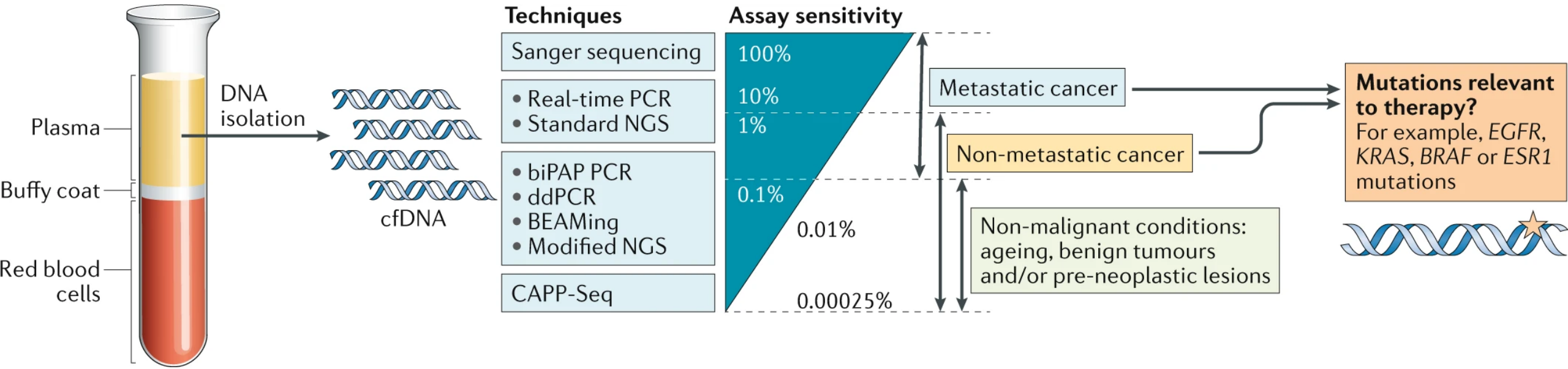


Pantel, Nature Reviews Clinical Oncology (2019)



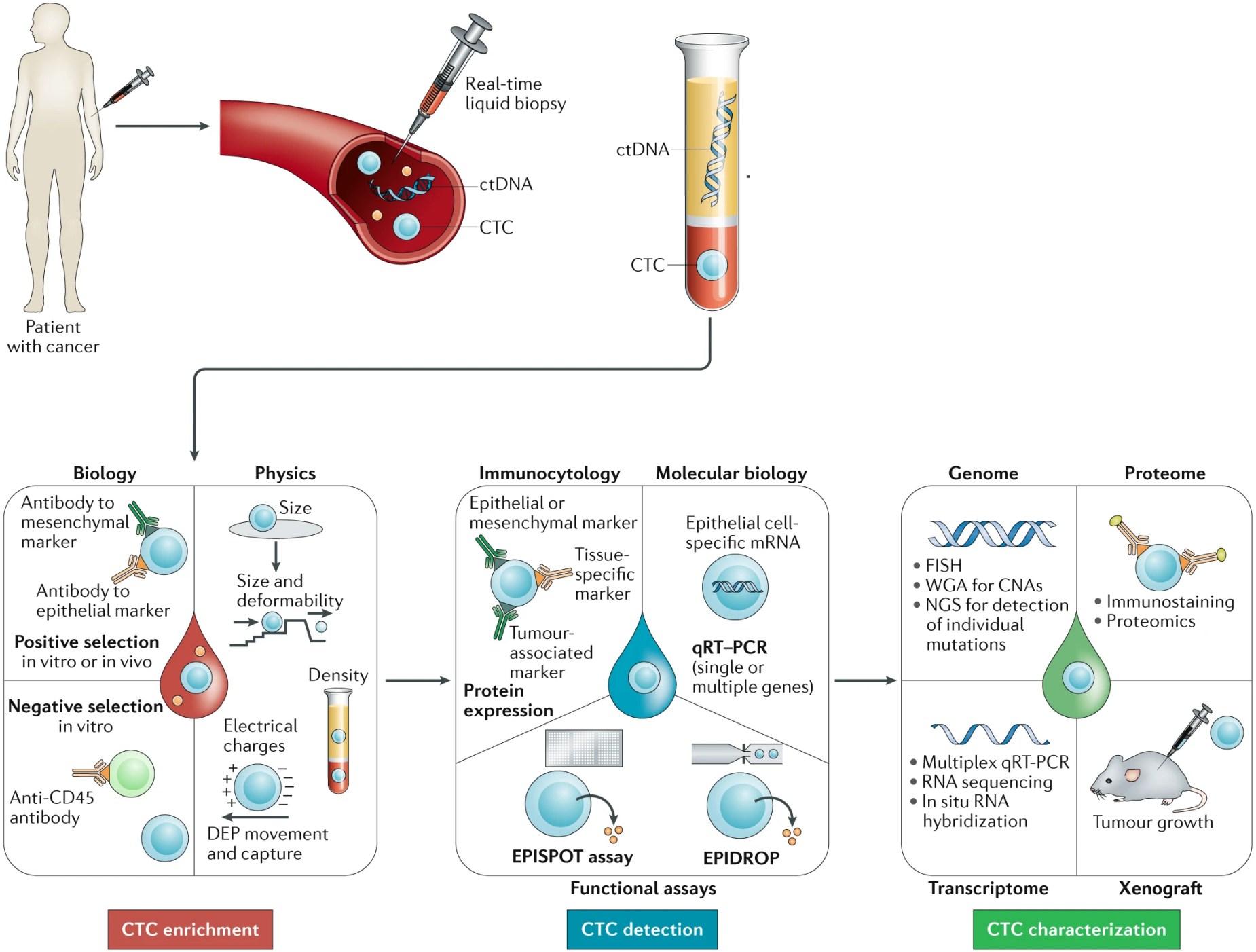
Pantel, Nature Reviews Clinical Oncology (2019)

Technology Development Has Unlocked ctDNA Analysis

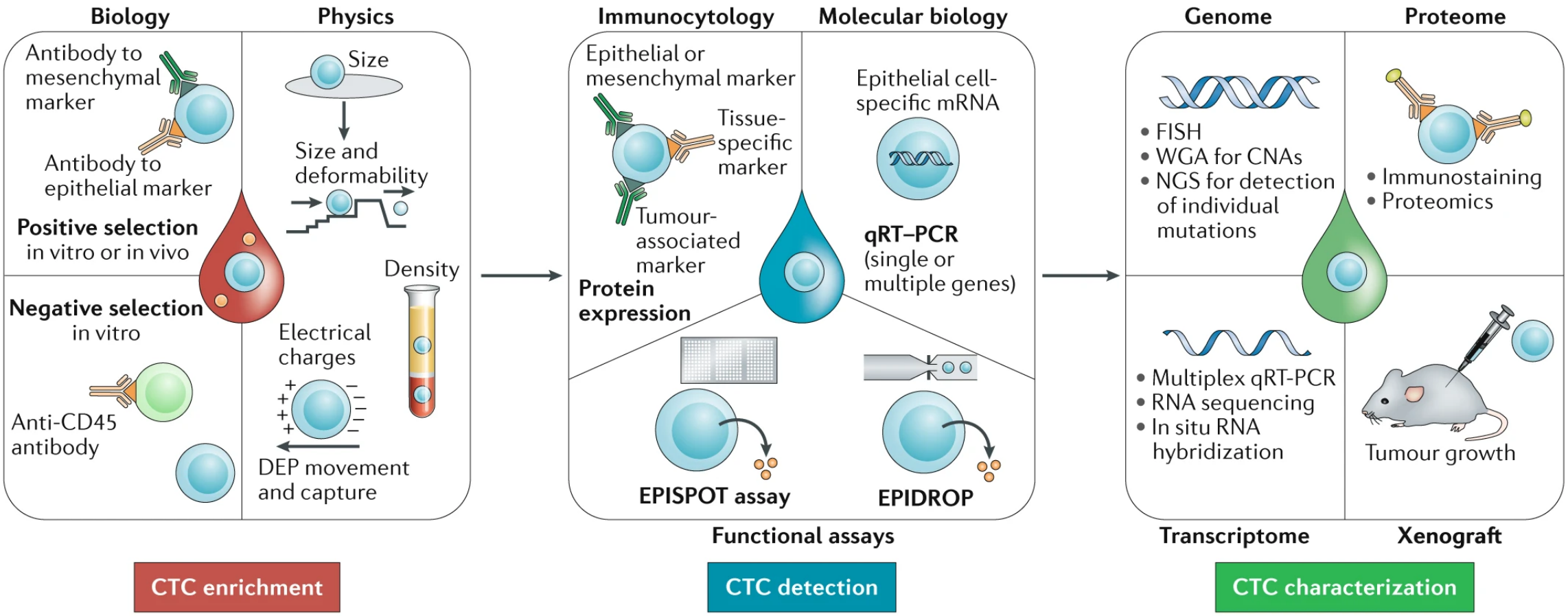


Pantel, Nature Reviews Clinical Oncology (2019)

CTCs

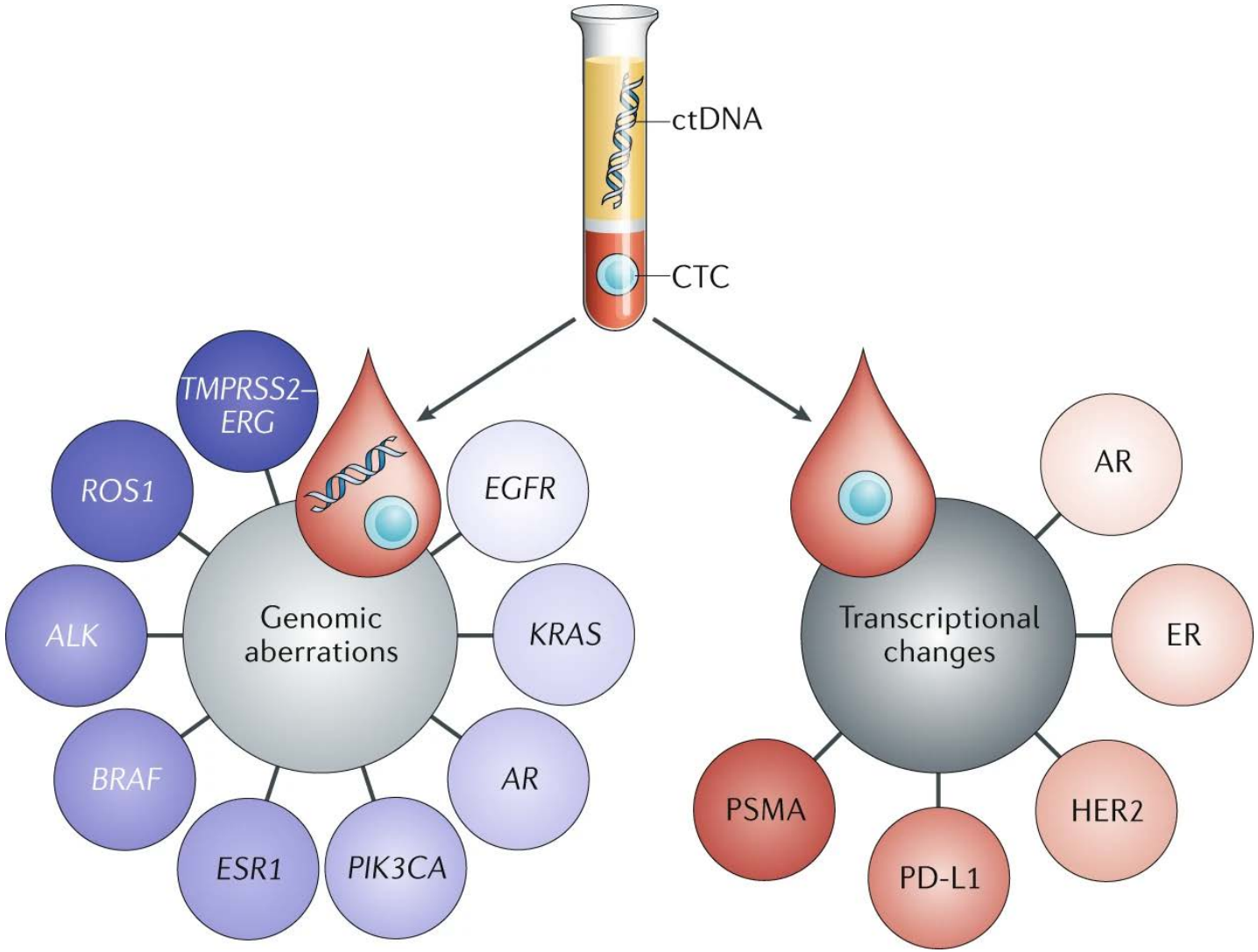

Patient with cancer
Real-time liquid biopsy
ctDNA
CTC
ctDNA
CTC
Biology
Physics
Antibody to mesenchymal marker
Antibody to epithelial marker
Positive selection in vitro or in vivo
Negative selection in vitro
Anti-CD45 antibody
Size
Size and deformability
Density
Electrical charges
DEP movement and capture
Immunocytology
Molecular biology
Epithelial or mesenchymal marker
Tissue-specific marker
Tumour-associated marker
Protein expression
Epithelial cell-specific mRNA
qRT–PCR (single or multiple genes)
EPISPOT assay
EPIDROP
Functional assays
Genome
Proteome
• FISH
• WGA for CNAs
• NGS for detection of individual mutations
• Immunostaining
• Proteomics
• Multiplex qRT-PCR
• RNA sequencing
• In situ RNA hybridization
Tumour growth
Transcriptome
Xenograft
CTC enrichment
CTC detection
CTC characterization

Methods for Enrichment, Detection and Characterization of CTCs



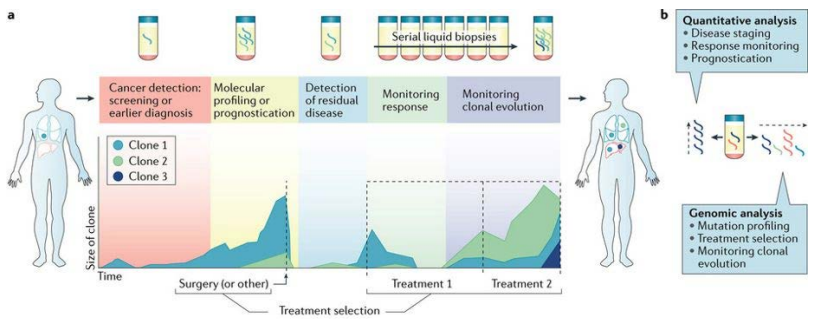
Pantel, Nature Reviews Clinical Oncology (2019)

Detectable Molecular Changes in ctDNA vs CTCs



Pantel, Nature Reviews Clinical Oncology (2019)

Liquid Biopsy Applications

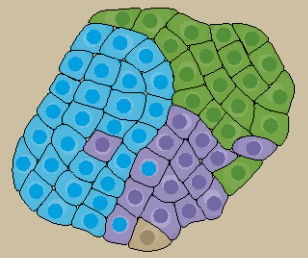
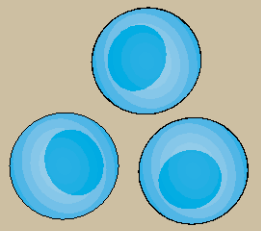
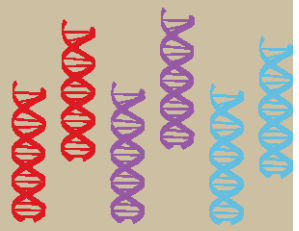


As Biomarkers, Liquid Biopsies Can Be:

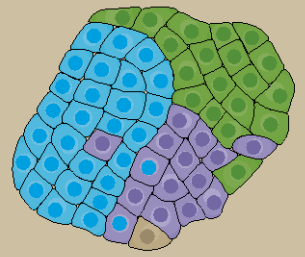
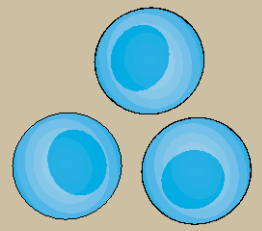
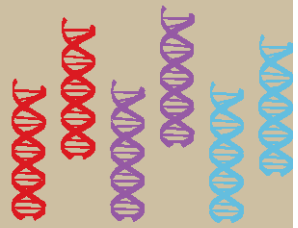
- Diagnostic
- Prognostic
- Predictive
- Surrogate

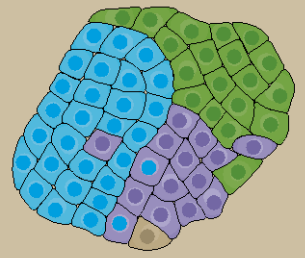
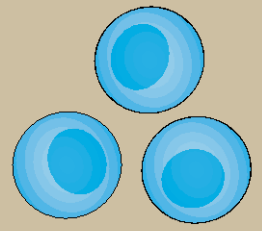
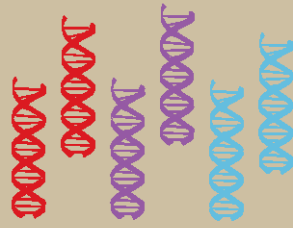
- Essential considerations for biomarkers apply to liquid biopsy:
  - Analytic Validity, Clinical Validity, Clinical Utility

Solid vs Liquid Biopsies

Analyte	Solid biopsy	Liquid biopsy	
	Tissue	CTCs	ctDNA
			
Origin			
Viable cells	Yes	Yes	No
Apoptotic cells	Yes	Yes	Yes
Components			
DNA	Yes	Yes	Yes
RNA	Yes	Yes	No
Proteins	Yes	Yes	No
Metabolites	Yes	Yes	No

Heitzer, Nature Reviews Genetics (2019)

Analyte	Solid biopsy	Liquid biopsy	
	Tissue	CTCs	ctDNA
			
Extractable information			
Mutations	Yes	Yes	Yes
Copy number alterations	Yes	Yes	Yes
Epigenetic alterations	Yes	Yes	Yes
Fusion genes	Yes	Yes	Yes
Splice variants	Yes	Yes	No
Information at single-cell level	Yes	Yes	No
Functional assays	Yes	Yes	No

Analyte	Solid biopsy	Liquid biopsy	
	Tissue	CTCs	ctDNA
			
Applications in precision oncology			
Prognostication	Yes	Yes	Yes
Identification of predictive marker	Yes	Yes	Yes
Classification of molecular subtypes	Yes	Possibly	Possibly
Tracking of clonal evolution over time	No	Yes	Yes
Early identification of resistance mechanisms	No	Yes	Yes
Monitoring treatment response	No	Possibly	Yes
Early detection of recurrence and MRD	No	Possibly	Yes
Early detection of cancer	No	Unknown	Possibly
FDA-approved assays	Yes[a]	Yes[b]	Yes[c]

“Conventional” vs Liquid Biopsy

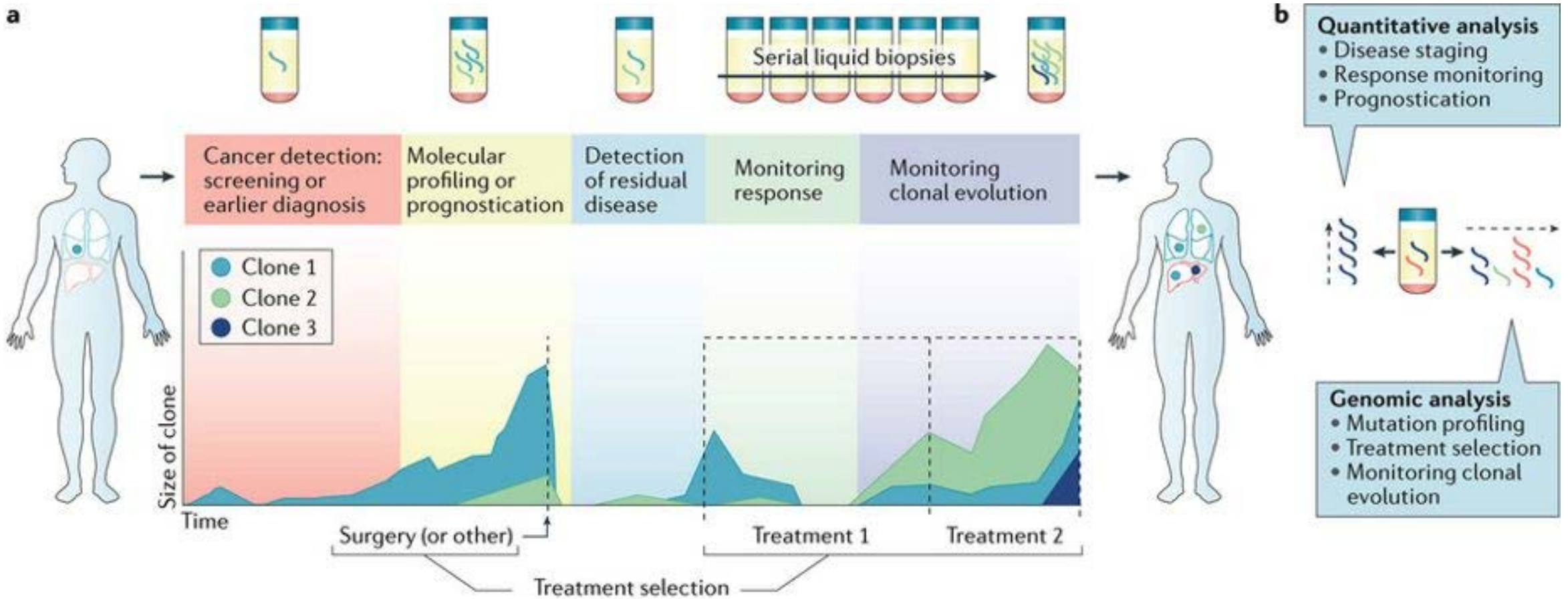
Table 3. Comparison of ctDNA Versus Tumor Tissue Testing

Consideration	ctDNA Assay	Tissue Assay
Logistics	Easy to draw * Variable venipuncture risks Easy serial testing *	Invasive, more challenging to obtain Variable biopsy risks Serial testing more difficult
Biology	Cannot directly correlate ctDNA results with histology or cellular phenotype More likely to represent whole tumor, but differential tumor cell turnover may bias representation	Can correlate with histology and cellular phenotype Represents one small tumor region
Pre-analytical	Easier to standardize across sites Requires special processing and handling unless using cell-stabilization tubes Limited data on confounding patient-related factors	More difficult to standardize across sites Uses existing, validated tissue processing and handling approaches
Clinical utility	Limited evidence for treatment selection in advanced cancer No evidence for other potential indications	Substantial evidence for treatment selection in multiple malignancies for early and advanced cancers

-Potential for rapid turnaround
-Inexpensive to collect/bank
-Does not require a “tumor”

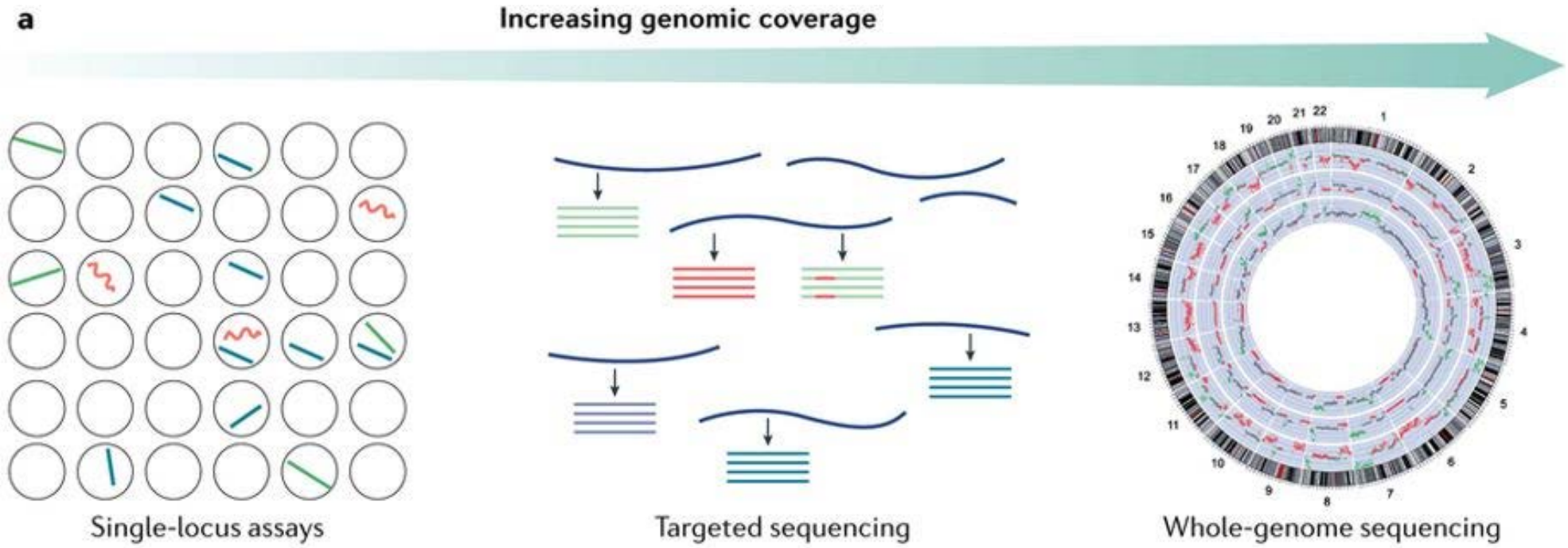
Merker et al. ASCO/CAP Review JCO 2018.

Liquid Biopsy: Opportunity for Serial Molecular Diagnostics



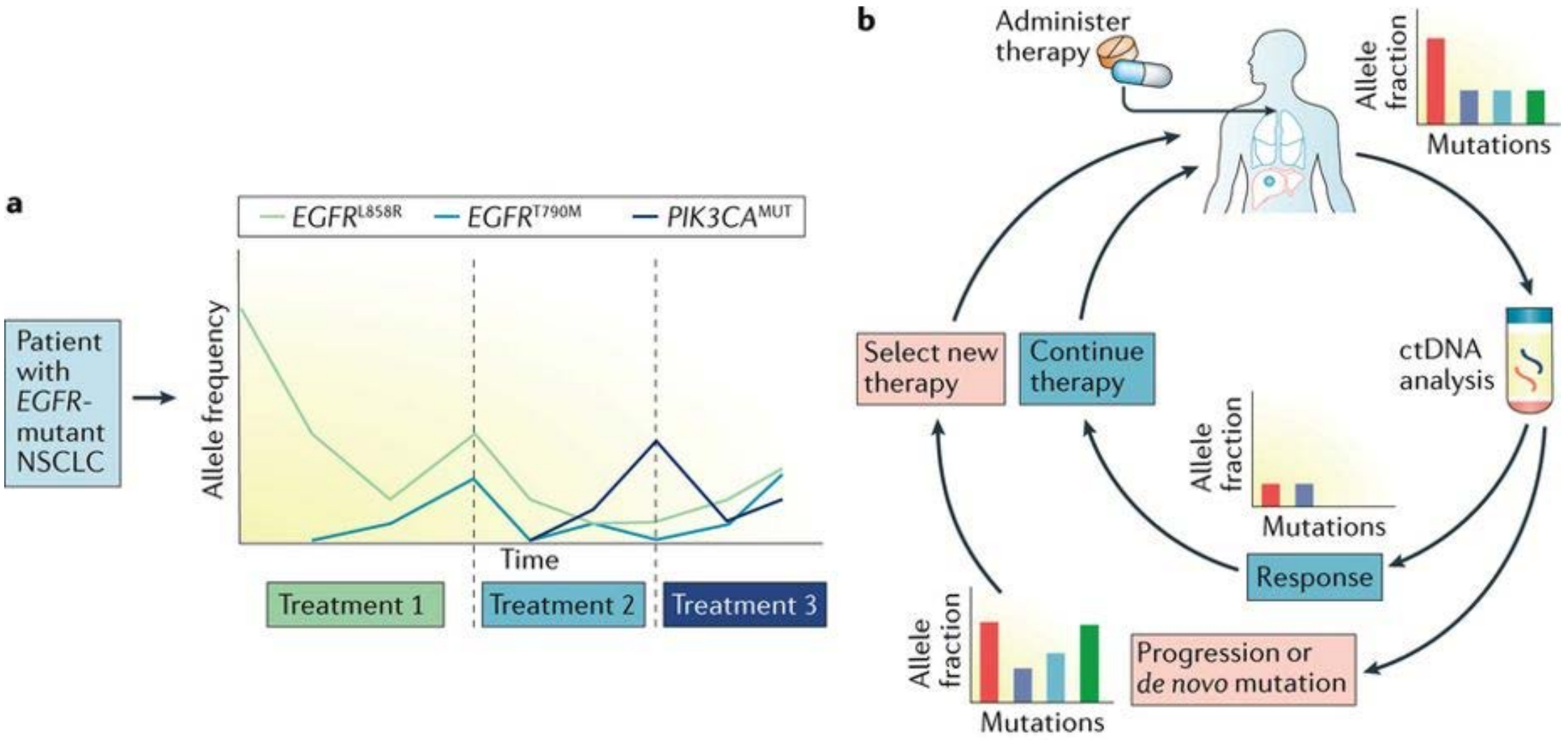
Wan et al. Nature Reviews Cancer, 2017

Genetic Assays for ctDNA



Wan et al. Nature Reviews Cancer, 2017

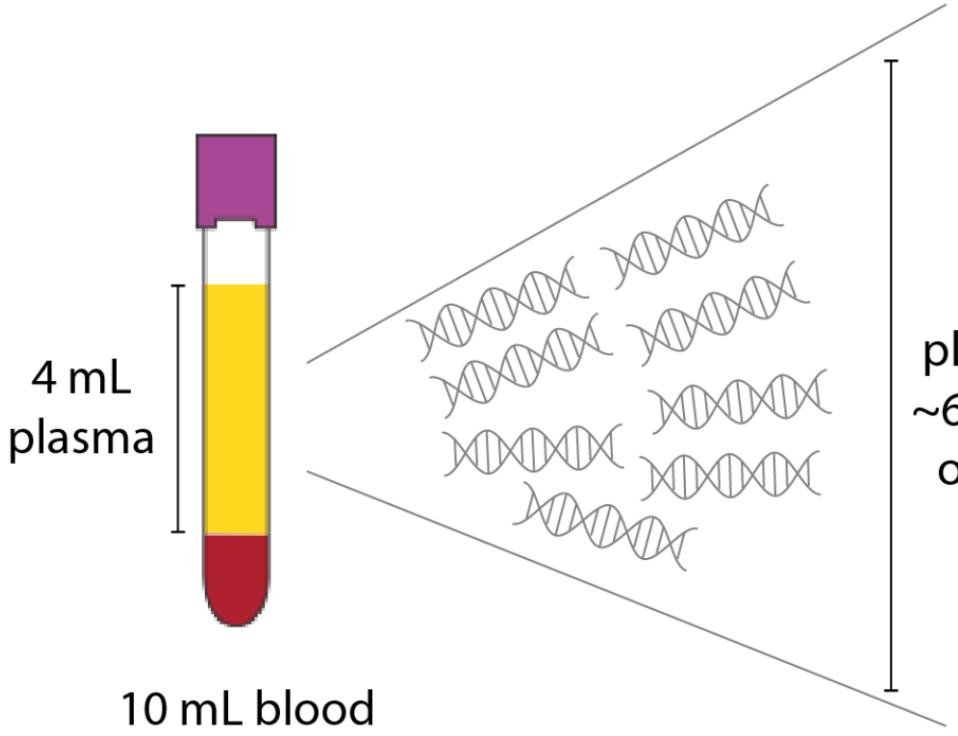
A Potential ctDNA-Guided Precision-Medicine Paradigm



Wan et al. Nature Reviews Cancer, 2017

Pre-requisites for ctDNA-guided Precision Medicine

Physical Limits on ctDNA Detection Sensitivity



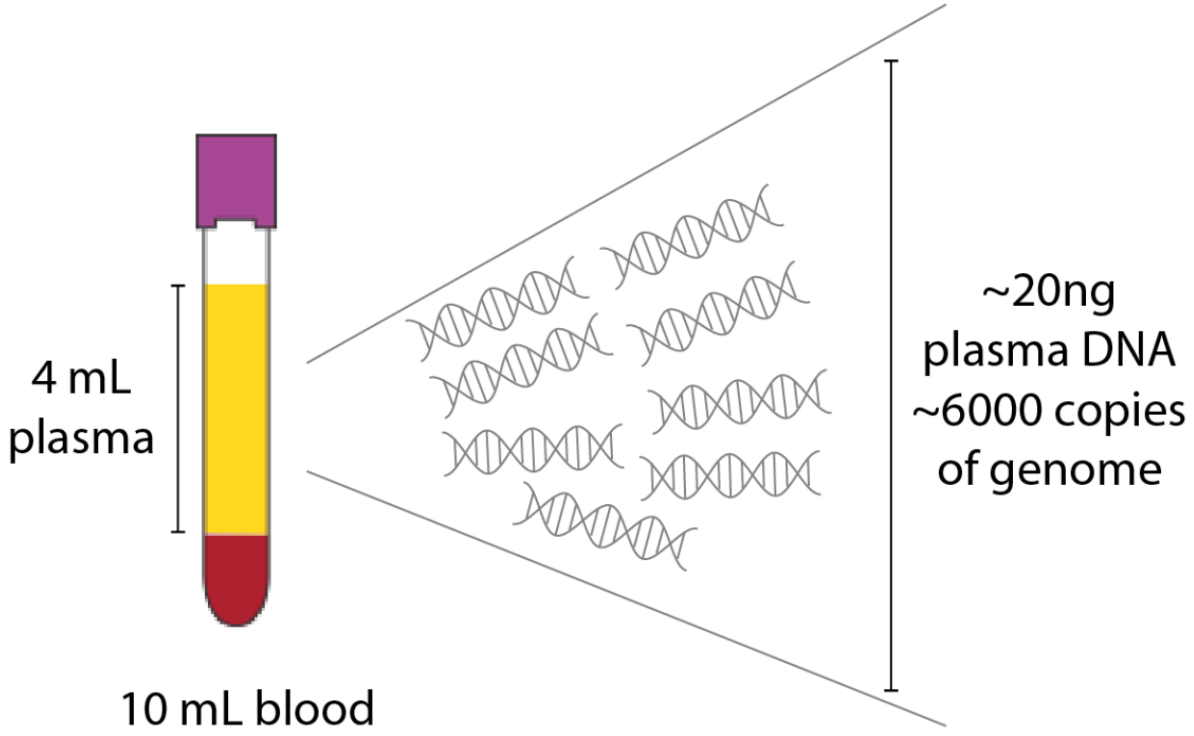
~20ng
plasma DNA
~6000 copies
of genome

tumor fraction		tumor genome copies
1%	=	60
0.1%	=	6
0.01%	=	0.6
0.001%	=	0.06

Median Tumor Fraction in ctDNA at Presentation
Metastatic Breast Cancer: 2.4%-12.5%
Localized Breast Cancer: 0.11%

Murtaza, AACR 2019

Physical Limits on ctDNA Detection Sensitivity

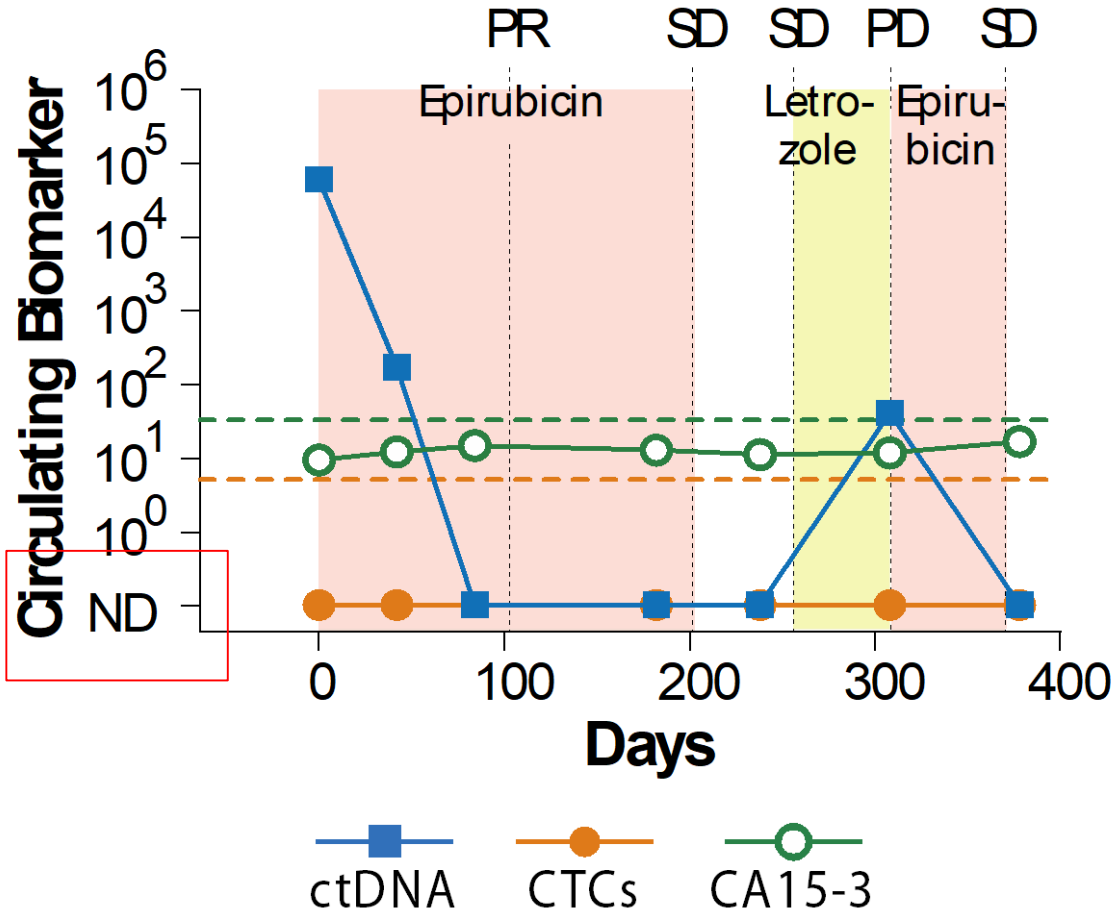


tumor fraction		tumor genome copies
1%	=	60
0.1%	=	6
0.01%	=	0.6
0.001%	=	0.06

Median Tumor Fraction in ctDNA at Presentation
Metastatic Breast Cancer: 2.4%-12.5%
Localized Breast Cancer: 0.11%

Murtaza, AACR 2019

Importance of Detection Limit

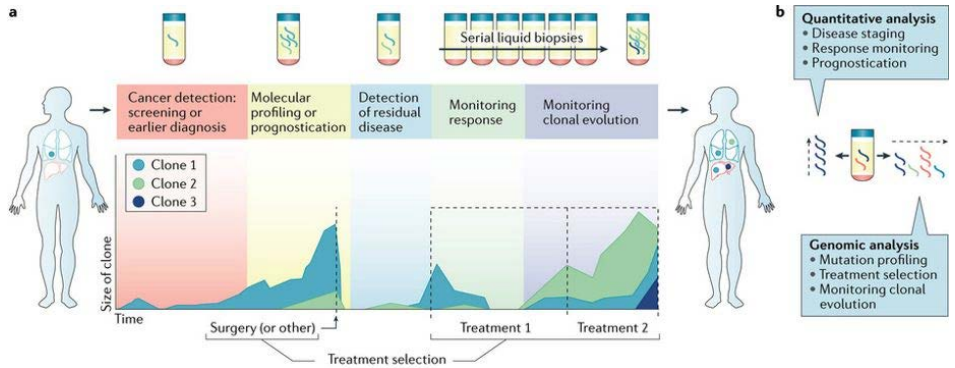


Dawson et al, NEJM 2013

Key Questions That Can be Addressed with ctDNA

Localized/locally advanced disease

- Predict relapse/patient selection (*prognostic +/- predictive*)
- Eliminate molecular residual disease (*surrogate*)
- Guide adjuvant treatment duration or selection



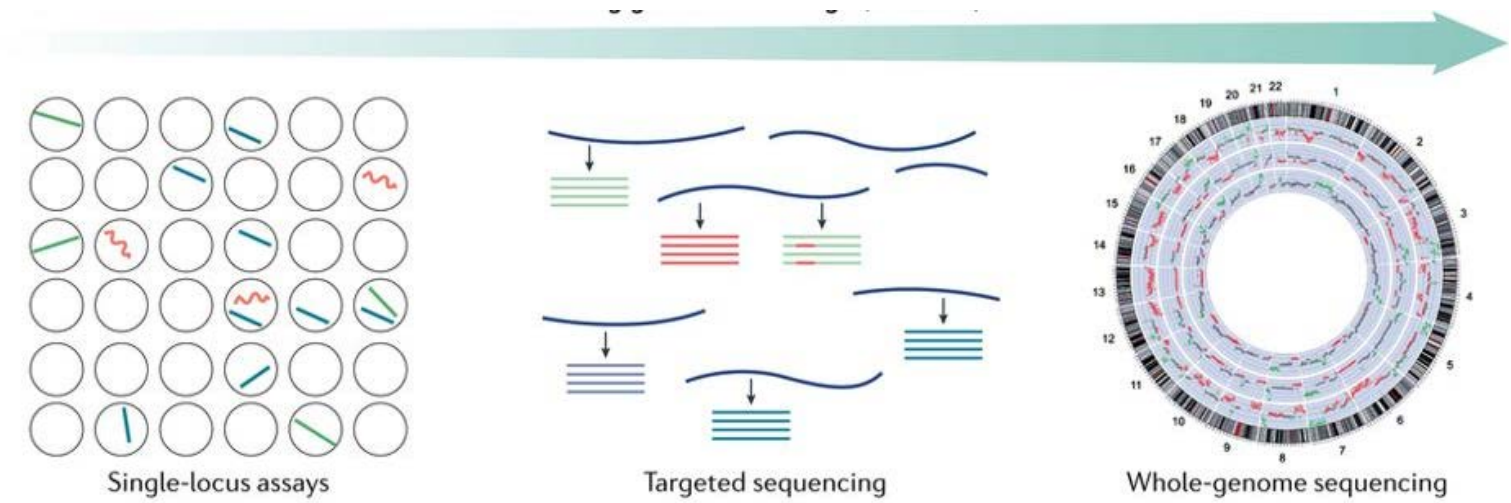
Recurrent or metastatic disease

- Patient Selection/Predictive Biomarker
- Prognostic Biomarker
- Early Surrogate/Dynamic Response Marker
- Treatment Duration for I/O, NED
- Identify Drug Resistance
- Inform Drug Mechanism of Action
- Guide Change of Therapy

How Can Liquid Biopsies Be Used in Clinical Trials?

- Patient Selection
- Correlative Studies
  - Integral
  - Integrated
  - Retrospective

ctDNA to Identify Actionable Alterations



Tumor Genotyping with ctDNA – Tissue Concordance

Table 2 Concordance between tumor-tissue analysis and cfDNA analysis (n = 95)

		Tumor-tissue analysis				
	KRAS	Mutant	WT	Sensitivity	Specificity	Accuracy
cfDNA analysis	Mutant	36	1	92%	98%	96%
	WT	3	55			
	Total	39	56			
	BRAF	Mutant	WT	Sensitivity	Specificity	Accuracy
cfDNA analysis	Mutant	5	0	100%	100%	100%
	WT	0	90			
	Total	5	90			
	All mutations	Mutant	WT	Sensitivity	Specificity	Accuracy
cfDNA analysis	Mutant	41	1	93%	98%	96%
	WT	3	50			
	Total	44	51			

KRAS indicates codon 12 and 13 mutations; *BRAF* indicates V600E mutation.

Theirry et al, Nature Medicine 2014

ctDNA for Actionable Alterations in NSCLC

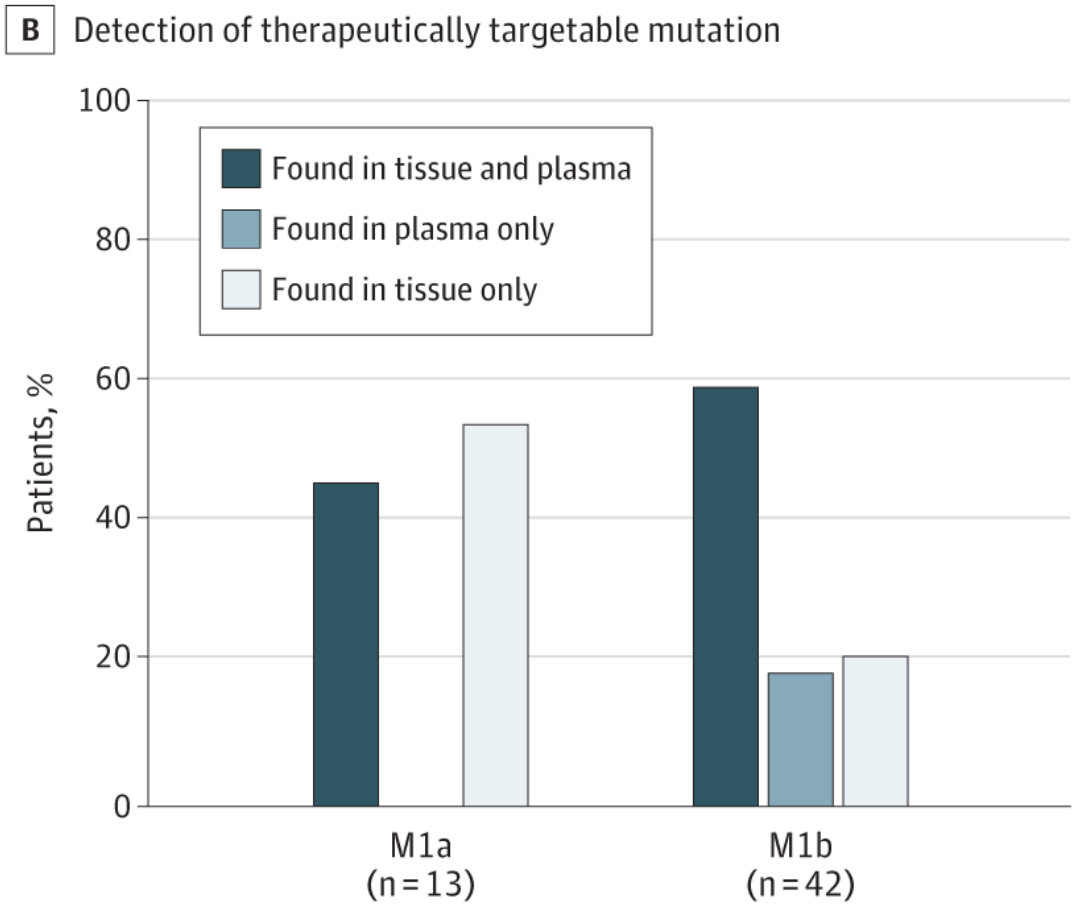
Table 1. Number of patients with driver or resistance mutations detected in tumor and plasma, as well as sensitivity and specificity. Sensitivity estimates are based on Group 1, in which tumor variants were detected in the plasma. Specificity estimates are based on Group 2, in which tumor samples tested negative for driver mutations by NGS

		Group 1 (n = 91) Driver positive on tissue genotyping		Group 2 (n = 19) Driver negative on tissue NGS	Group 3 (n=17) Driver unknown insufficient tissue
		Detected in tumor	Tumor variant detected in plasma, N (sensitivity)	Detected in plasma, N (specificity)	Detected in plasma
Driver	Total Subjects	91	68 (75%)	0 (100%)	4
	EGFR	37	29 (78%)	0	0
	KRAS	29	23 (79%)	0	4
	ALK	8	5 (62%)	0	0
	MET	6	3 (50%)	0	0
	ERBB2	4	4 (100%)	0	0
	BRAF	3	3 (100%)	0	0
	ROS1	3	1 (33%)	0	0
	RET	1	0 (0%)	0	0
Resistance	EGFR	13	11 (85%)	NA	NA
	MET	2	0 (0%)	NA	NA

NGS, next-generation sequencing.

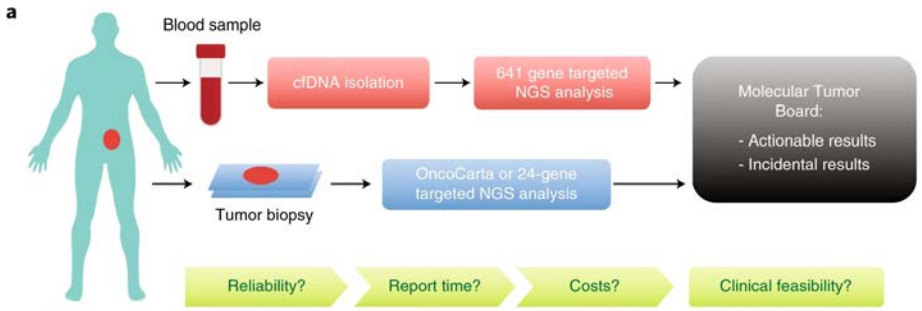
Li et al, Annals of Oncology 2019

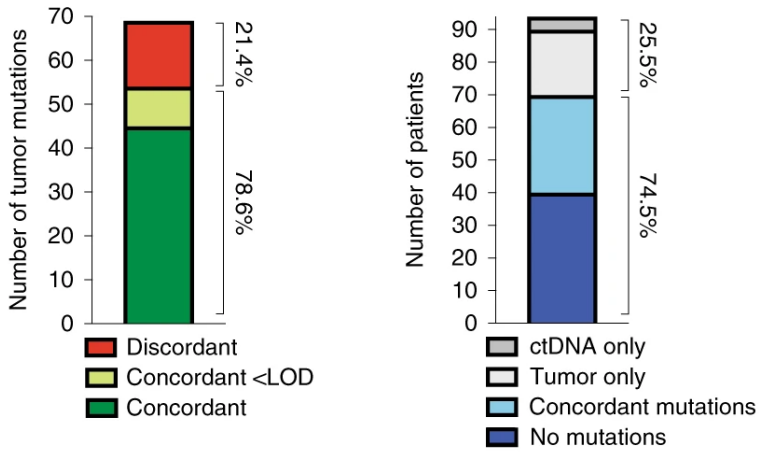
Sensitivity of ctDNA Mutation Detection Depends on Clinical Features (NSCLC)

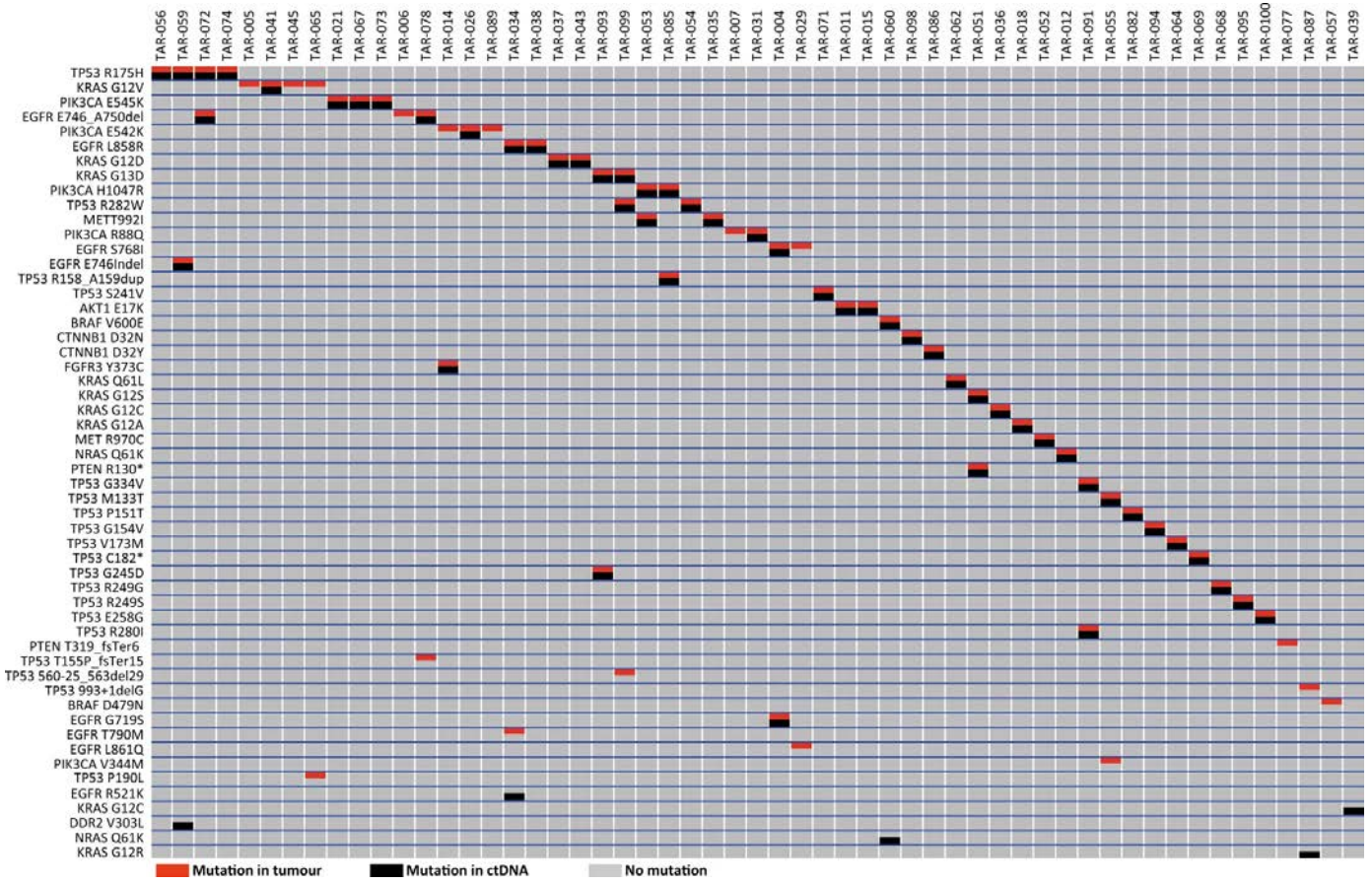


Aggarwal et al, JAMA Oncology 2018

Identifying Actionable Alterations: TARGET

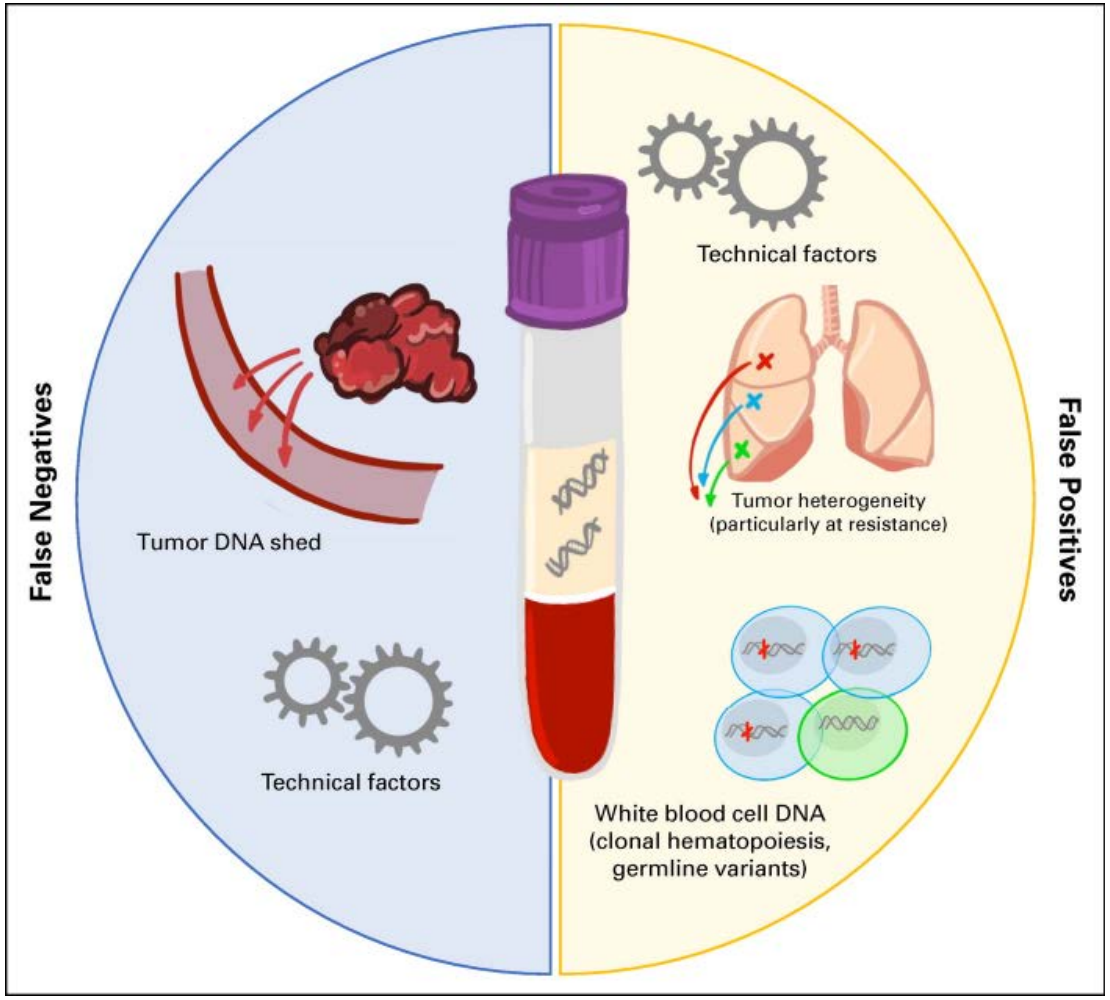






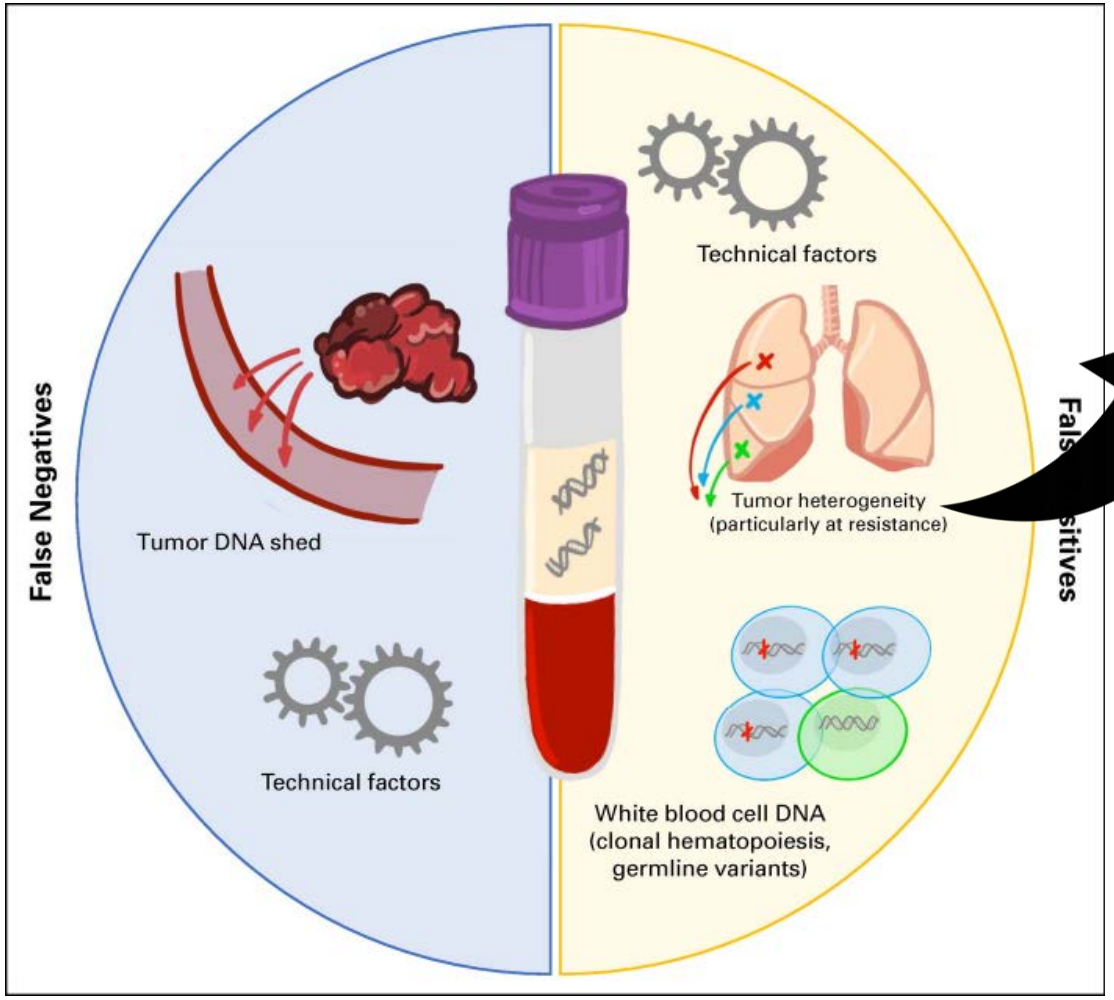
Rothwell et al, Nature Medicine 2019

Disease and Technical Factors Contribute to Differences in Blood and Tissue Findings



Paweletz et al. JCO Precision Oncology, 2019

Disease and Technical Factors Contribute to Differences in Blood and Tissue Findings



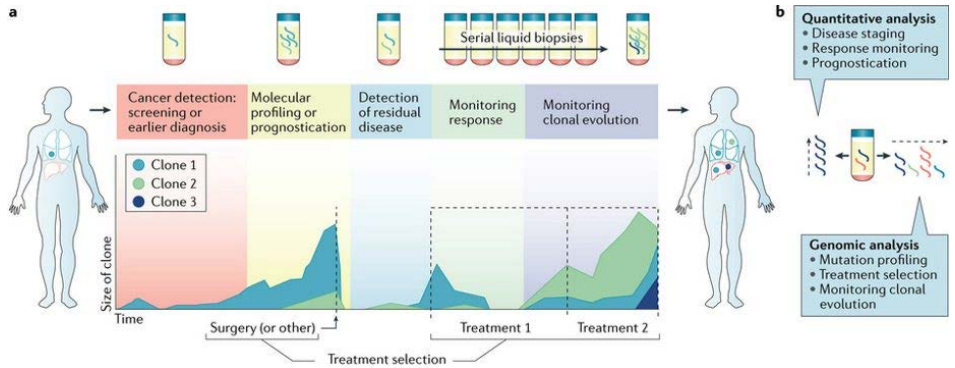
Acquired Drug Resistance
(Intervening Therapy)

Paweletz et al. JCO Precision Oncology, 2019

Key Questions That Can be Addressed with ctDNA

Localized/locally advanced disease

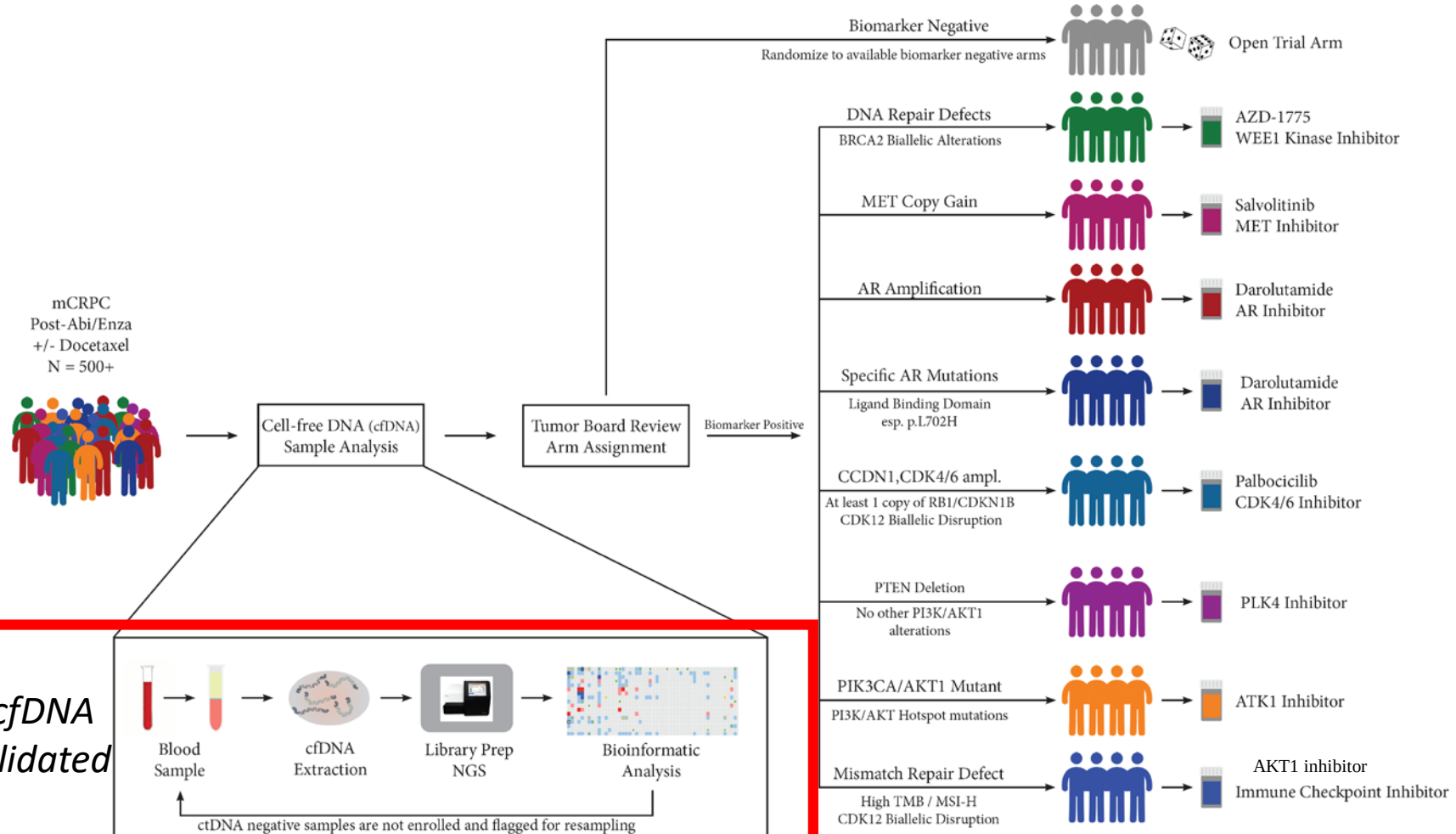
- Predict relapse/patient selection (*prognostic +/- predictive*)
- Eliminate molecular residual disease (*surrogate*)
- Guide adjuvant treatment duration or selection



Recurrent or metastatic disease

- *Patient Selection/Predictive Biomarker*
- Prognostic Biomarker
- Early Surrogate/Dynamic Response Marker
- Treatment Duration for I/O, NED
- Identify Drug Resistance
- Inform Drug Mechanism of Action
- Guide Change of Therapy

IND.234 – Metastatic Prostate Cancer ctDNA Umbrella Trial



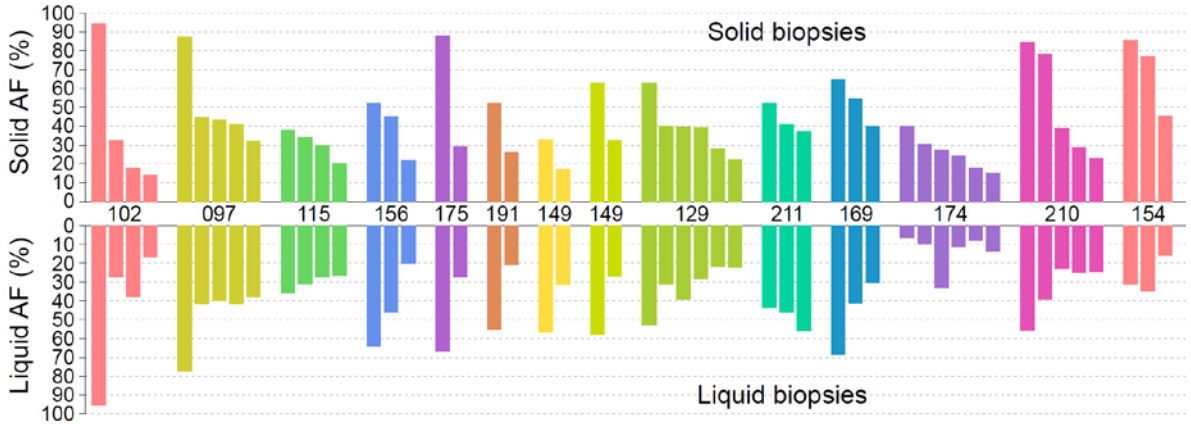
Strategy: targeted cfDNA sequencing with validated research approach

Slides Courtesy of Alex Wyatt, BC Cancer

Kim Chi, Martin Smoragiewicz, Lesley Seymour, et al.

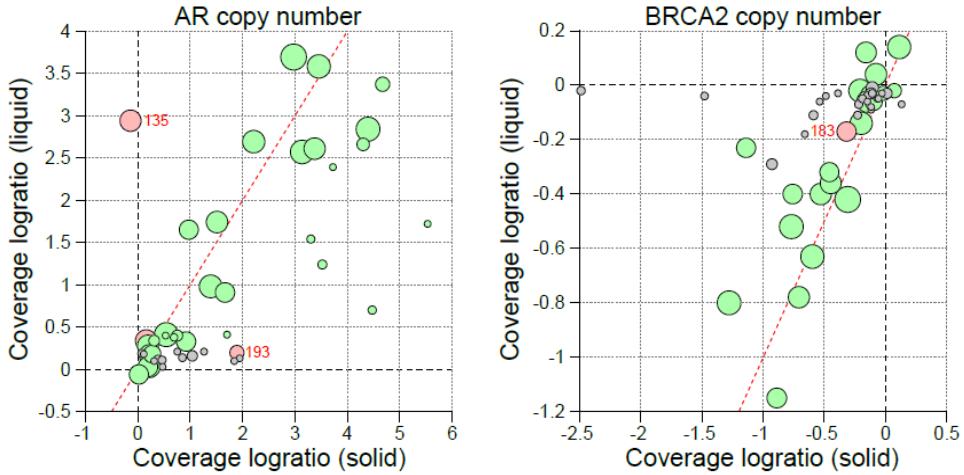
High concordance between ctDNA and matched metastatic tissue biopsy (in mCRPC)

- 45 plasma cfDNA samples collected at time of metastatic tissue biopsy (SU2C / PCF West Coast Dream Team, Eric Small *et al*.)



Similar mutation profiles, ctDNA vs tissue

Similar gene copy numbers, ctDNA vs tissue

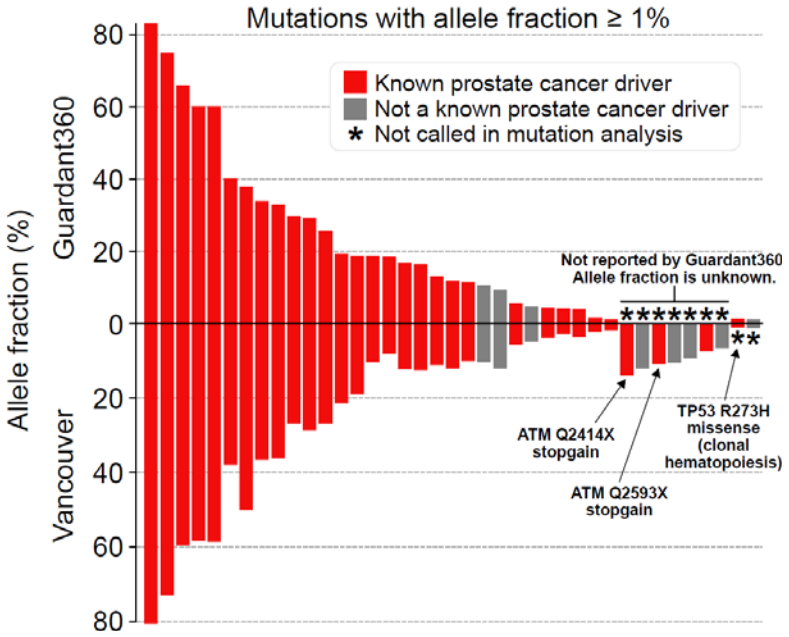


Slides Courtesy of Alex Wyatt, BC Cancer

Wyatt, Annala, *et al*., J Natl Cancer Inst. 2017

Existing commercial assays are adequate for mutation calling (at high allele frequency)

- Analyzed 24 same-day cfDNA samples with Guardant360 and academic targeted sequencing
- Confirmed 94% of somatic mutations identified by Guardant360 at >1% allele fraction

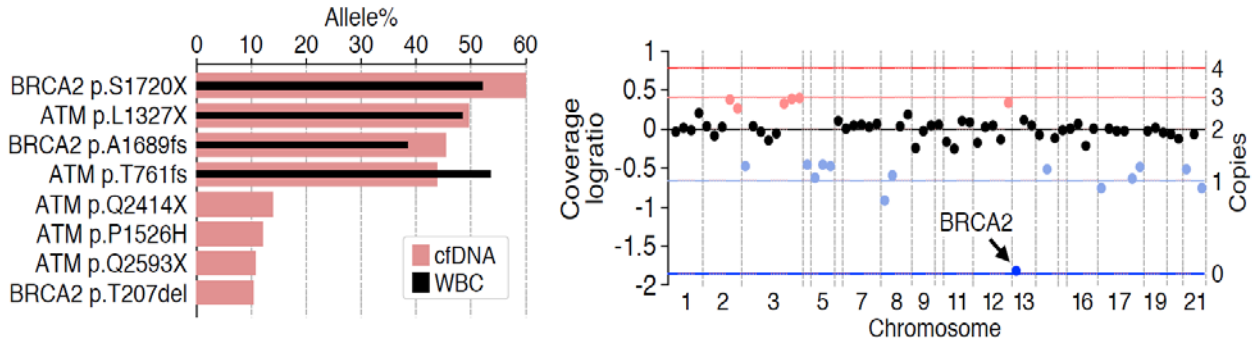


Caveat: many somatic mutations identified by Guardant360 at <1% allele fraction appeared to represent subclonal passenger events or non-prostate derived clones

Slides Courtesy of Alex Wyatt, BC Cancer

Commercial Assay Utility is limited by lack of reporting on deletions, rearrangements and germline

- Several DNA repair gene defects were not reported by Guardant360
  - Germline and somatic truncating *BRCA2/ATM* mutations
  - *BRCA2* biallelic deletions
  - *BRCA2* reversion mutations
  - Hypermutator phenotype (mismatch repair defect / high TMB)

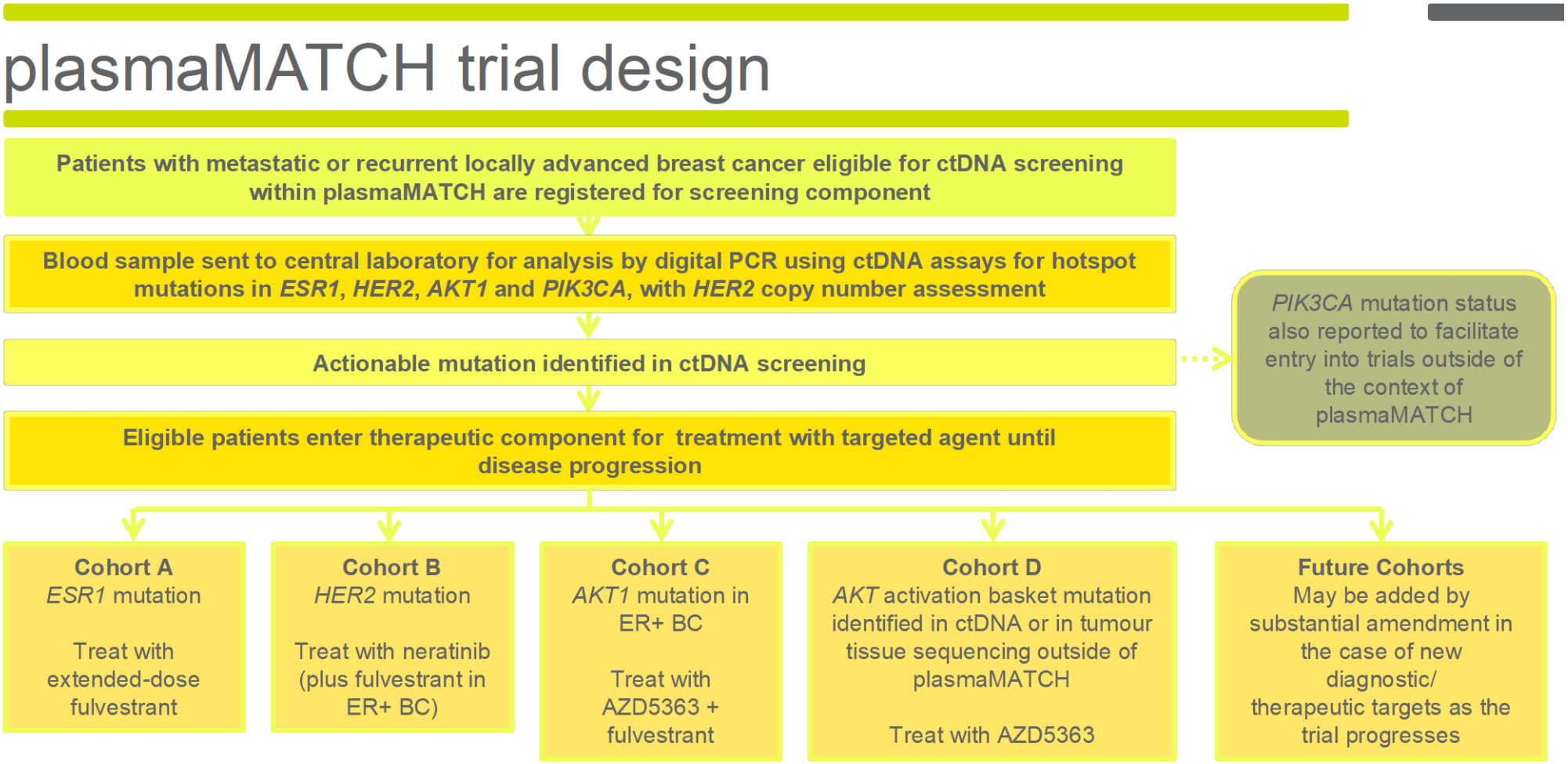


Note: critical prostate-specific genes such as FOXA1, SPOP, CDK12, PIK3R1 and ERG are also rarely present in existing pan-cancer commercial assays

Slides Courtesy of Alex Wyatt, BC Cancer

Taavitsainen, Sartor, et al., JCO Precision Oncology

Many Other ctDNA Umbrella Trials Underway



Number of centres ~50 UK Screening Sites, of which ~25 sites will also be Treatment Sites

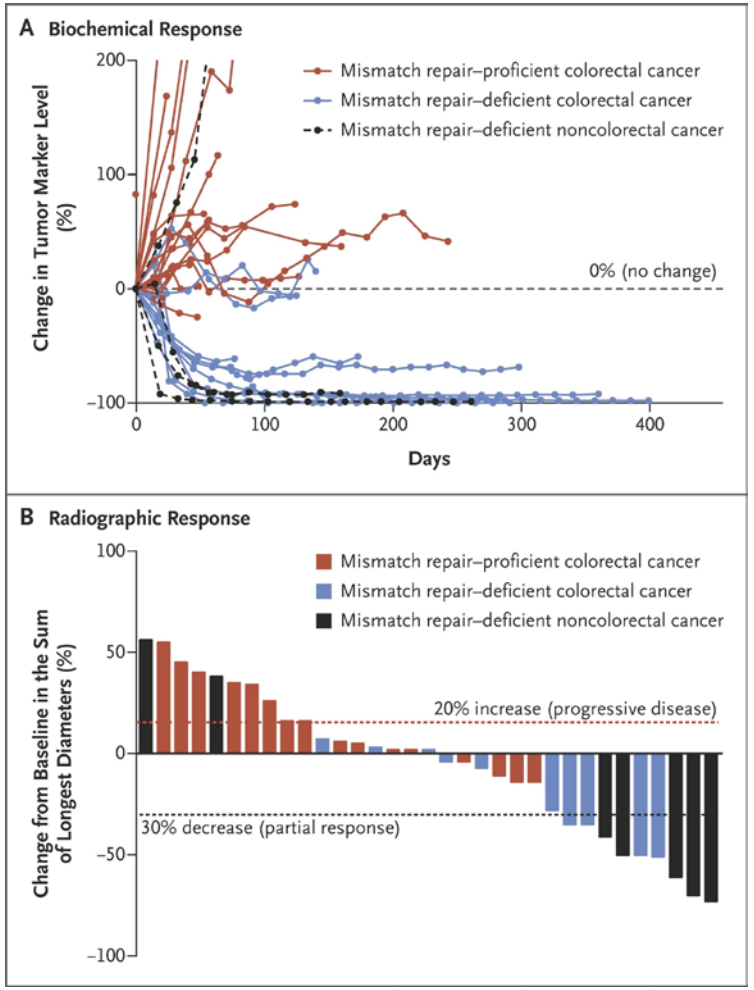
Recruitment target

- ctDNA screening component: ~1000 patients with metastatic or recurrent locally advanced BC who have received prior systemic treatment in the advanced setting
- Therapeutic component: Cohort A – 40 patients; Cohorts B–D – 16 patients in each

Identification of Other “Genotypes” in ctDNA

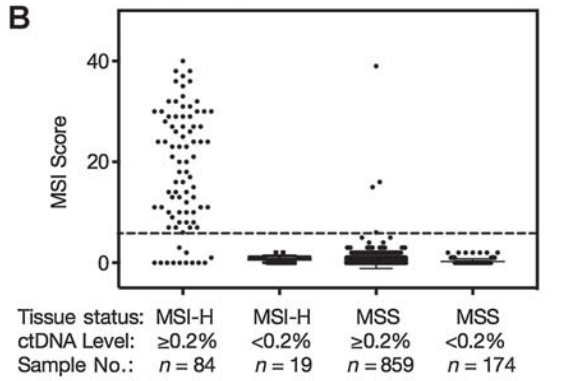
ORIGINAL ARTICLE

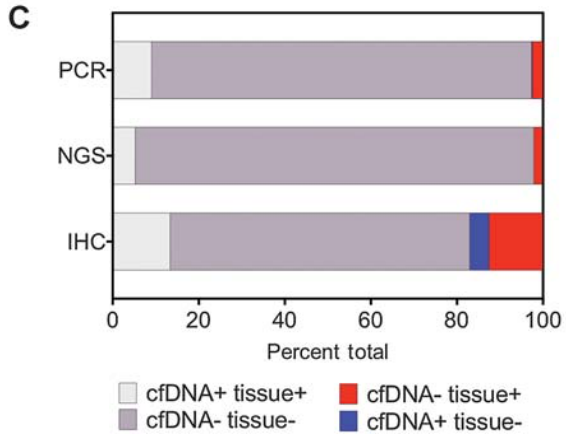
PD-1 Blockade in Tumors with Mismatch-Repair Deficiency



A

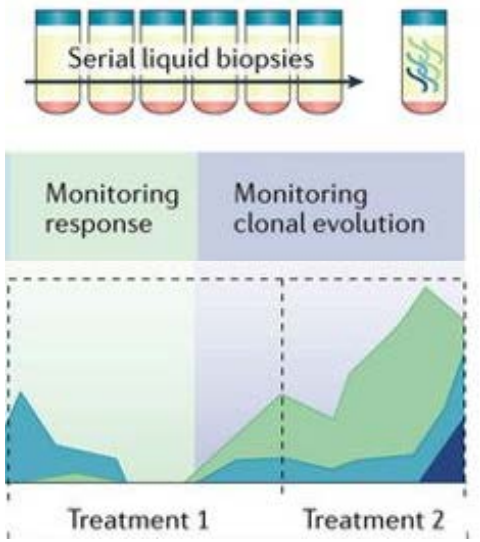
Total samples	1,145
Unique evaluable patients	949
PPA	87% (71/82, 77%–93%)
NPA	99.5% (863/867, 98.7%–99.8%)
PPV	95% (71/75, 86%–98%)
Accuracy	98.4% (934/949, 97.3%–99.1%)





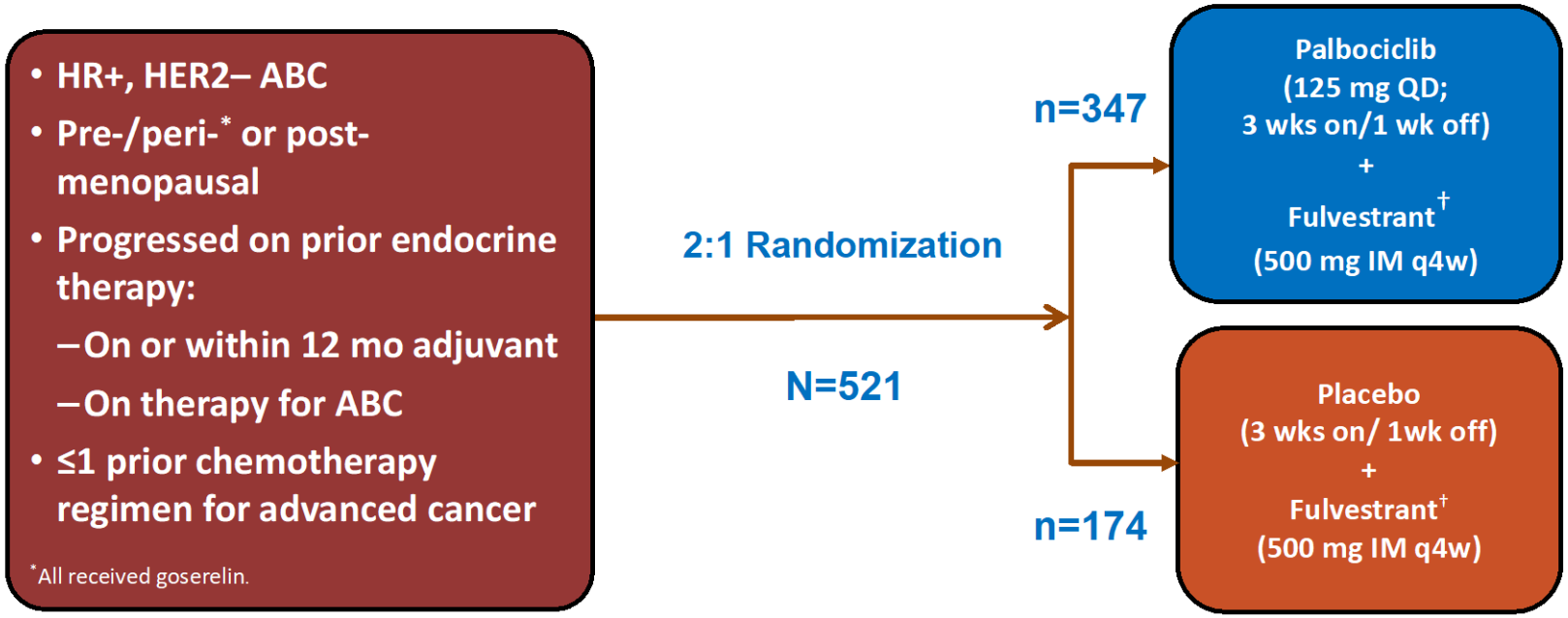
Willis et al. CCR 2019

Quantitative ctDNA for Prognosis or Monitoring



ctDNA Tumor Fraction and Prognosis in 2nd Line ER+ Breast Cancer

PALOMA-3 Study Design

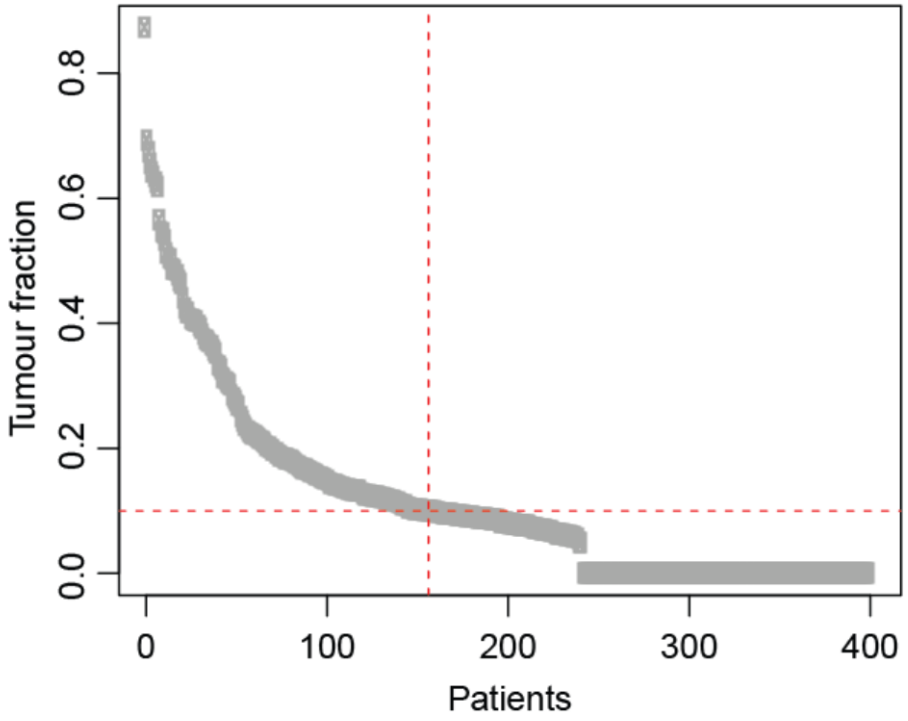


Plasma collected at baseline for ctDNA analysis n = 459 (88.1% of ITT population)

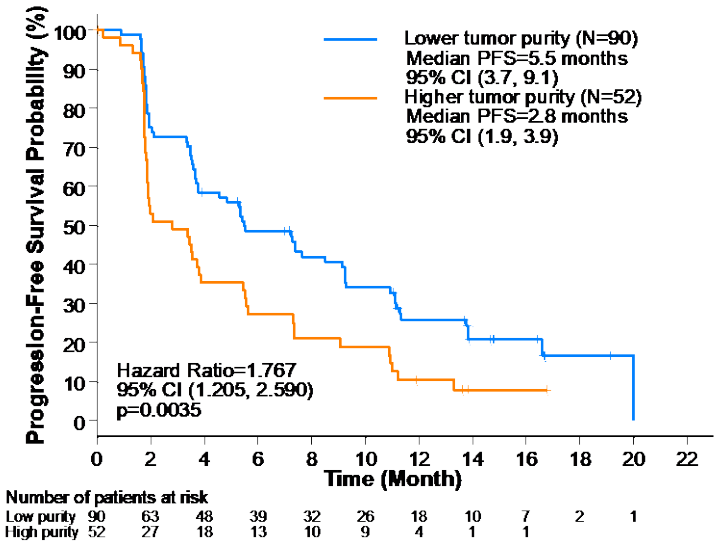
O'Leary et al. ASCO 2019

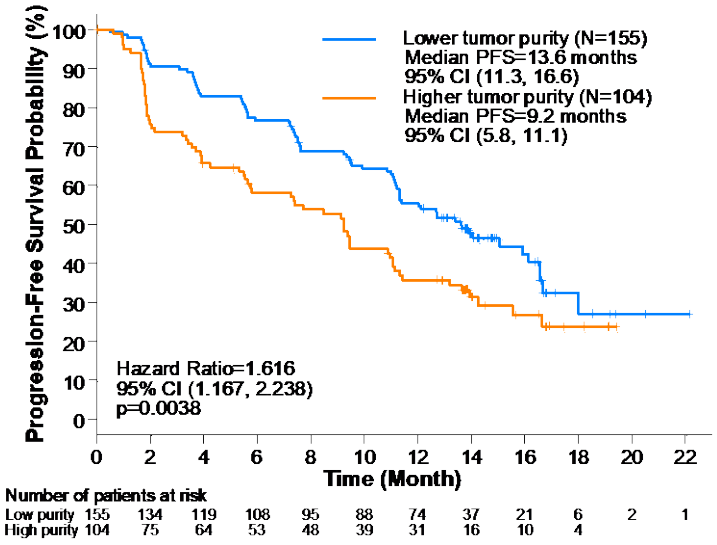
ctDNA Tumor Fraction and Prognosis in 2nd Line ER+ Breast Cancer

Tumor fraction (n = 401)



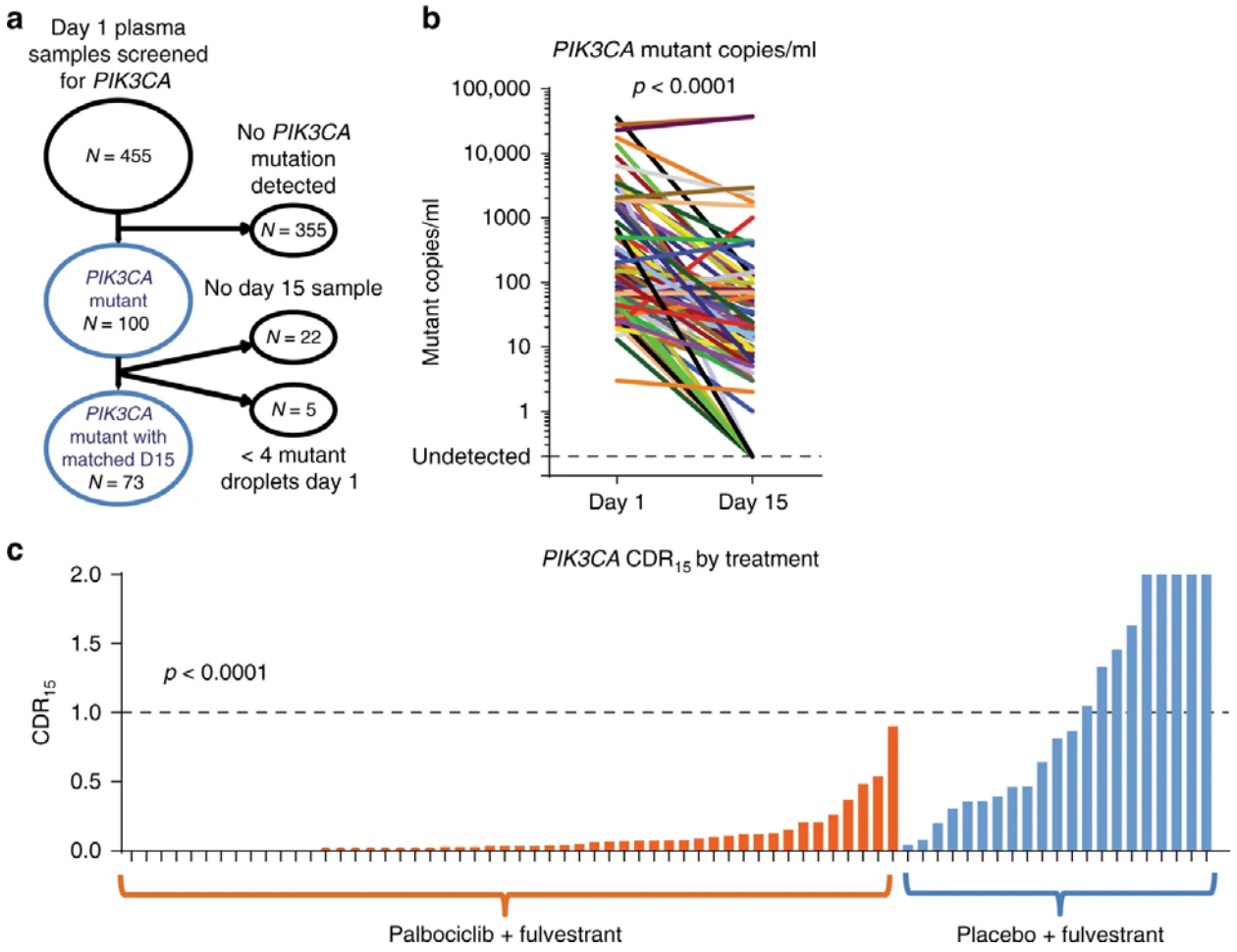
Tumor fraction >10% associates with early progression





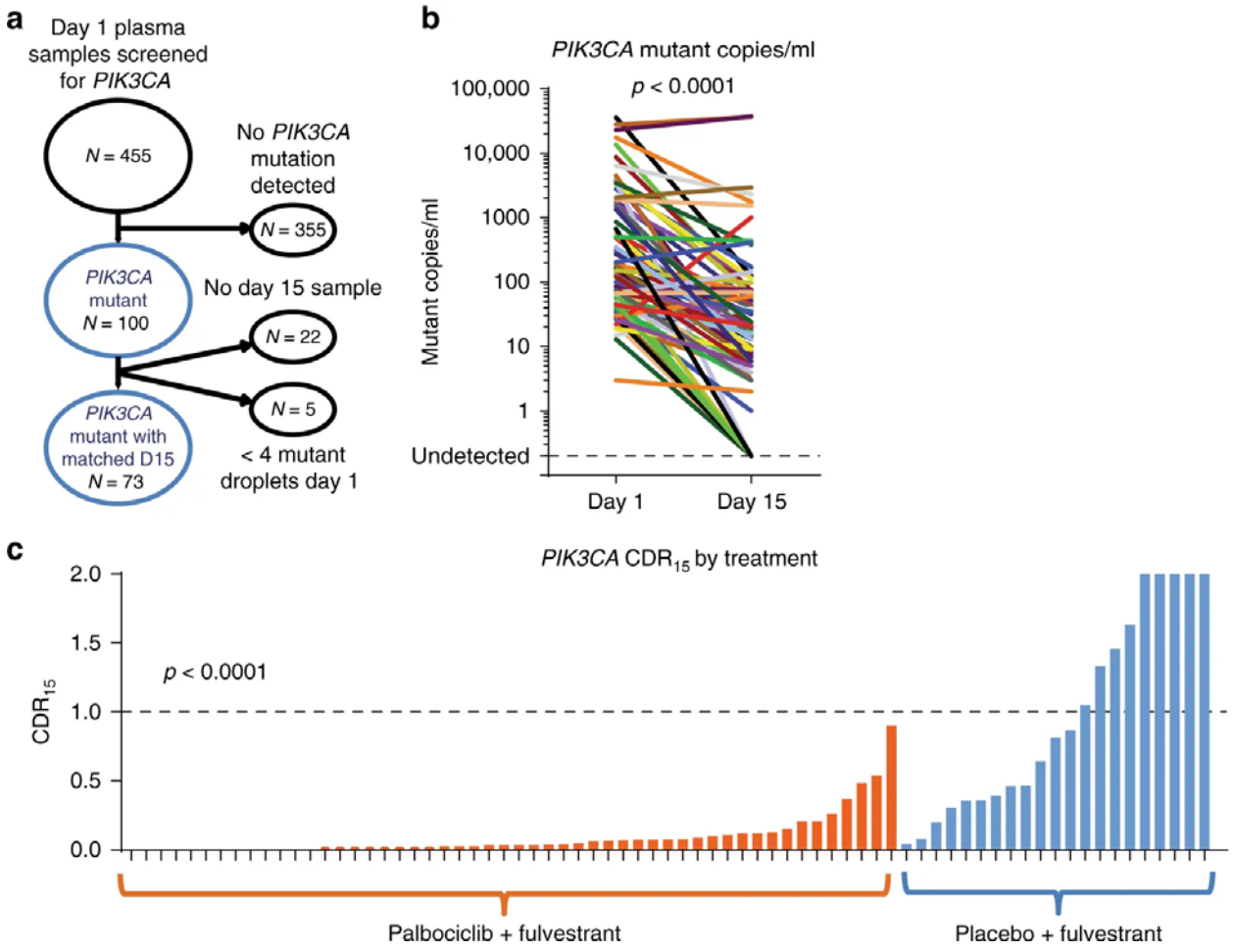
O'Leary et al. ASCO 2019

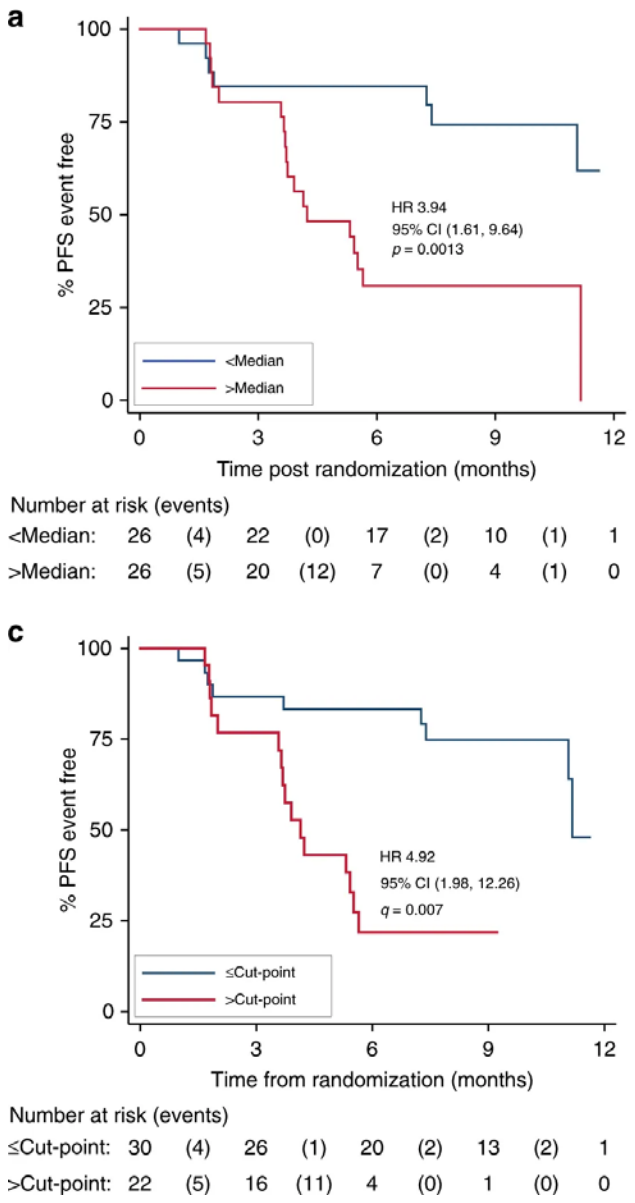
ctDNA Early Response in PALOMA-3



O'Leary, Nature Communications 2018

ctDNA Early Response in PALOMA-3

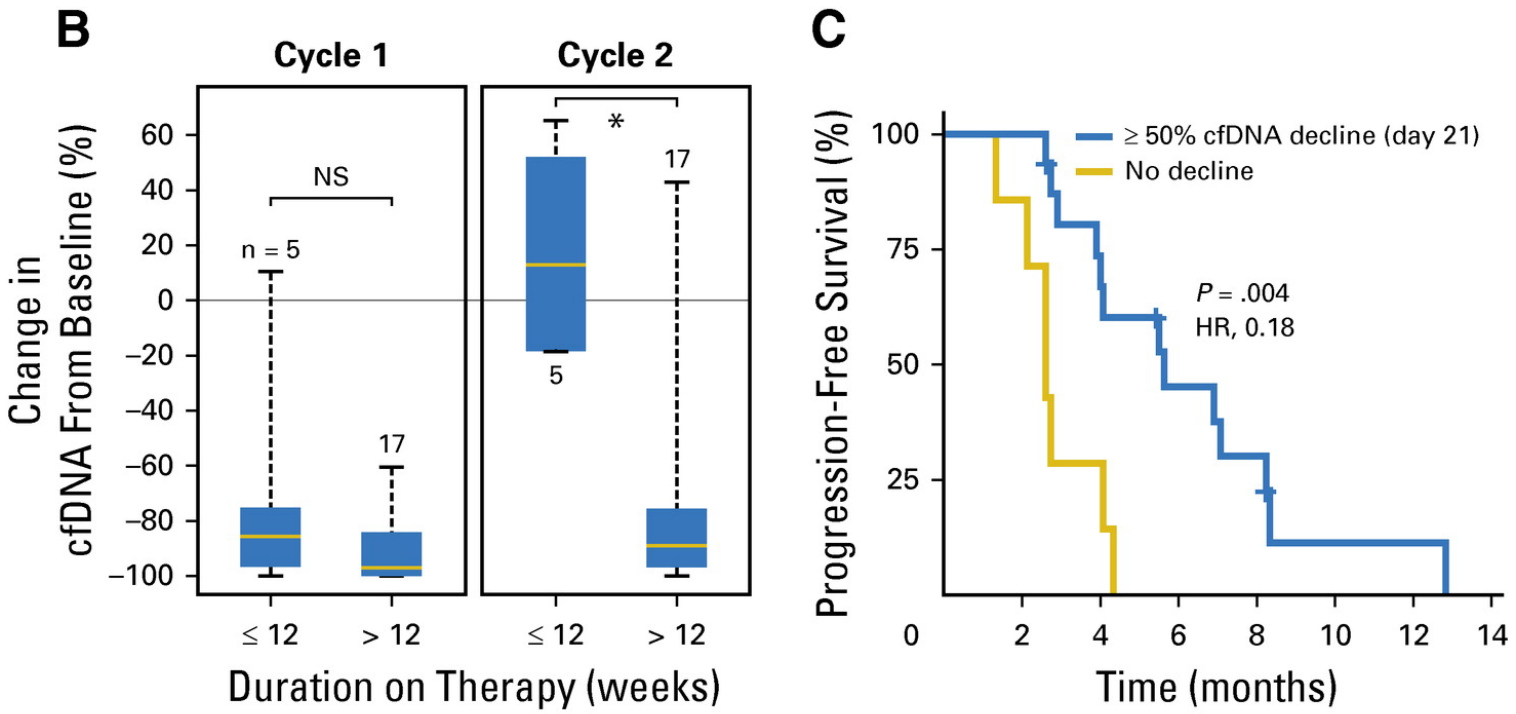




O'Leary, Nature Communications 2018

ctDNA Dynamics in AKT1-mutated Metastatic Breast Cancer Treated with an AKT Inhibitor (AZD 5363)

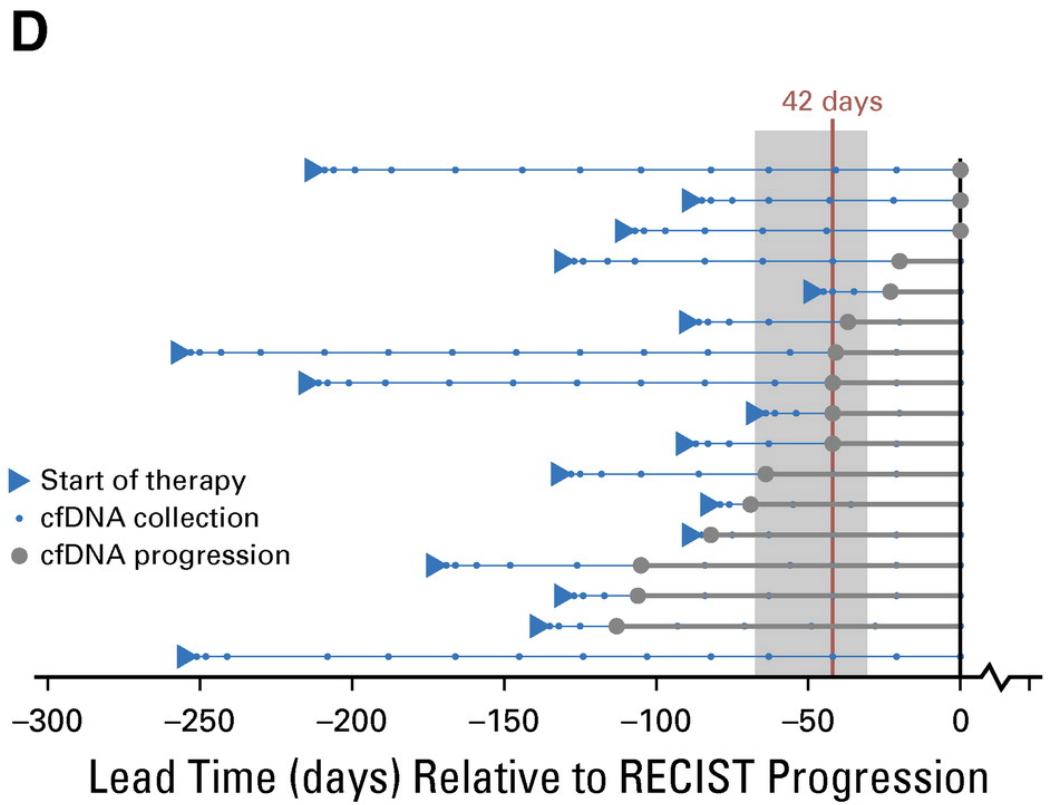
cfDNA decline at Day 21 is associated with response



Hyman et al. JCO 2017

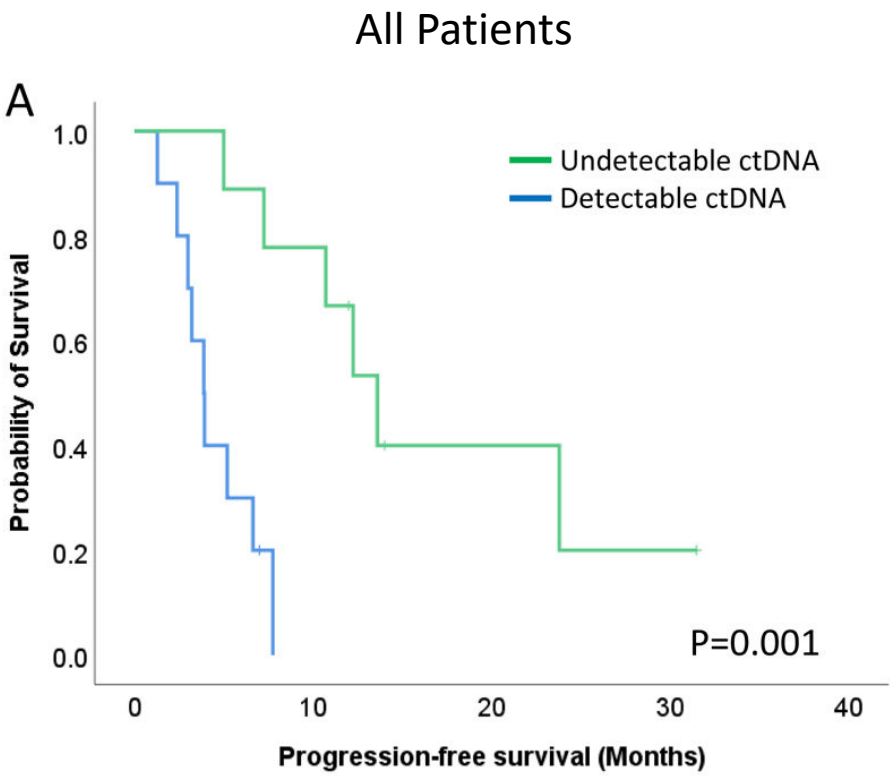
ctDNA Dynamics in AKT1-mutated Metastatic Breast Cancer Treated with an AKT Inhibitor (AZD 5363)

Progression



Hyman et al. JCO 2017

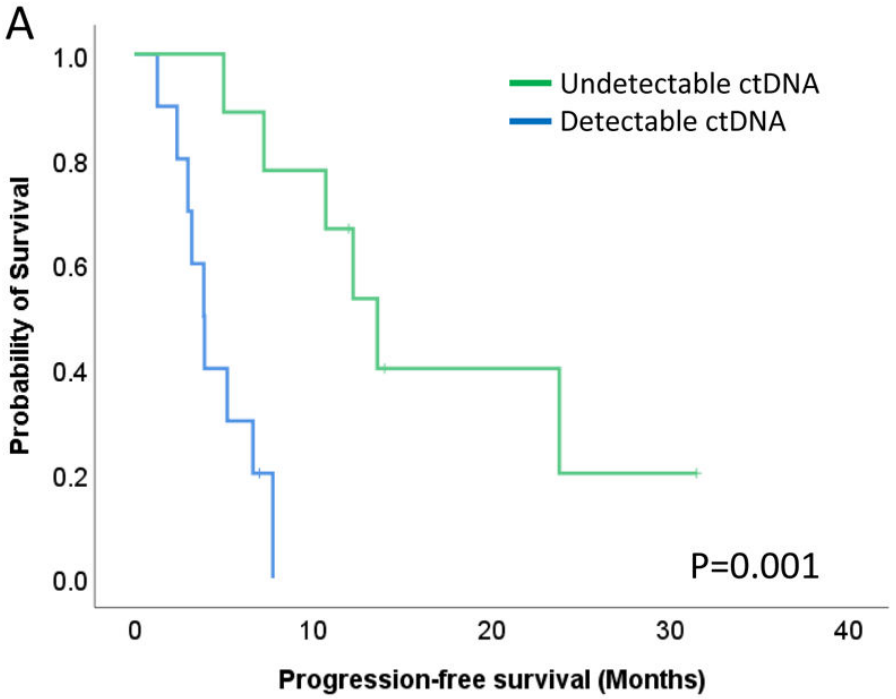
Early ctDNA Clearance at 4 wks Correlates with Clinical Outcome for Immunotherapy in NSCLC



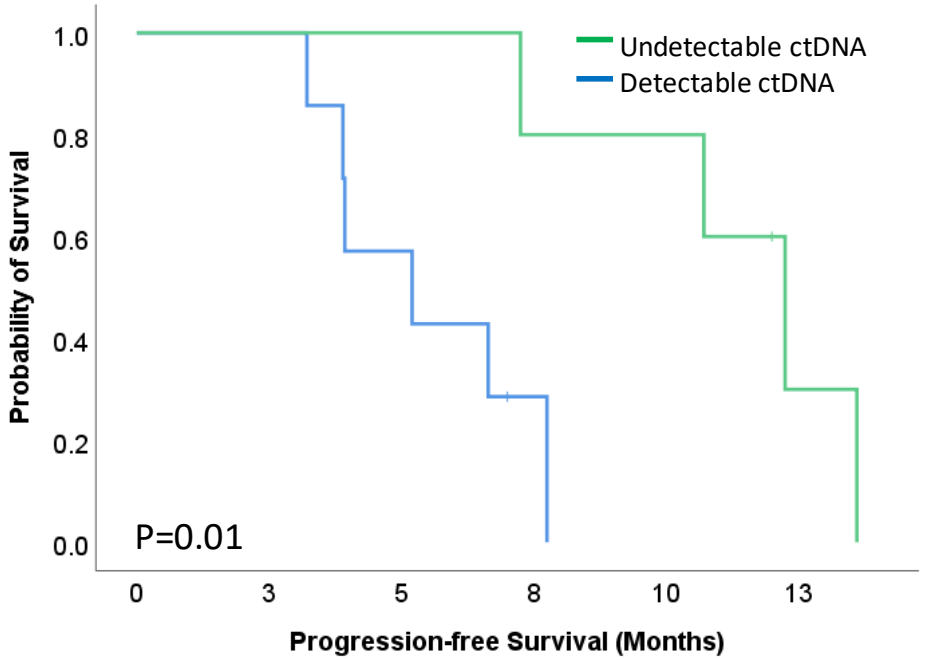
Anagnostou, Cancer Research 2019

Early ctDNA Clearance at 4 wks Correlates with Clinical Outcome for Immunotherapy in NSCLC

All Patients

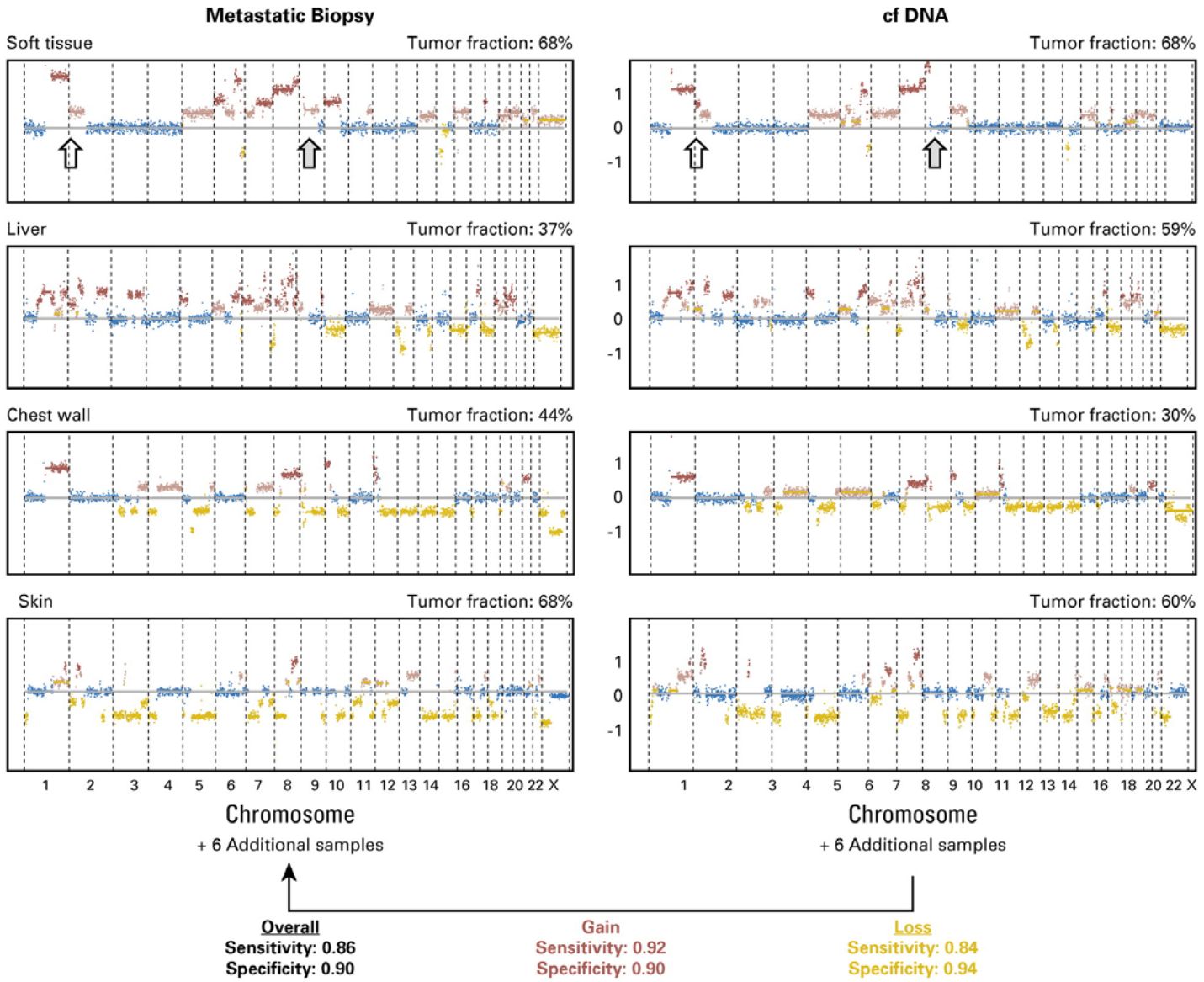


Patients with RECIST **Stable Disease**



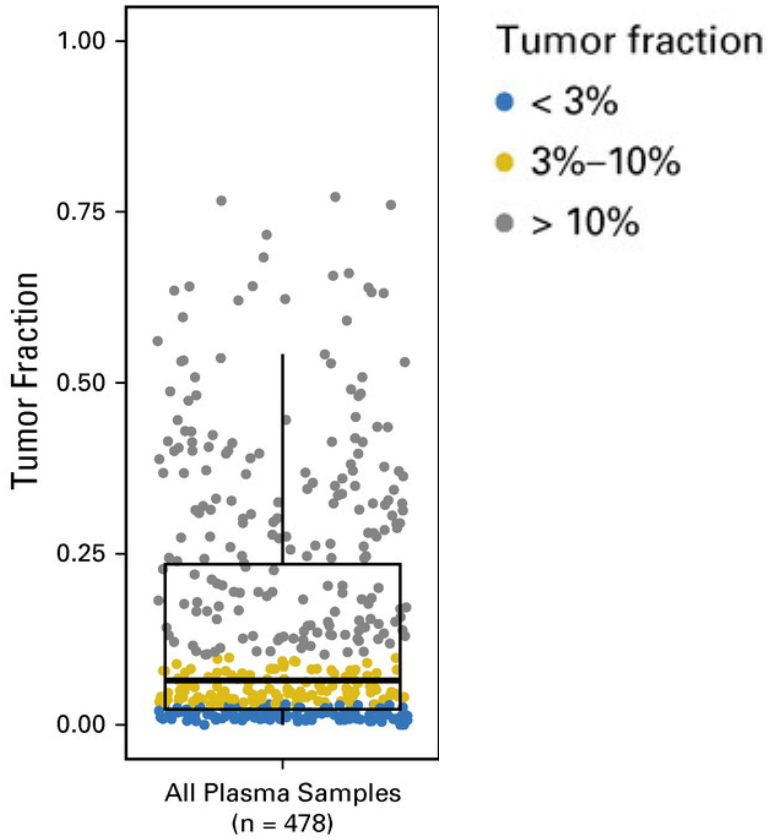
Anagnostou, Cancer Research 2019

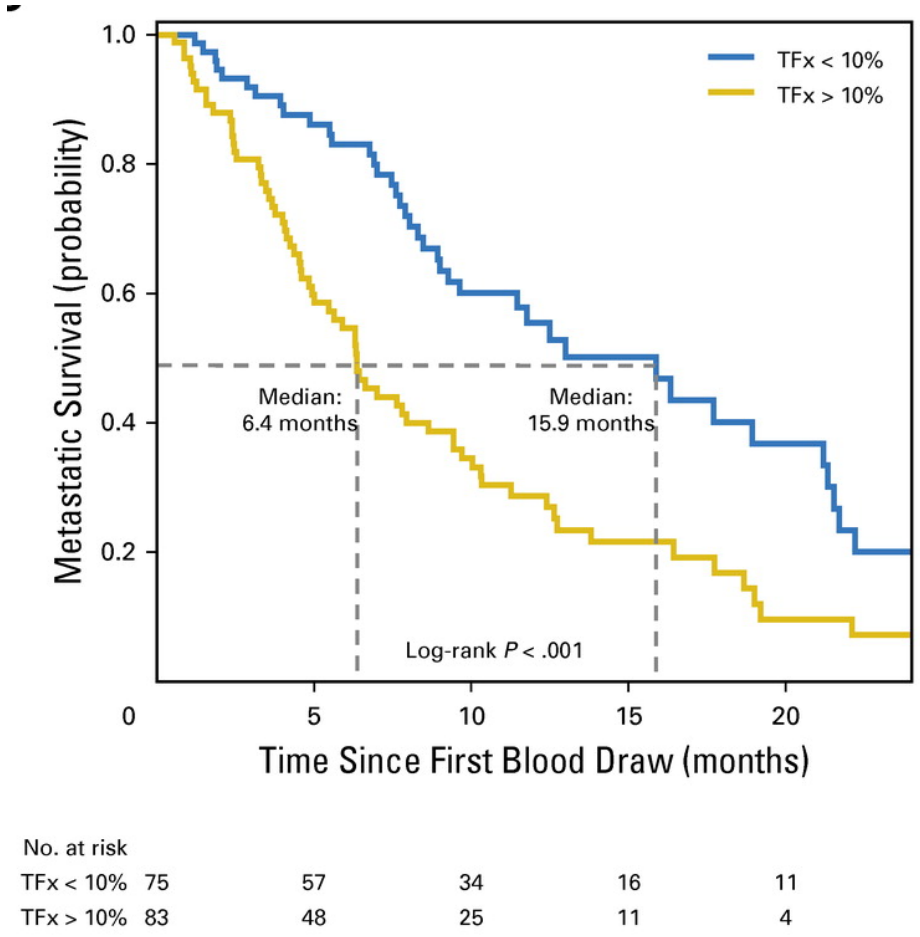
Copy Number Alterations in ctDNA (mTNBC)



Stover et al, JCO 2017

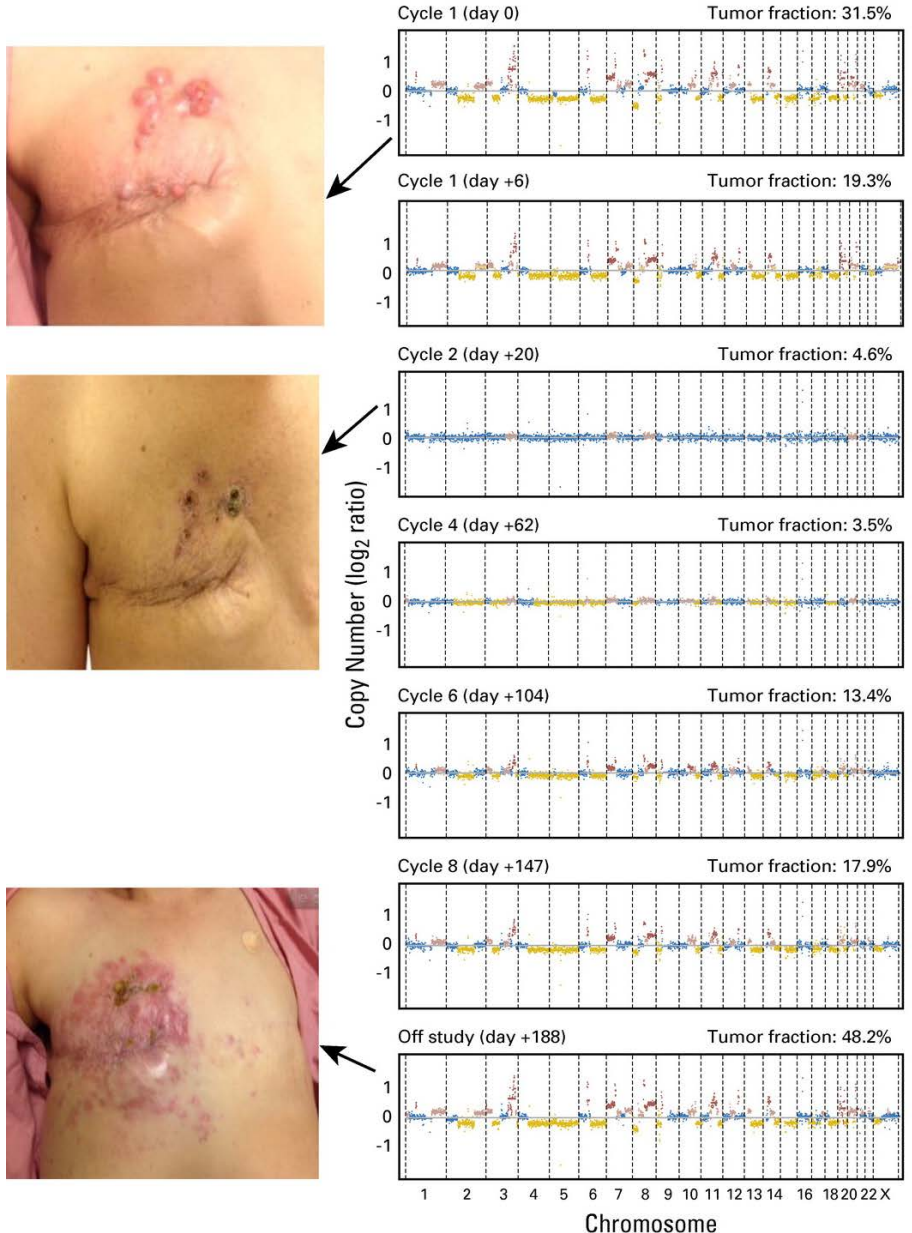
Tumor Fraction by CNA is Prognostic in TNBC





Stover et al, JCO 2017

Tumor Fx Correlates with Response and Progression

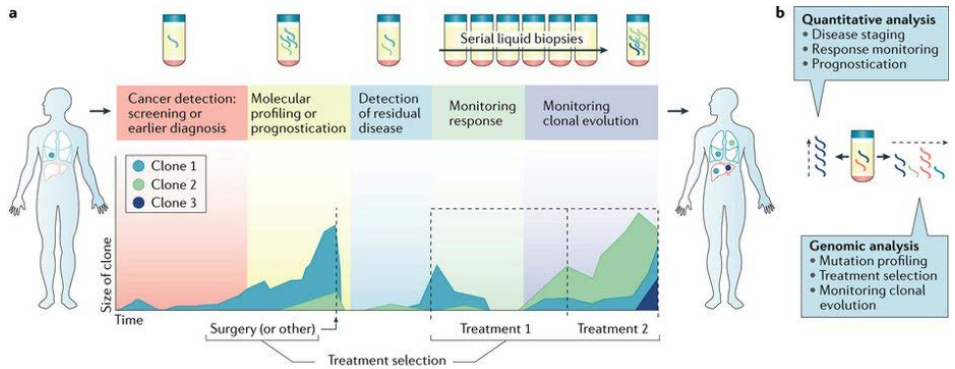


Stover et al, JCO 2017

Key Questions That Can be Addressed with ctDNA

Localized/locally advanced disease

- Predict relapse/patient selection (*prognostic +/- predictive*)
- Eliminate molecular residual disease (*surrogate*)
- Guide adjuvant treatment duration or selection

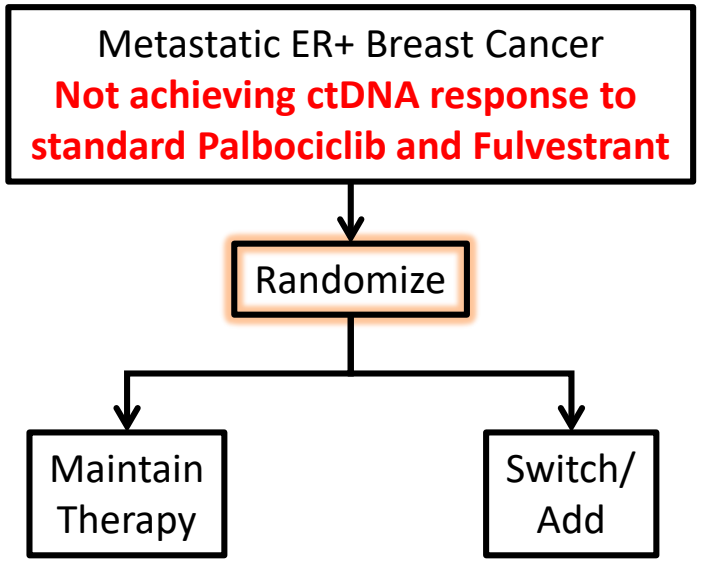


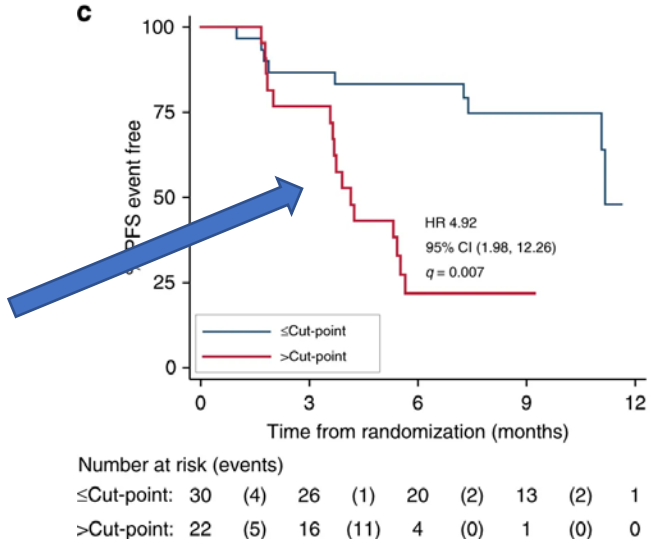
Recurrent or metastatic disease

- *Patient Selection/Predictive Biomarker*
- *Prognostic Biomarker*
- *Early Surrogate/Dynamic Response Marker*
- *Treatment Duration for I/O, NED*
- *Identify Drug Resistance*
- *Inform Drug Mechanism of Action*
- *Guide Change of Therapy*

Applications of ctDNA monitoring

- Proof of concept/surrogate endpoint for novel therapy
- Response-adaptive therapy (eg. switch or add)



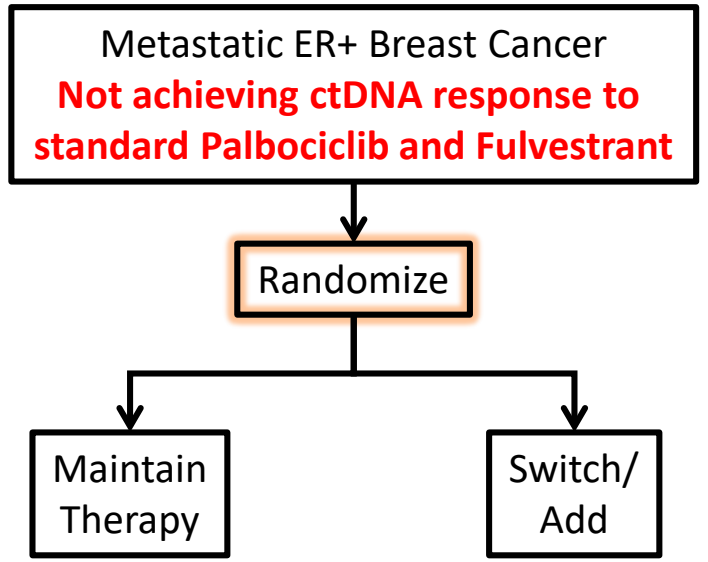


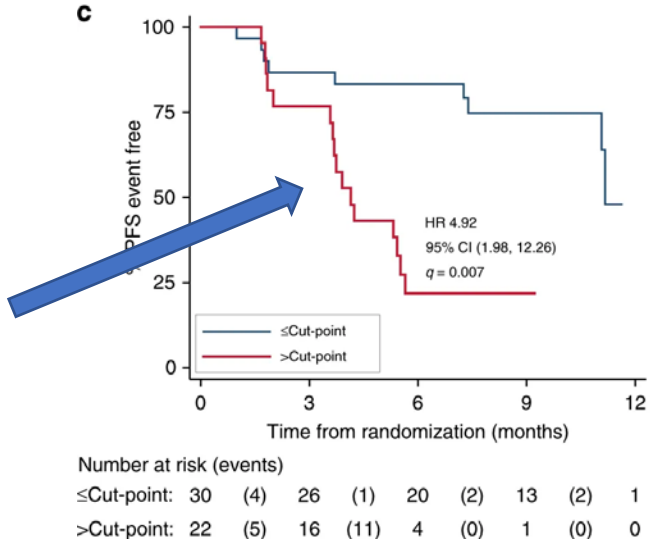
Applications of ctDNA monitoring

- Proof of concept/surrogate endpoint for novel therapy
- Response-adaptive therapy (eg. switch or add)

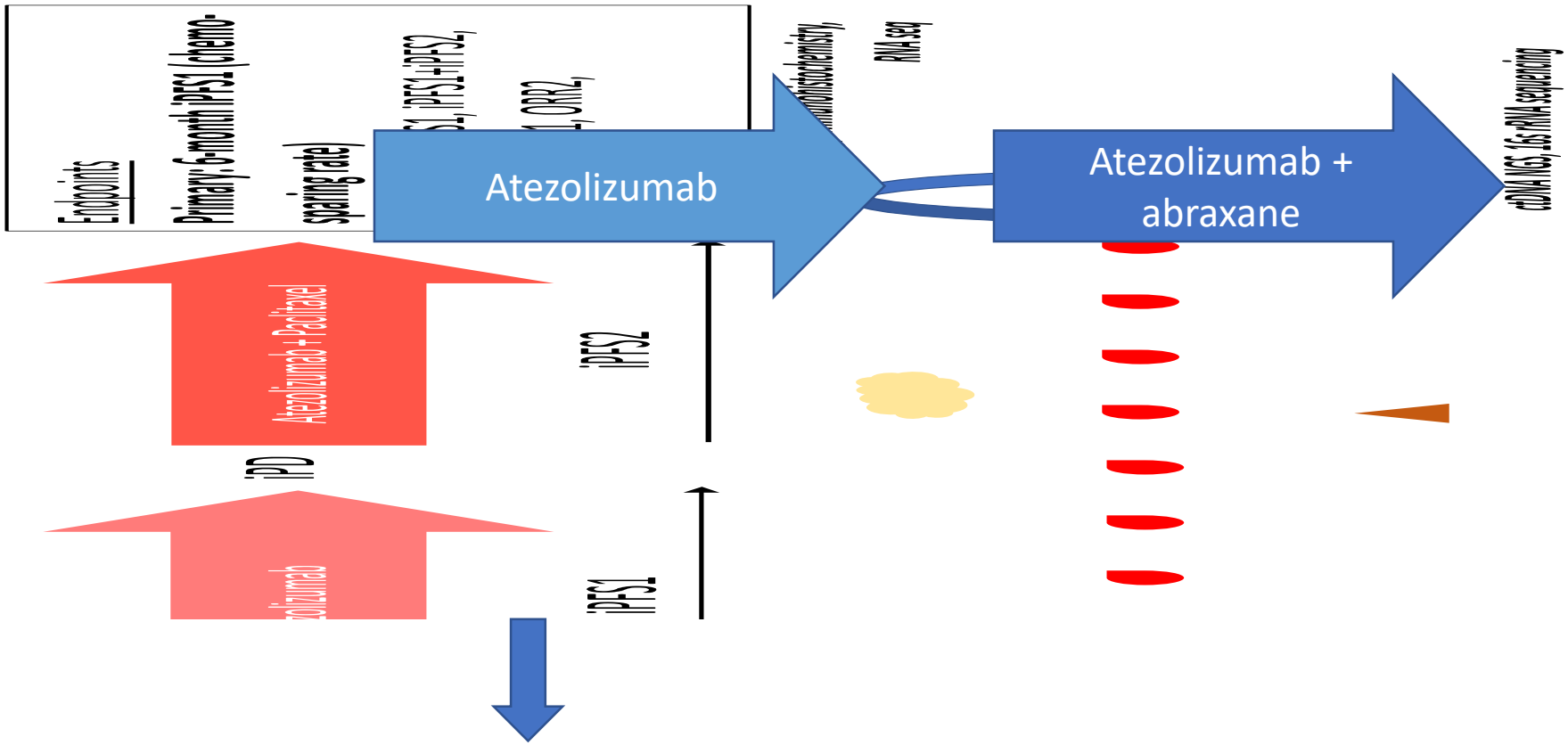
Prognostic

Predictive?



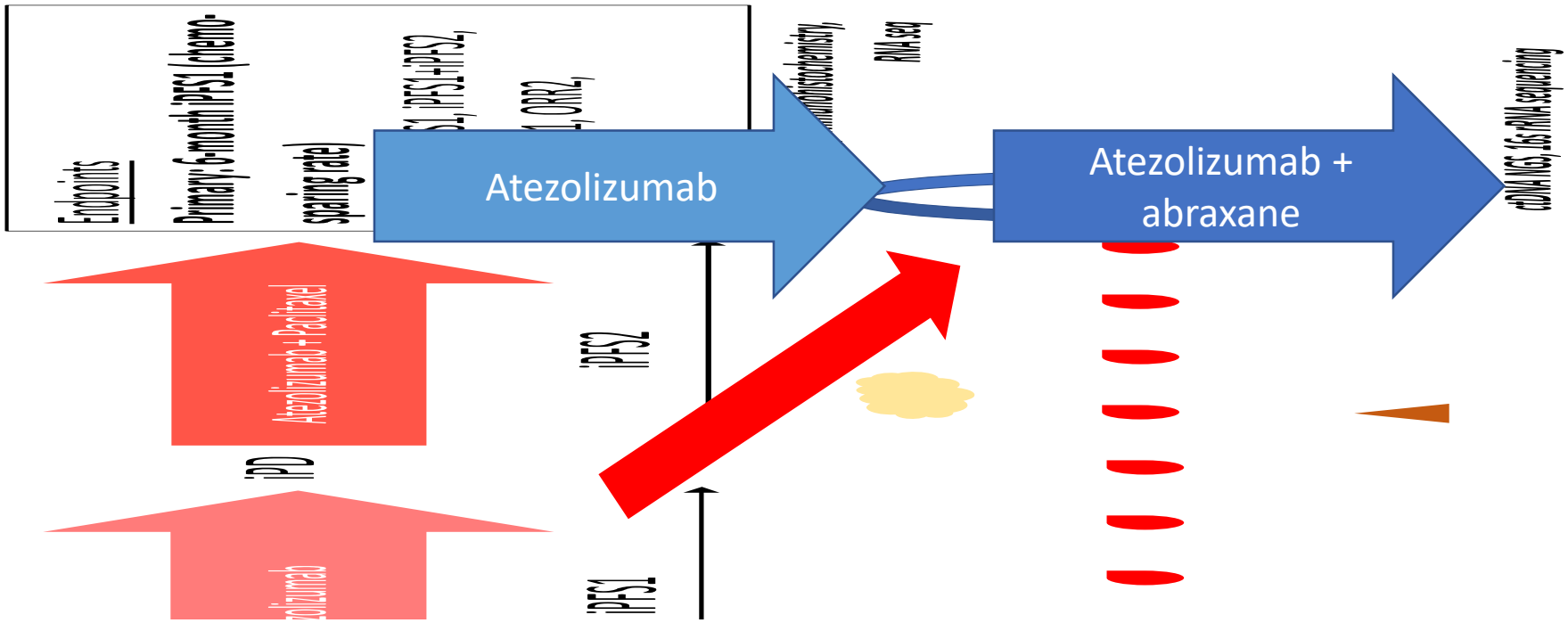


Response-adaptive chemo-sparing in TNBC



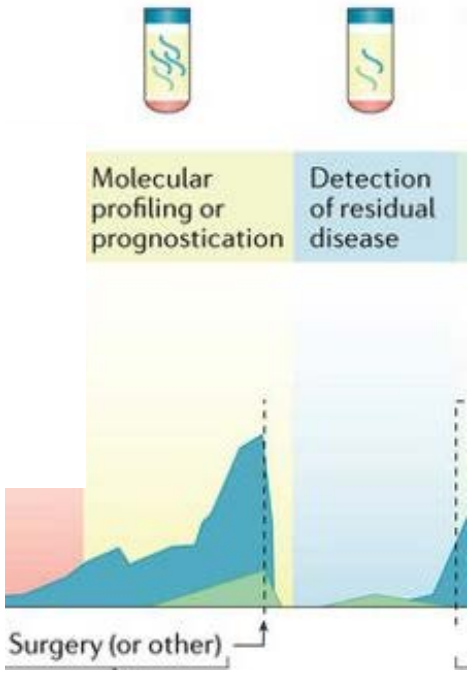
Part 1: Validation of early ctDNA response marker

Response-adaptive chemo-sparing in TNBC

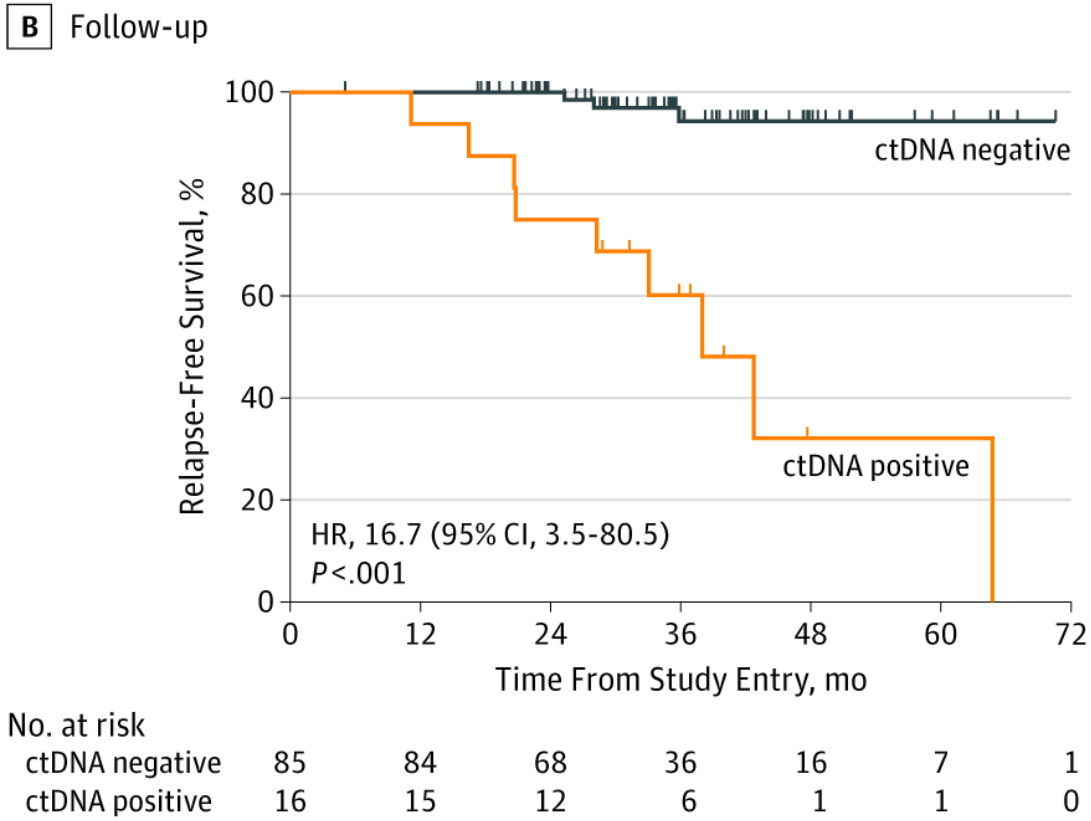


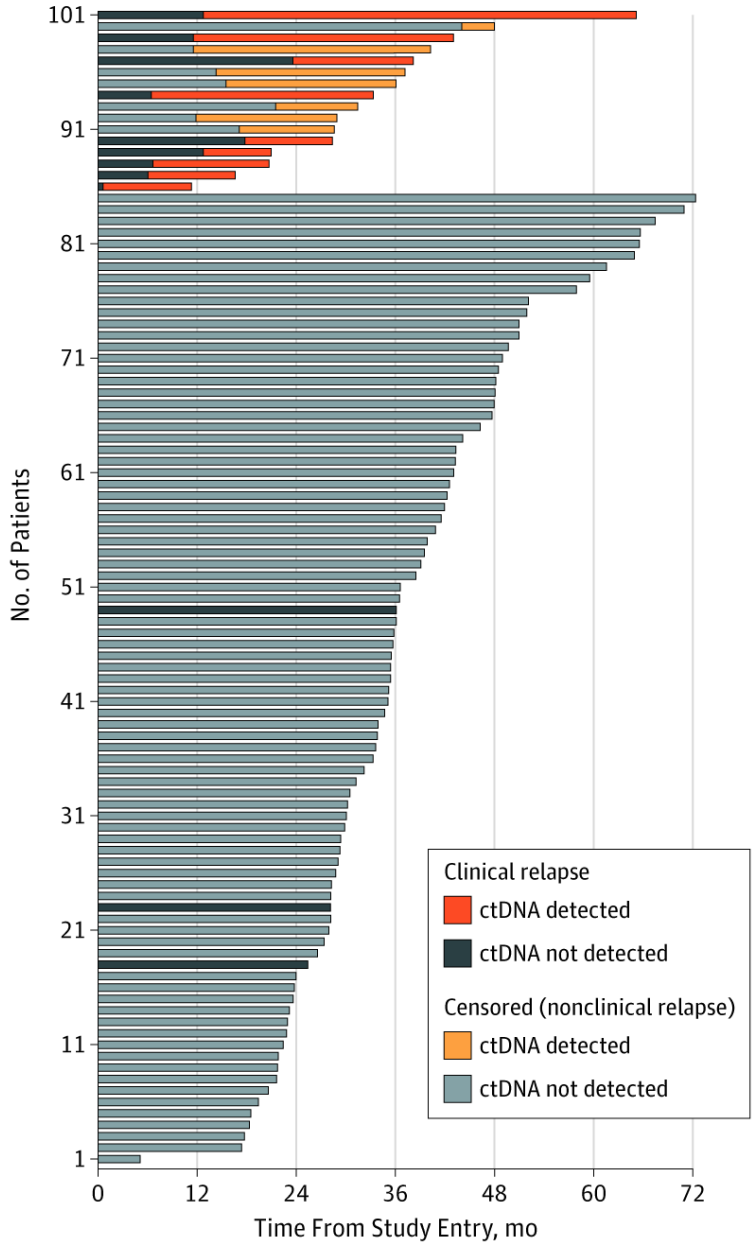
Part 2: Early cross-over in ctDNA non-respoders

ctDNA to Detect Minimal Residual Disease Following Curative-Intent Therapy



Detectable ctDNA Anticipates Relapse in Early BC



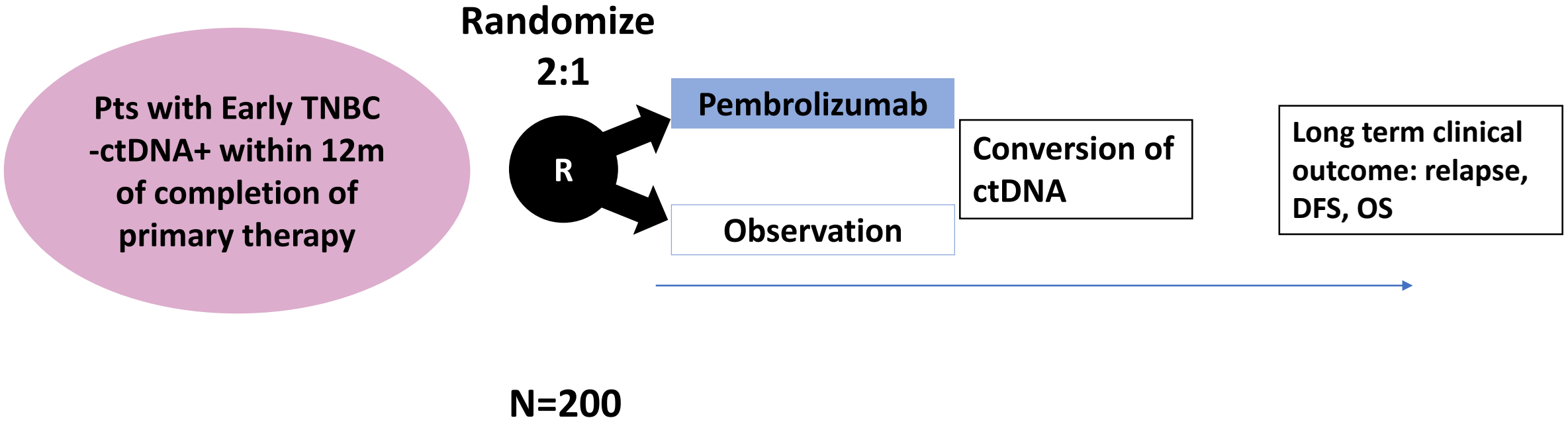


ddPCR detection in subset of patients with identified mutations on tumor panel

Garcia-Murillas I et al, JAMA Oncology 2019

MRD-guided Adjuvant Therapy: C-TRAK TN

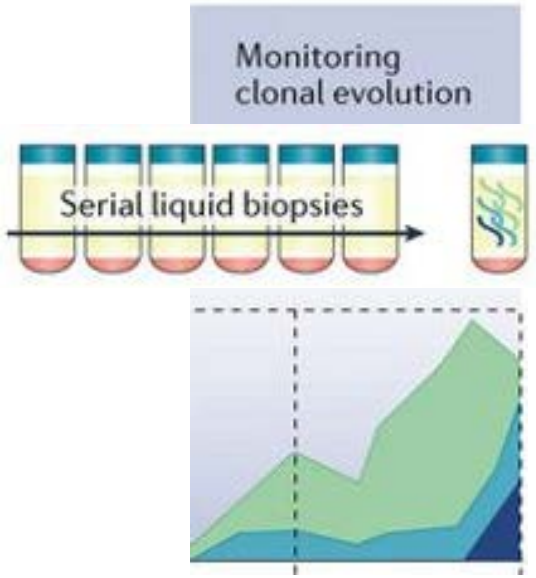
A Trial Using ctDNA Blood Tests to Detect Cancer Cells After Standard Treatment to Trigger Additional Treatment in Early Stage Triple Negative Breast Cancer Patients *(NCT03145961)*



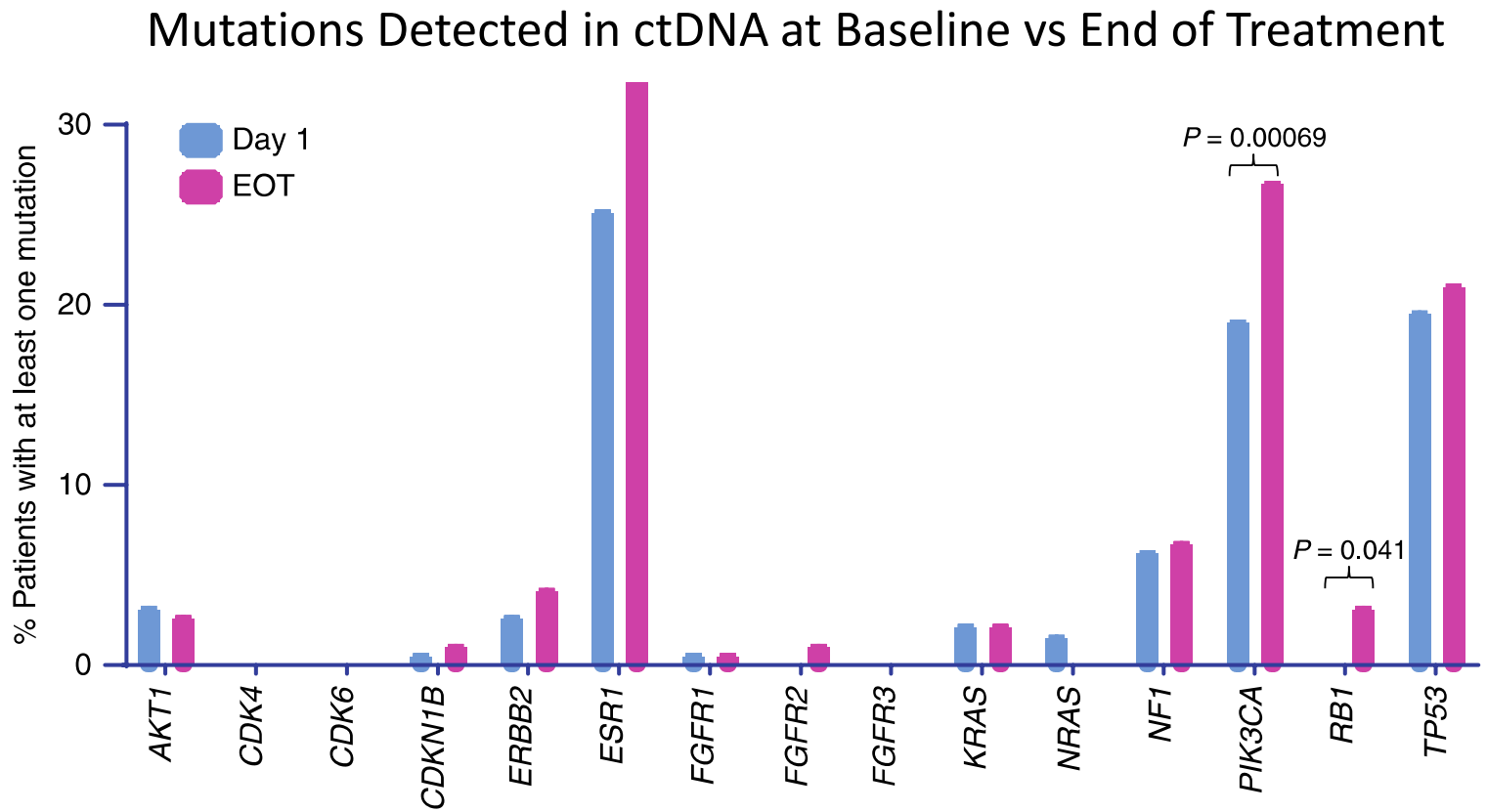
N=200

Primary Endpoint: ctDNA Detection or disease recurrence at 12 and 24 months

Evaluation of Acquired Resistance Using ctDNA

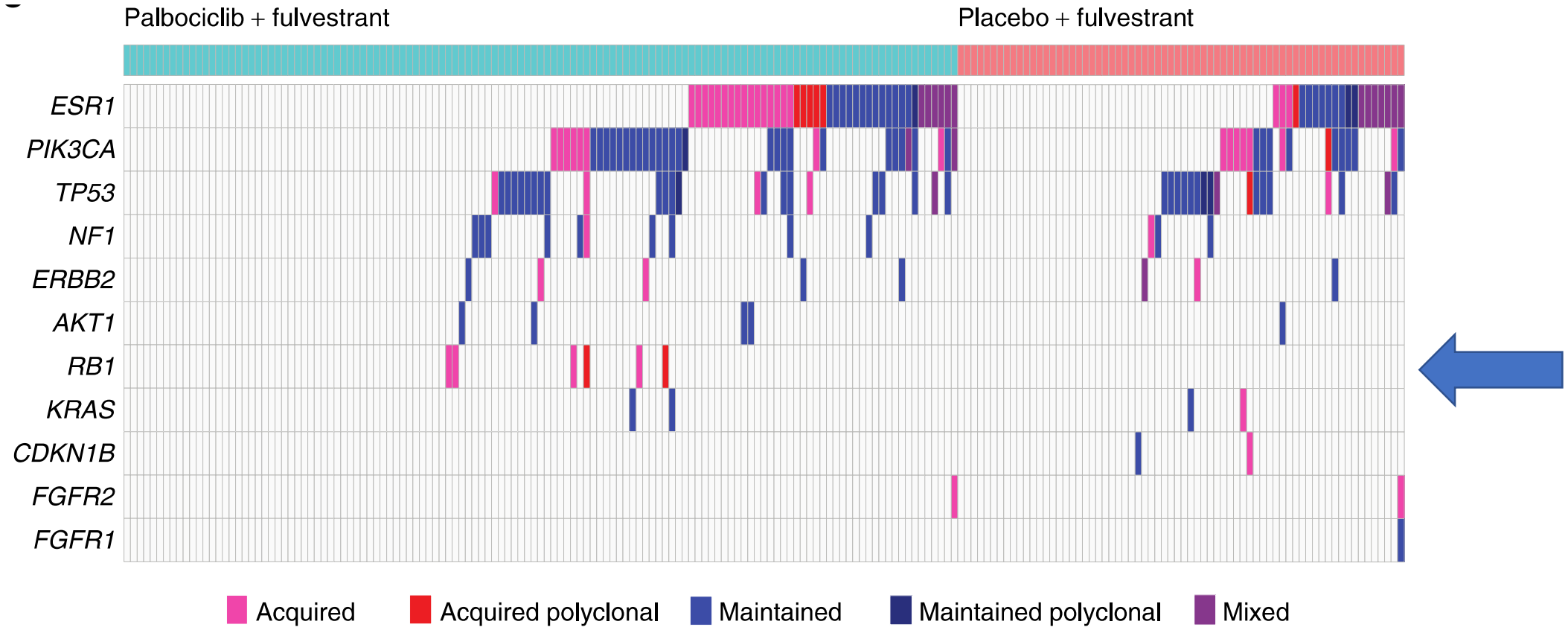


ctDNA to Identify Acquired CDK4/6i Resistance – PALOMA 3



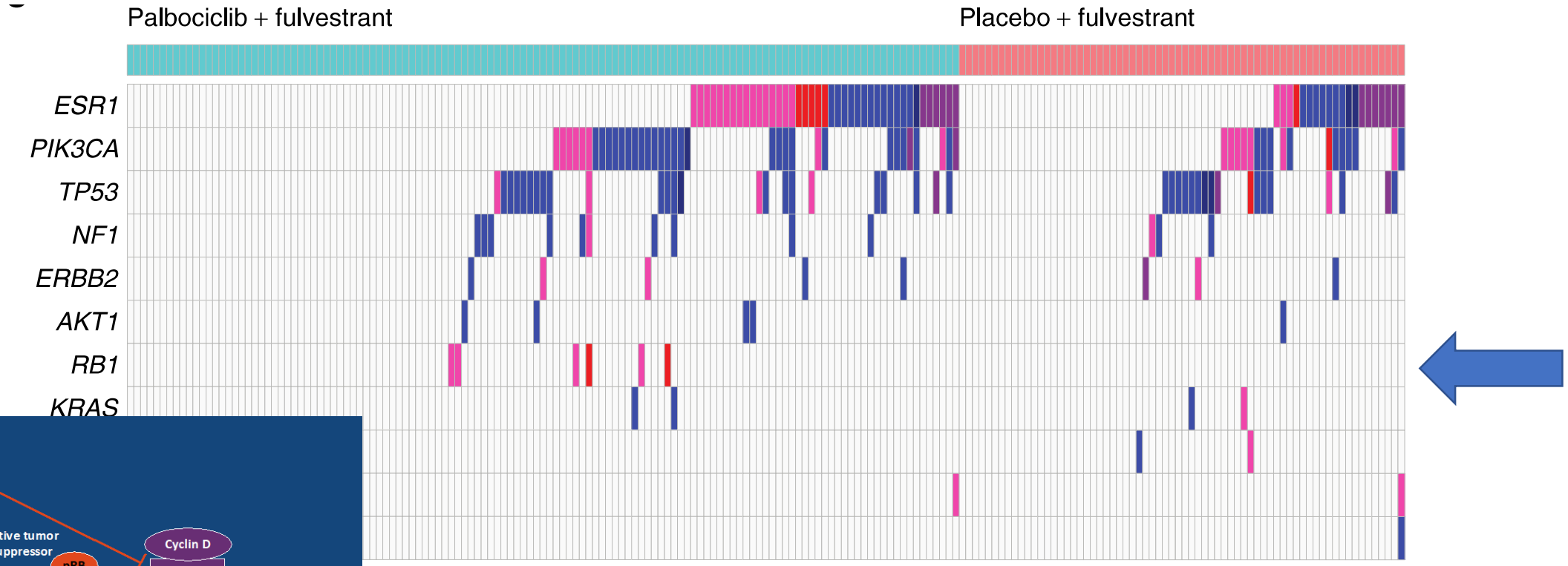
O'Leary, Cancer Discovery 2018

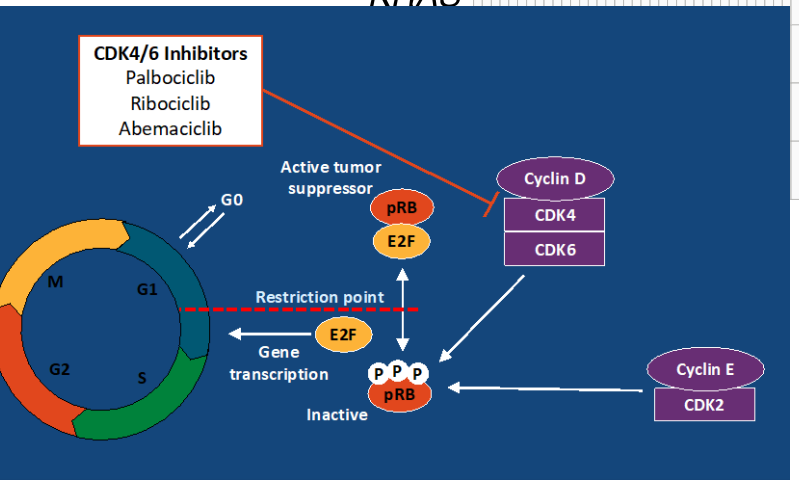
RB1 Mutations are Exclusive to CDK4/6 Arm at Progression



O'Leary, Cancer Discovery 2018

RB1 Mutations are Exclusive to CDK4/6 Arm at Progression

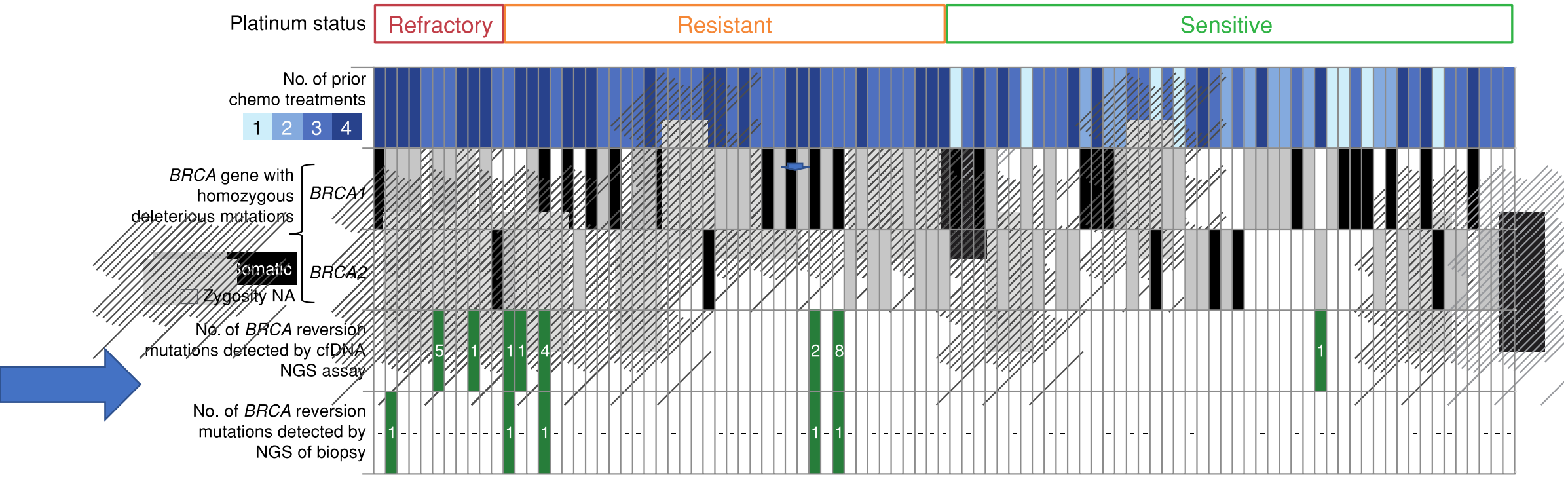




Opportunity for context-specific umbrella study

O'Leary, Cancer Discovery 2018

BRCA Reversions in Ovarian Cancer & Rucaparib

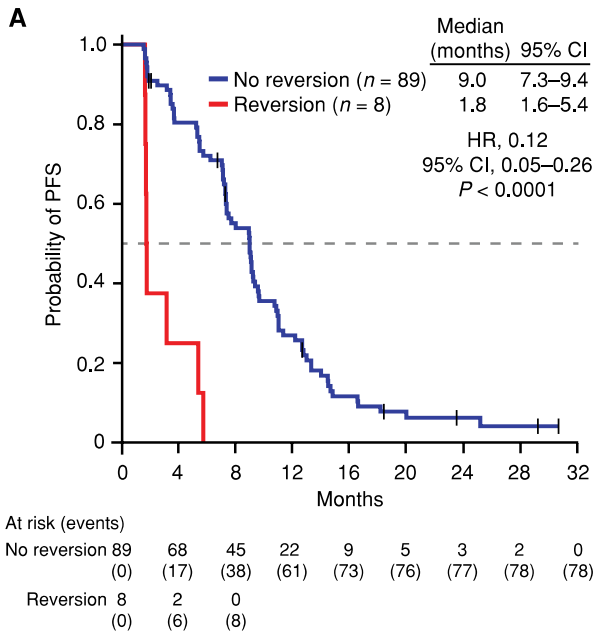


Lin et al, Cancer Discovery 2018

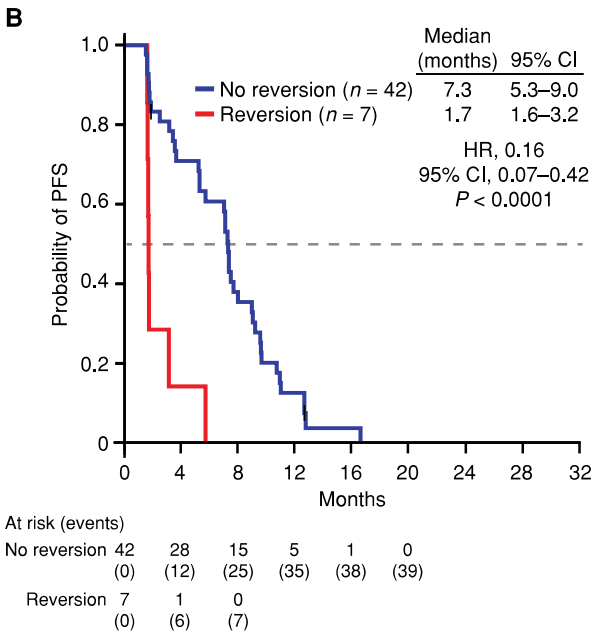
BRCA Reversions in Ovarian Cancer & Rucaparib

PFS According to BRCA Reversion Status in:

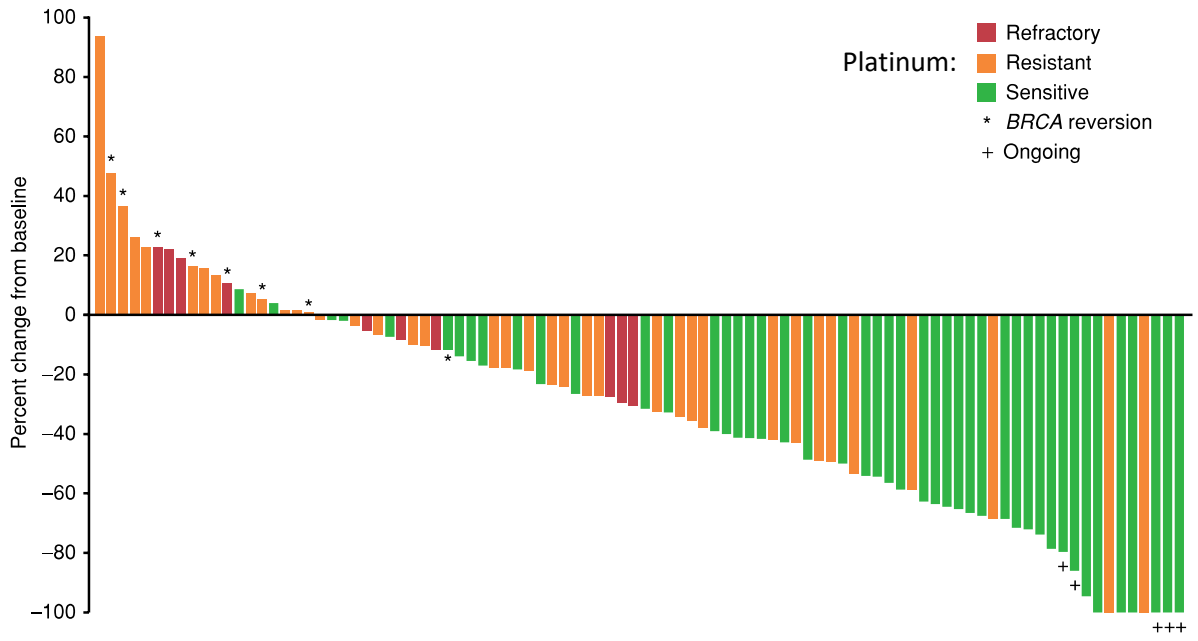
All Patients



Platinum Resistant/Refractory



Best Response To Rucaparib (RECIST)

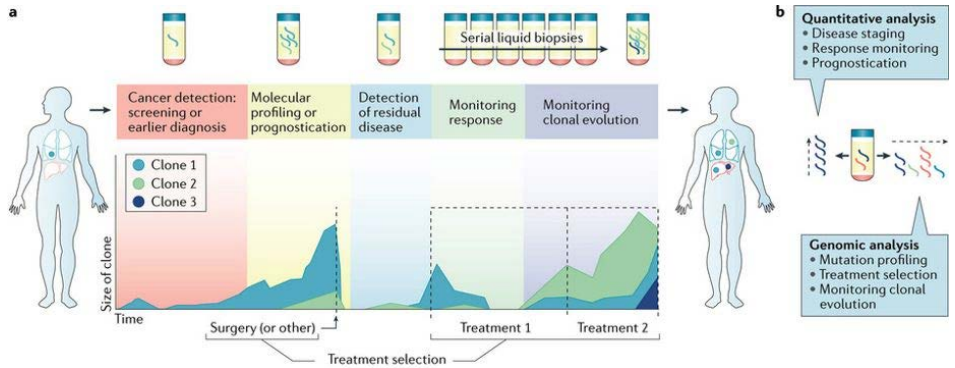


Lin et al, Cancer Discovery 2018

Key Questions That Can be Addressed with ctDNA

Localized/locally advanced disease

- Predict relapse/patient selection (*prognostic +/- predictive*)
- Eliminate molecular residual disease (*surrogate*)
- Guide adjuvant treatment duration or selection



Recurrent or metastatic disease

- Patient Selection/Predictive Biomarker
- Prognostic Biomarker
- Early Surrogate/Dynamic Response Marker
- Treatment Duration for I/O, NED
- Identify Drug Resistance
- Inform Drug Mechanism of Action
- Guide Change of Therapy

Summary – Take Home Messages

- Evaluation of ctDNA has multiple potential applications for cancer therapy and clinical trials
- Samples can be banked at relatively low cost for future analysis
- Technologies (measurement and analysis) are rapidly evolving and remain to be standardized
  - Basic principles of biomarker design and evaluation apply
- Careful consideration of study designs to set stage for future clinical utility is critical
- Next wave of interventional studies will evaluation novel strategies
  - High bar for assay performance and trial design

Table 2. Summary of Key Findings on the Use of ctDNA Analysis in Patients with Cancer

Topic	Key Findings
Pre-analytical variables for ctDNA specimens	• Evidence suggests that plasma is the optimal specimen type for ctDNA analysis. • Evidence supports the use of either cell-stabilizing tubes or EDTA anticoagulant tubes. However, EDTA tubes need to be processed as expediently as possible within 6 hours of collection. Leukocyte stabilization tubes allow up to 48 hours from collection to processing, and longer with some tubes. • Further studies are required to address other pre-analytical variables that may affect ctDNA testing, including specimen collection, handling variables, storage condition and time, and patient-related biologic factors.
Analytical validity	• Analytical validity needs to be clearly established for any clinical ctDNA assay, with particular attention paid to detection of variants near the reported lower limit of detection of the assay. Ideally, validation will include evaluation of standardized samples that facilitate cross-assay comparisons. • Evidence has not established optimal lower limits of detection for various types of somatic variants. Optimal lower limits of detection may vary depending on the intended use of the ctDNA assay, but are lower than for tumor genotyping assays. • Different ctDNA assays may not give the same results because of different assay performance characteristics, such as differing limits of detection. • Future studies should focus on cross-assay comparisons, assay robustness, and the development of proficiency testing mechanisms.
Interpretation and reporting	• Evidence demonstrates the importance of integrating clinical information, and available information from tumor analysis, with the identification of an actionable somatic variant in a ctDNA assay, to inform the appropriate selection of therapy. • The proportion of ctDNA as a fraction of total cell-free DNA in plasma varies substantially between different patients, and the potential prognostic and therapeutic implications of variant allele fractions from ctDNA assays need further study. • Caution is important when interpreting ctDNA variants found in genes that are mutated in clonal hematopoiesis of indeterminate potential. Additional research is necessary to determine how to interpret and report variants in these genes. • ctDNA assays in which a somatic variant is or is not identified should be reported in a way that conveys the potential for discordance with tumor tissue testing.
Clinical validity and utility	• Aside from assays that have received regulatory approval, most assays have insufficient evidence to demonstrate clinical validity, and most have no evidence of clinical utility. Well-designed clinical trials or equivalence studies are needed to demonstrate clinical utility for most assays. • Evidence shows discordance in results between ctDNA assays and tumor tissue genotyping and supports value of tumor tissue genotyping to confirm undetected ctDNA findings. • For advanced cancer, the evidence indicates that more reliable test results occur when the ctDNA assay is performed at the time of disease progression and not when responding to prior therapy. There is evidence that positive findings from well-validated ctDNA assays may support initiation of a targeted therapy option where an assay for the relevant genomic marker has demonstrated clinical utility when performed in tissue. For monitoring therapy effectiveness, evidence of clinical validity is still emerging, and there is currently no evidence of clinical utility to suggest that ctDNA assays are useful in this context, outside of a clinical trial. For early-stage cancer, evidence of clinical validity is still emerging, and there is currently no evidence of clinical utility to suggest that ctDNA assays are useful at diagnosis or in the adjuvant setting after completing treatment, outside of a clinical trial. • For cancer screening, there is no evidence of clinical validity and clinical utility to suggest that ctDNA assays are useful in this context, outside of a clinical trial.

Technology Assessment
ASCO/CAP 2018

Merker et al. ASCO/CAP Review JCO 2018.

Questions/Discussion

dave.cescon@uhn.ca