Advantages and Disadvantages of ctDNA vs CTC Assays: how to move the needle forward towards clinical application

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Circulating tumor (ctDNA) assays

- Nucleic acids shed into the bloodstream in patients with cancer are linked to apoptosis and necrosis of cancer cells in the tumor microenvironment. Vast amount wt DNA.
- Cell free DNA (cfDNA) has been identified in the peripheral blood plasma fraction of healthy individuals but patients with cancerous tumors have higher quantities of ctDNA and detection is associated with poorer prognosis
- Nucleic acids have a half-life in the circulation ranging from 15 minutes to several hours

Is ctDNA the ideal circulating biomarker?

- Technically easier to isolate than CTCs
- DNA is more stable than cells or RNA
- Proportion of ctDNA related to tumor burden and overall survival
- High sensitivity and dynamic range
- Pool of cancer cells/ disease sites – represents heterogeneity
  - repertoire of genetic alterations
  - level of genetic instability
  - number and properties of subclones

Potential disadvantages of ctDNA

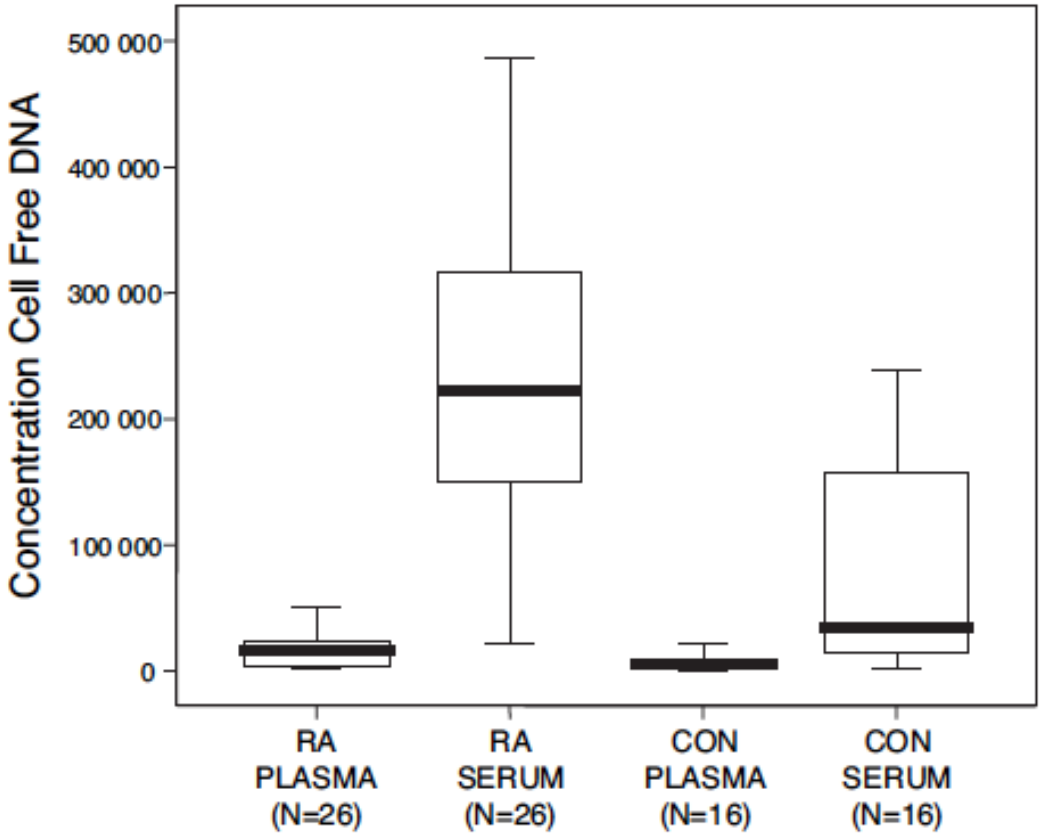
- Requires *a priori* knowledge of the target of interest in most cases
- Somatic mutations in only three genes (*TP53*, *PIK3CA* and *GATA3*) occurred at >10% incidence across all primary breast cancers*
- Not all DNA mutations are expressed
- Limitation of available material (NGS detection of mutations < 1% AF challenging)
- Blood cell death under therapy could spike ctDNA fraction (not reflecting cancer cell death)
- Source not clear – lytic, apoptotic tumor cells or are they derived from CTCs?
- Large background of 'normal' cfDNA (detected in healthy volunteers)

*TCGA, Nature, 2012

Potential confounders for ctDNA

- The majority of specific DNA markers is in the cell bound fraction
- Cytotoxic chemotherapy leads to leukocyte and erythrocyte apoptosis, which may release cell bound DNA into plasma. An increase in these markers could be from the death of these cells rather than tumor cells.
- Unclear if ctDNA is released from cancer cells because they are dying from therapy or if because they are resistant to therapy.

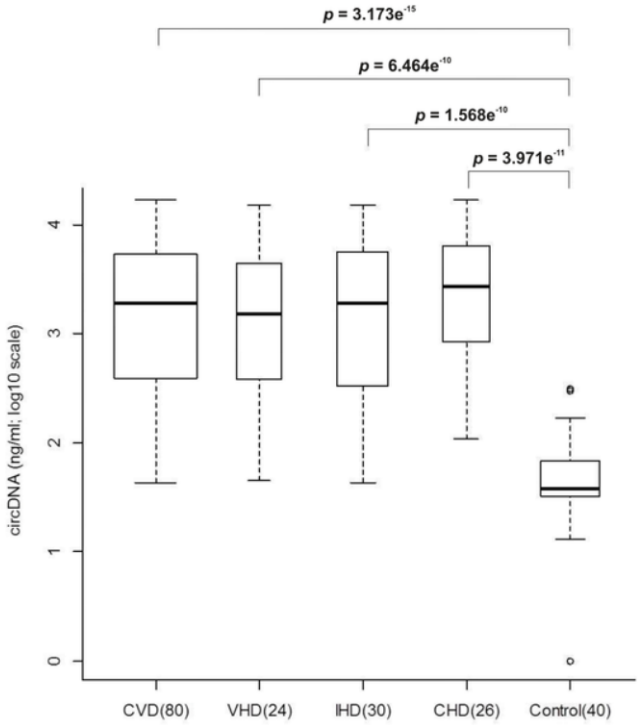
Certain medical conditions may cause elevation of circulating DNA – need to focus on tumor specific genes/mutations



Zhong et al, Clinical Chemistry 2007

Elevated Levels of Circulating DNA in Cardiovascular Disease Patients

Figure 1. Levels of circDNA in the plasma of CVD patients and healthy control samples (in Log10 scale).



Dinakaran V, Rathinavel A, Pushpanathan M, Sivakumar R, Gunasekaran P, et al. (2014) Elevated Levels of Circulating DNA in Cardiovascular Disease Patients: Metagenomic Profiling of Microbiome in the Circulation. PLoS ONE 9(8): e105221. doi:10.1371/journal.pone.0105221
http://journals.plos.org/plosone/article?id=info:doi/10.1371/journal.pone.0105221

PLOS | ONE

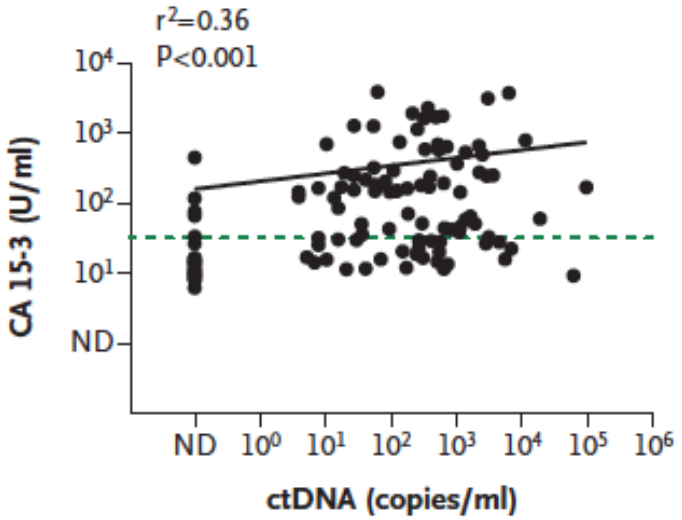
Examples of ctDNA assays

- Digital droplet PCR – requires knowledge of gene mutation of interest (i.e., PIK3CA). Up to 5 mutations may be assayed (typically).
- CAPP-Seq – hundreds of known recurrent mutations (139 genes, SNVs in 521 exons and 13 introns for NSCLC)
- Guardant 360 - 70 genes including SNVs, CNVs, indels, and rearrangements across more than 150,000 base pairs

ctDNA is more sensitive than CA15-3

A **CA 15-3 vs. ctDNA**

CA 15-3	ctDNA		
	Detected	Not detected	Total
Patients			
Elevated (>32.4 U/ml)	21	0	21
Not elevated (≤32.4 U/ml)	5	1	6
Total	26	1	27
ctDNA sensitivity, 26/27 (96%) CA 15-3 sensitivity, 21/27 (78%)			
Samples			
Elevated (>32.4 U/ml)	67	4	71
Not elevated (≤32.4 U/ml)	27	16	43
Total	94	20	114
ctDNA sensitivity, 94/114 (82%) CA 15-3 sensitivity, 71/114 (62%)			

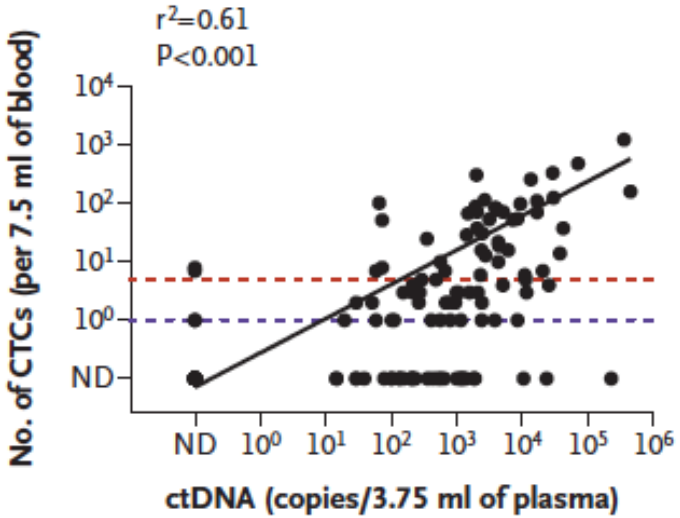


Dawson et al, NEJM 2013

ctDNA is more sensitive than Cell Search CTC Assay

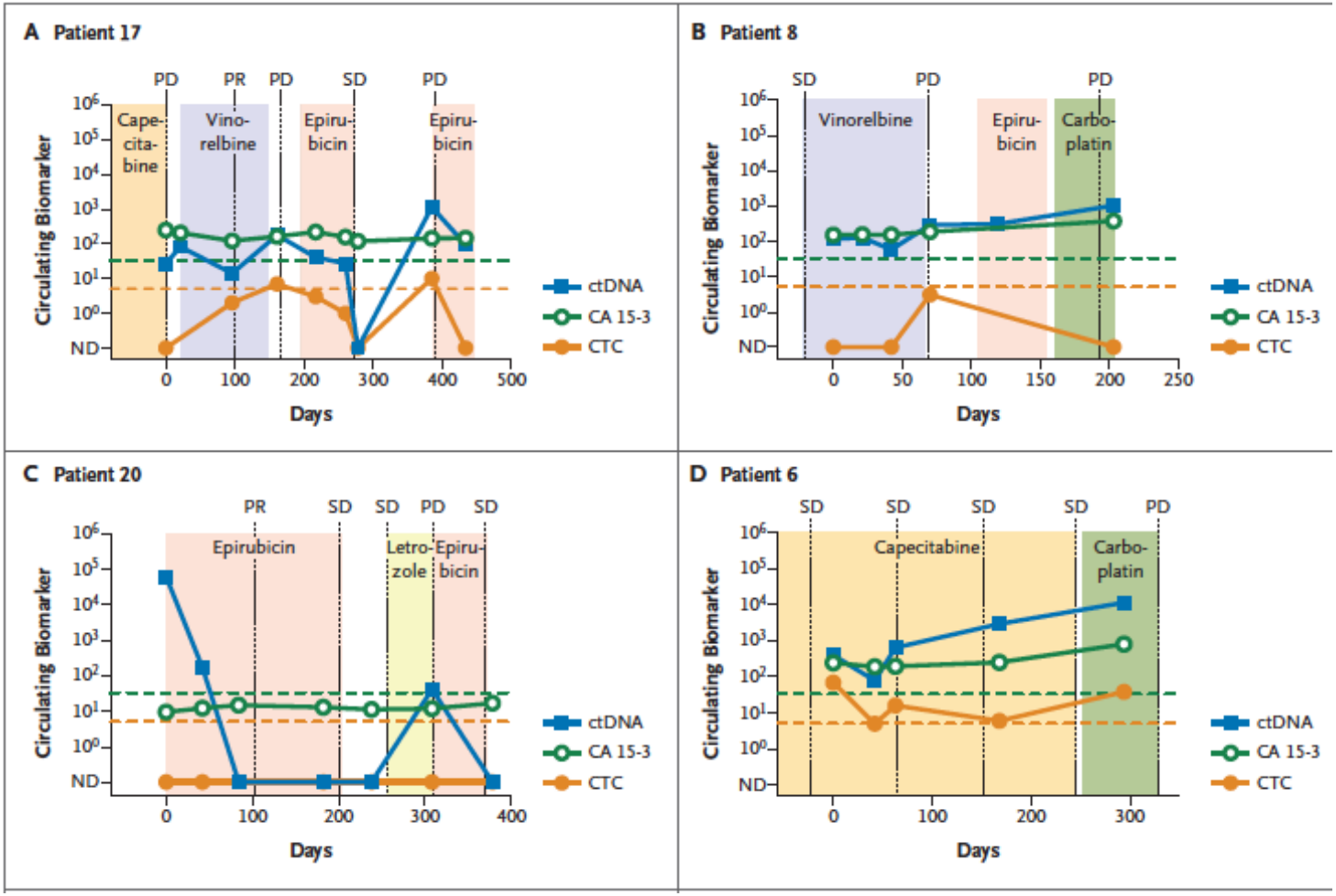
B CTC vs. ctDNA

CTC	ctDNA		
	Detected	Not detected	Total
Patients			
Elevated (≥5)	18	0	18
Detected (1–4)	7	1	8
Not detected (0)	4	0	4
Total	29	1	30
ctDNA sensitivity, 29/30 (97%) CTC sensitivity (detected, >0), 26/30 (87%) CTC sensitivity (elevated, ≥5), 18/30 (60%)			
Samples			
Elevated (≥5)	45	1	46
Detected (1–4)	28	2	30
Not detected (0)	33	17	50
Total	106	20	126
ctDNA sensitivity, 106/126 (84%) CTC sensitivity (detected, >0), 76/126 (60%) CTC sensitivity (elevated, ≥5), 46/126 (37%)			
Median ratio of ctDNA copy numbers (per 3.75 ml of plasma) to number of CTCs (per 7.5 ml of whole blood)=133 (interquartile range, 51–528)			



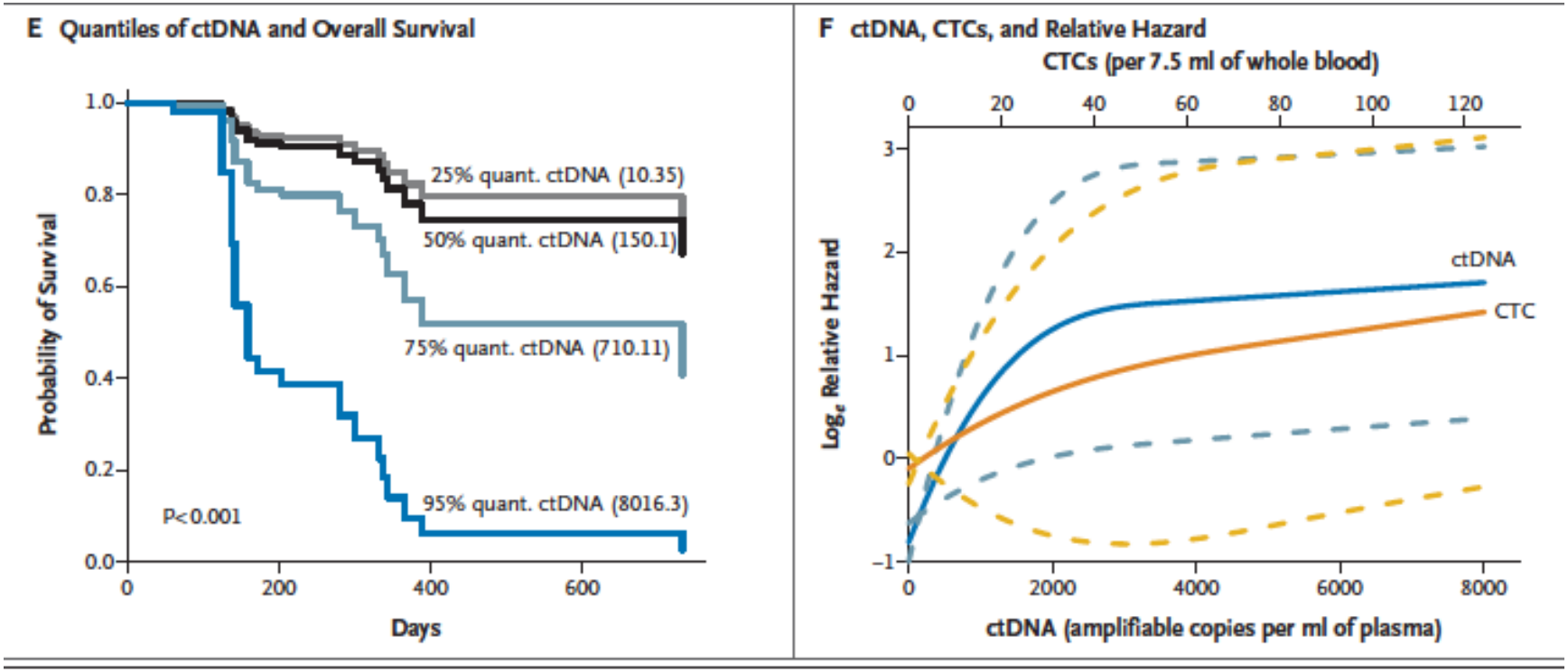
Dawson et al, NEJM 2013

Comparison of Circulating Biomarkers to Monitor Tumor Dynamics



Dawson et al, NEJM 2013

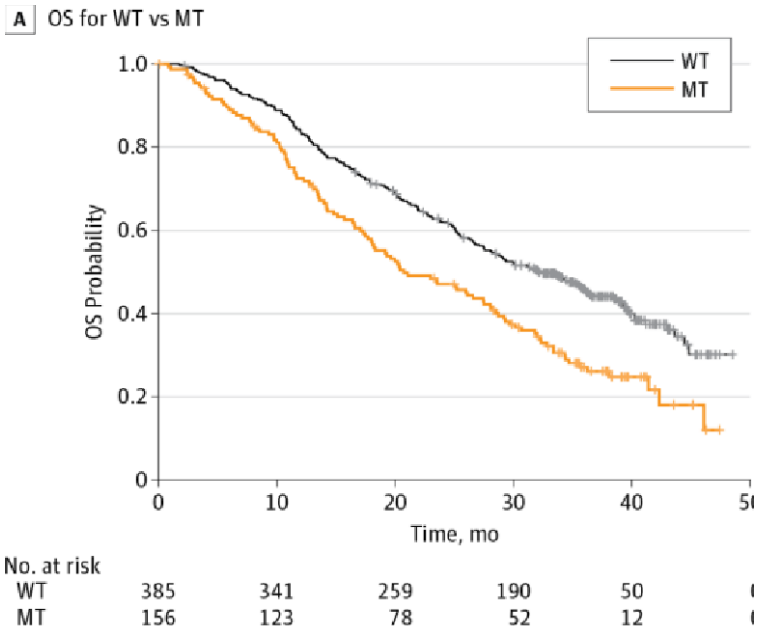
ctDNA is prognostic of survival

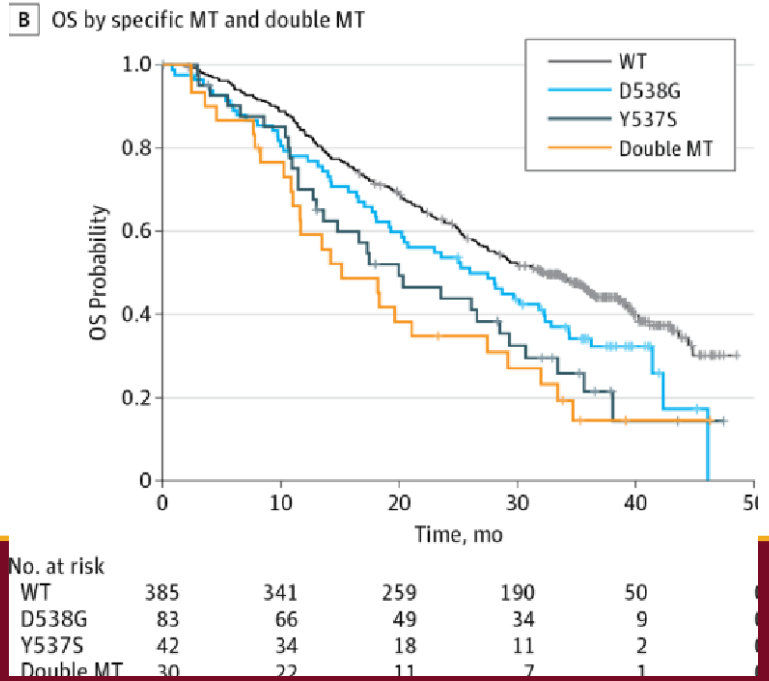


Dawson et al, NEJM 2013

Prevalence of ESR1 Mutations in Cell-Free DNA and Outcomes in Metastatic Breast Cancer: A Secondary Analysis of the BOLERO-2 Clinical Trial

According to Mutation Status **A**, Superior overall survival (OS) is shown for patients without mutation (MT) in ESR1 (wild-type [WT]) compared with those with D538G and/or Y537S mutation. **B**, Overall survival results are shown for WT or D538G alone or Y537S alone or both D538G and Y537S (double MT).





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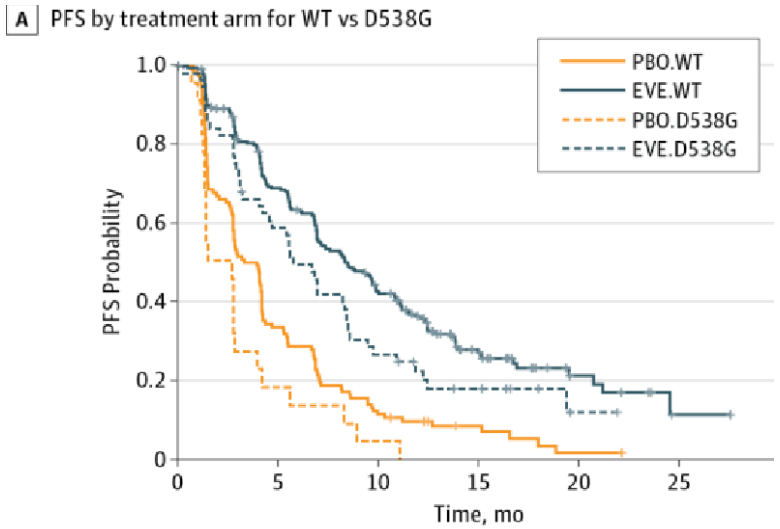
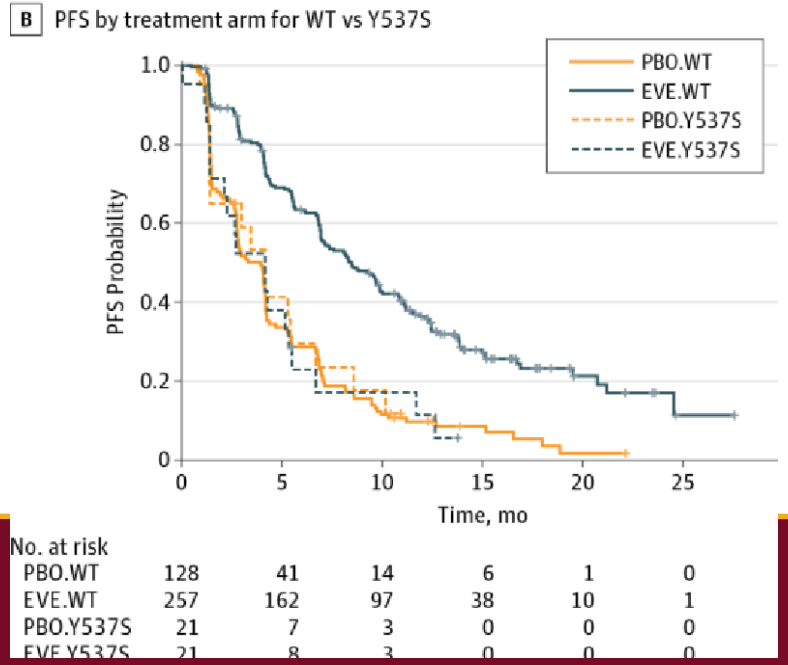
Chandarlapaty et al, JAMA Oncology 2016

Prevalence of ESR1 Mutations in Cell-Free DNA and Outcomes in Metastatic Breast Cancer: A Secondary Analysis of the BOLERO-2 Clinical Trial

Kaplan-Meier Curves for Effect of Addition of Everolimus to Exemestane **A**, Progression-free survival (PFS) for patients without ESR1 mutation (wild-type [WT]) or with the D538G mutation (D538G). **B**, Results for patients without ESR1 mutation (WT) or with the Y537S mutation (Y537S). Addition of everolimus (EVE) was associated with improved progression-free survival (PFS) for patients with WT or D538G mutation but not for those with Y537S mutation. PBO indicates placebo.

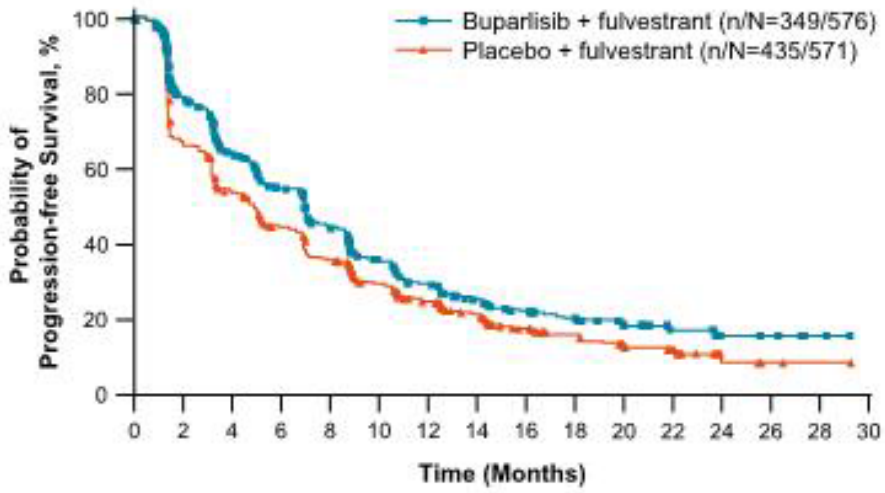




Chandarlapaty et al, JAMA Oncology 2016

ctDNA in clinical trials: use as a companion biomarker

BELLE-2 Met the Primary Endpoint for PFS Improvement in the Full Population

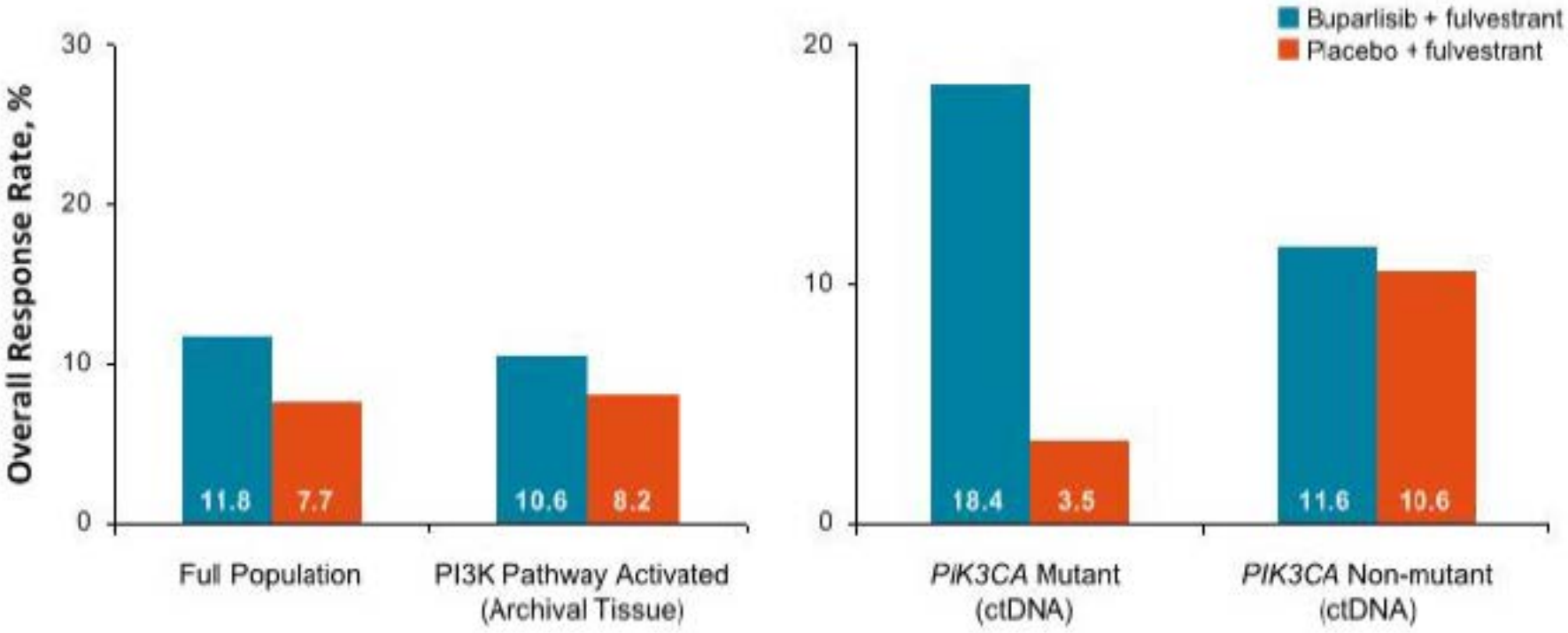


Full Population (N=1147)	Buparlisib + Fulvestrant n=576	Placebo + Fulvestrant n=571
Median PFS, months (95% CI)	6.9 (6.8–7.8)	5.0 (4.0–5.2)
HR (95% CI)	0.78 (0.67–0.89)	
One-sided *P* value	<0.001	

- A similar PFS improvement was observed in the main population (HR 0.80 [95% CI: 0.68–0.94]; one-sided *P* value 0.003)
- Follow-up for OS analysis is ongoing, with a pre-specified target of 588 deaths in the full population
  - At the time of primary PFS analysis, OS data were immature (281 deaths in the full population), with a trend in favor of the buparlisib arm

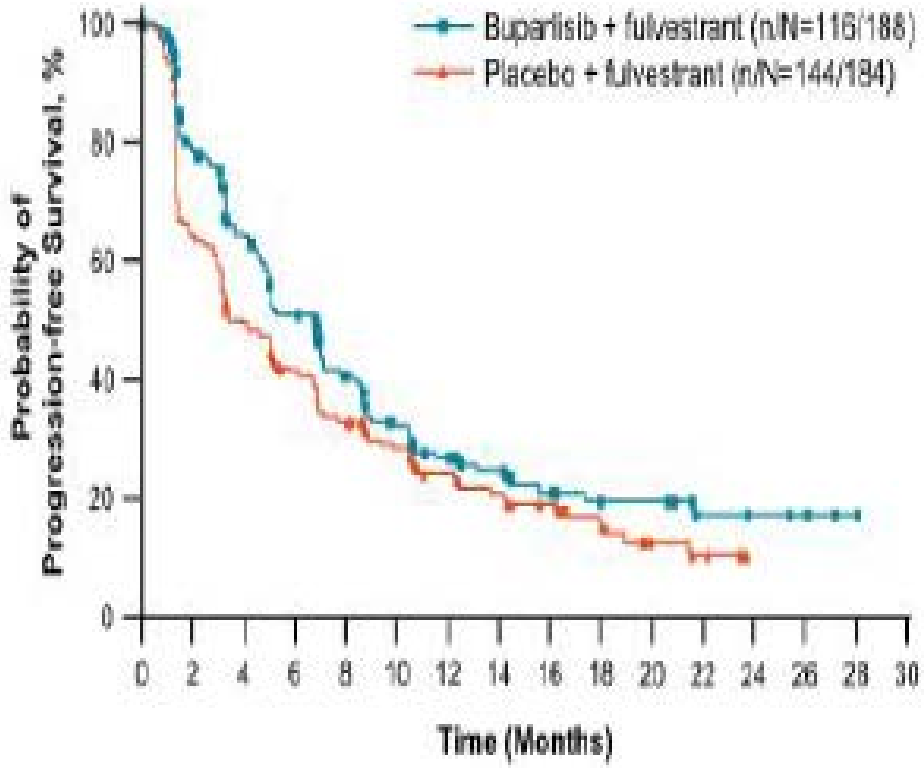
Baselga et al, SABCS 2015

Buparlisib Plus Fulvestrant Resulted in Higher Response Rates in Patients With ctDNA *PIK3CA* Mutations



Baselga et al, SABCS 2015

PFS Improvement in the PI3K Activated Group Was Not Statistically Significant



PI3K Activated Group (N=372)	Buparlisib + Fulvestrant n=188	Placebo + Fulvestrant n=184
Median PFS, months (95% CI)	6.8 (4.9–7.1)	4.0 (3.1–5.2)
HR (95% CI)	0.76 (0.60–0.97)	
One-sided *P* value*	0.014	

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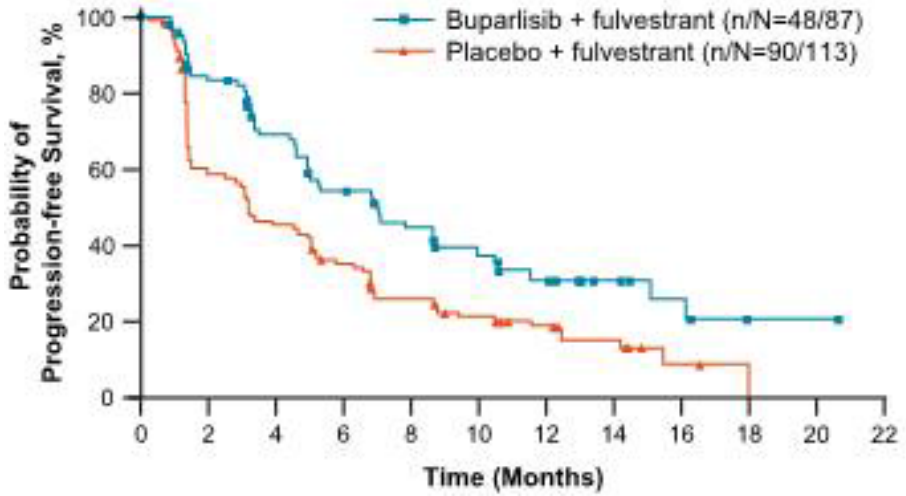
Baselga et al, SABCS 2015

ctDNA in clinical trials

Buparlisib Plus Fulvestrant Produced a Clinically Meaningful PFS Improvement in Patients With ctDNA *PIK3CA* Mutations

ctDNA *PIK3CA* Mutant n=200	Buparlisib + Fulvestrant n=87	Placebo + Fulvestrant n=113
Median PFS, months (95% CI)	7.0 (5.0–10.0)	3.2 (2.0–5.1)
HR (95% CI)	0.56 (0.39–0.80)	
One-sided nominal *P* value	<0.001	



Baselga et al, SABCS 2015

Accuracy of ctDNA vs tissue

- Matched tissue for sequencing is often not available:
- Carpenter et al, Clin Cancer Research 2016 lung cancer: ctDNA NGS on 102 patients but only 49% successful NGS of matching tumor tissue.
- Guardant 360 ASCO 2016 presentation by Zill et al: 2.6% (386/15,000) patients included had matched tumor tissue for comparison.
- Allelic frequencies in plasma tumor DNA derived from low-level subclonal variants present in a metastatic site are extremely low (lower than current sensitivity of NGS).

Carpenter et al, Clin Cancer Research 2016.

Zill et al, ASCO 2016.

Feasibility of A Prospective Study of Molecular Profiling of Tissue and Blood in Metastatic Triple Negative Breast Cancer

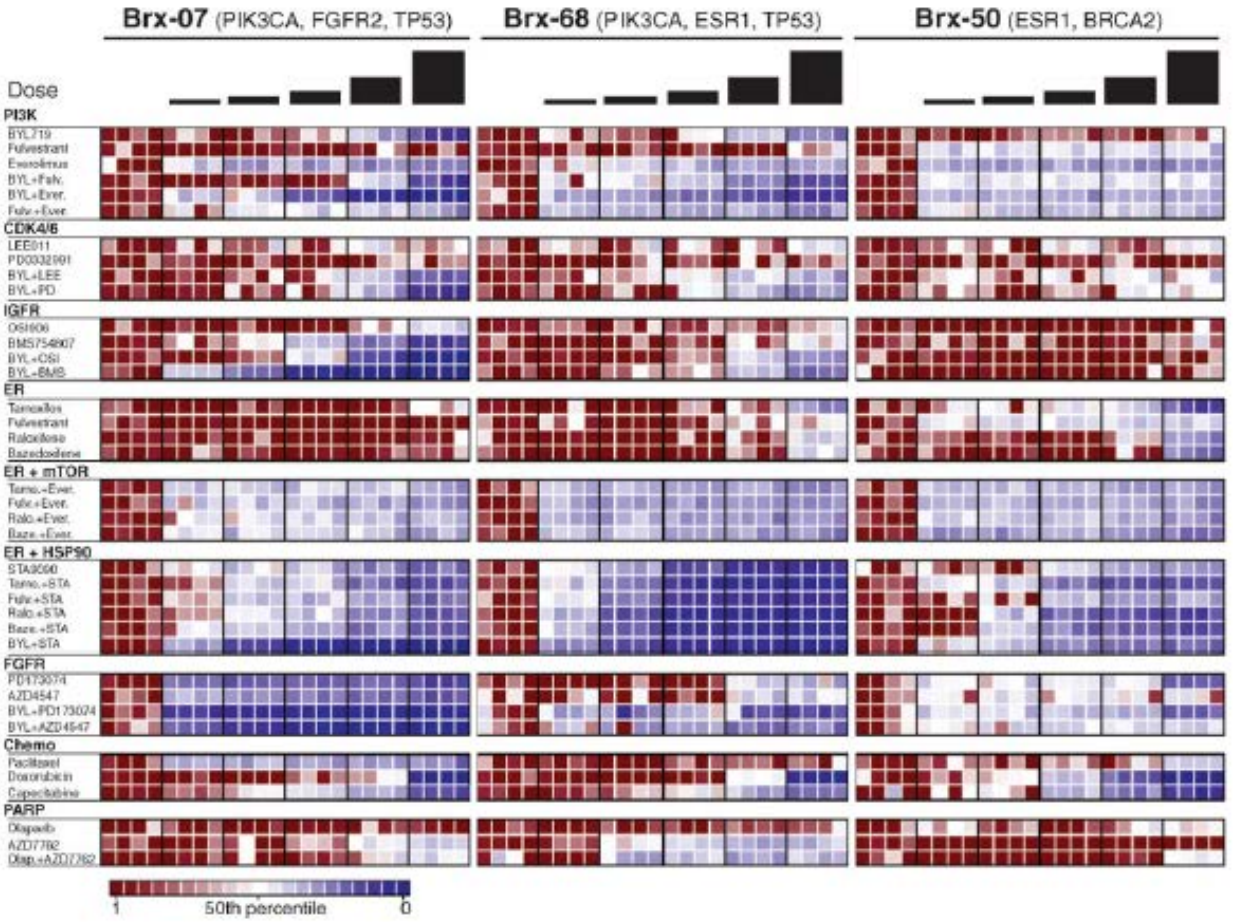
- Single institutional study with the goal of molecularly profiling of tumor tissue and plasma DNA with hybrid capture based NGS
- Goal: perform molecular profiling on a recent biopsy and blood, convey results within 28 days.
- Met interim analysis for futility, study stopped.
- Did show high concordance of mutations found in liquid biopsy with tissue biopsy, but it was challenging to perform NGS of prospective tissue biopsies rapidly enough to impact clinical decision making.

Parsons et al, Clinical Cancer Research 2016

Are CTCs the ideal circulating biomarker?

- CTCs are prognostic in all stages of breast cancer
- Deep sequencing revealed matching mutation with tumor sub clones – represents heterogeneity
- Intact cells could be resistant clones (DNA not in ctDNA) – isolation and characterization could give valuable information for therapeutic decisions
- Can be used for functional assays (DNA, RNA, protein)
- Can be cultured to evaluate drug resistance *in vitro* or *in vivo*

CTCs may be used for functional assays



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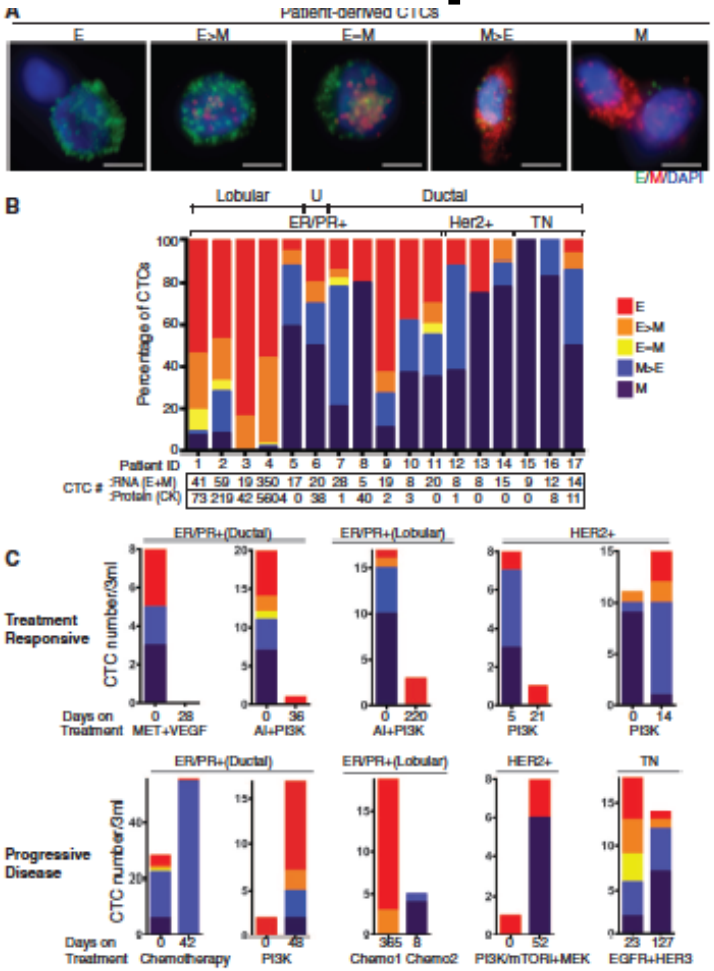
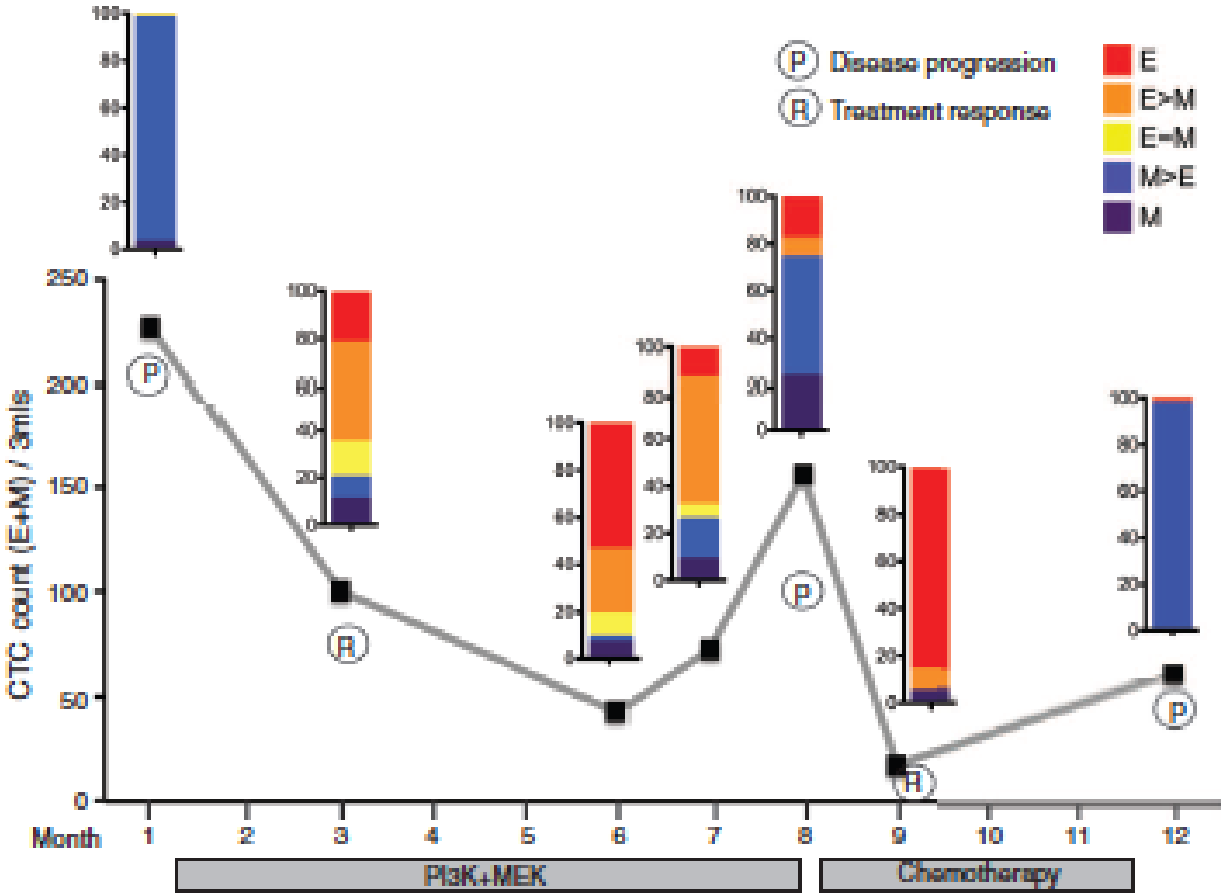
Yu et al, Science 2014

CTCs Exhibit Dynamic Changes in EMT Composition

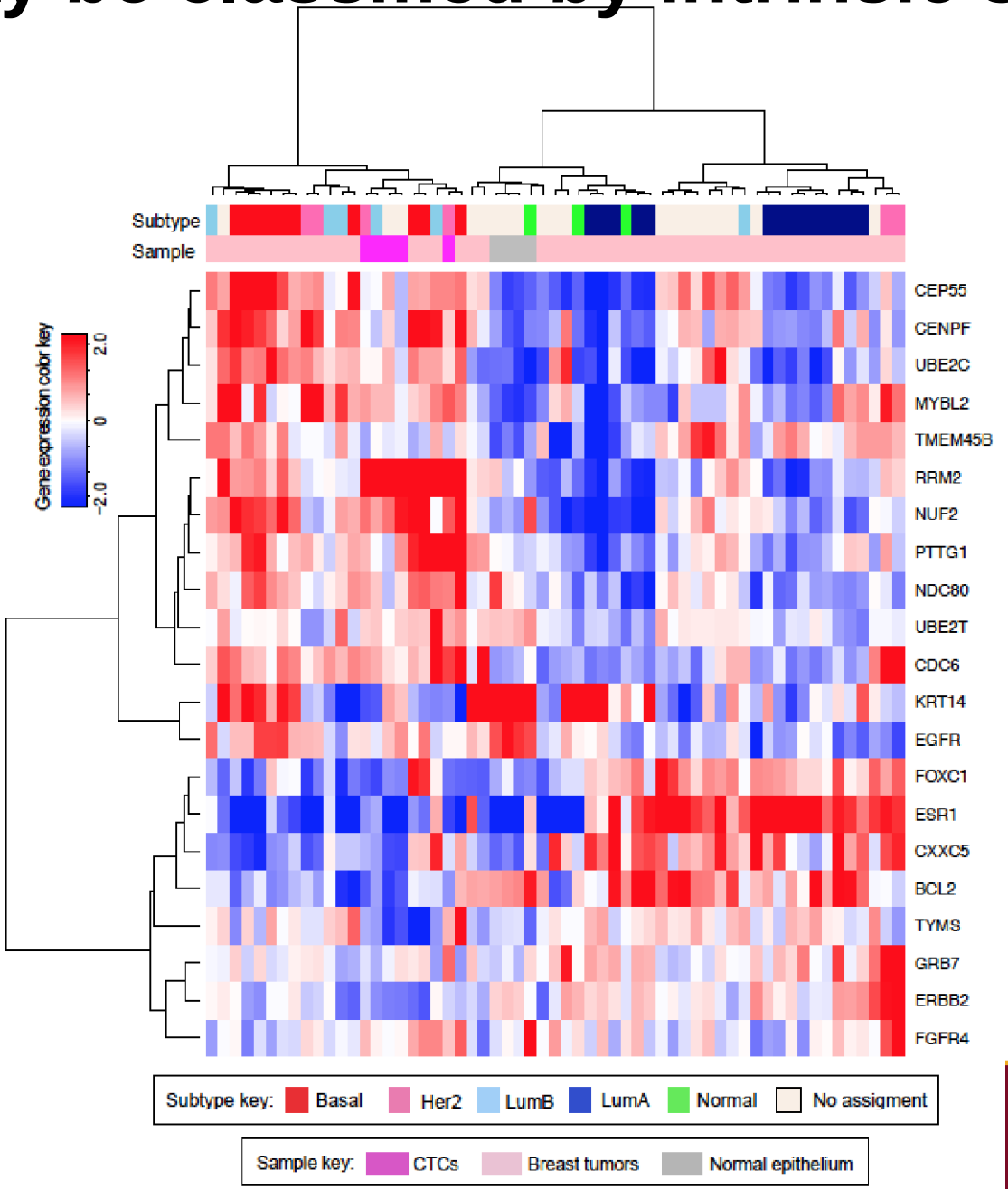


CTC as a liquid biopsy: N of one design

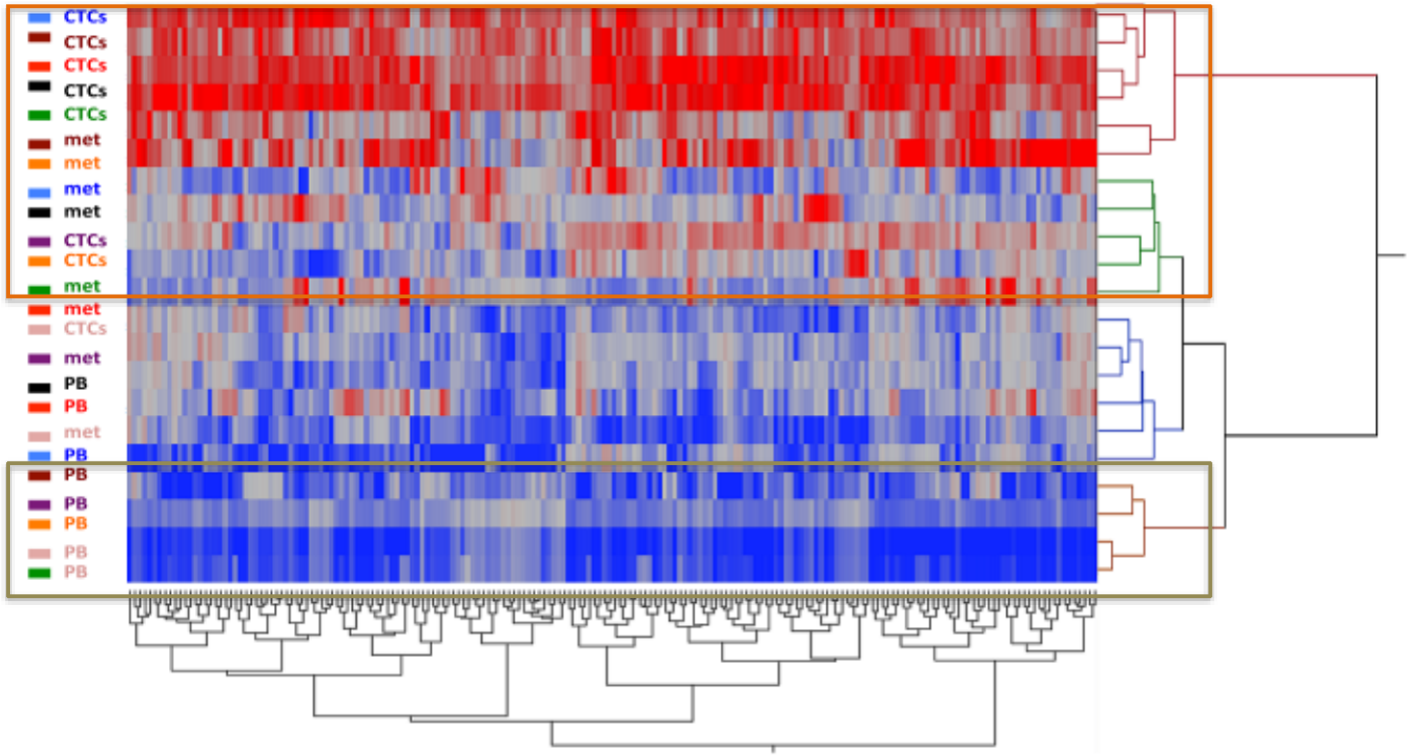


Yu et al, Science 2013

CTCs may be classified by intrinsic subtype



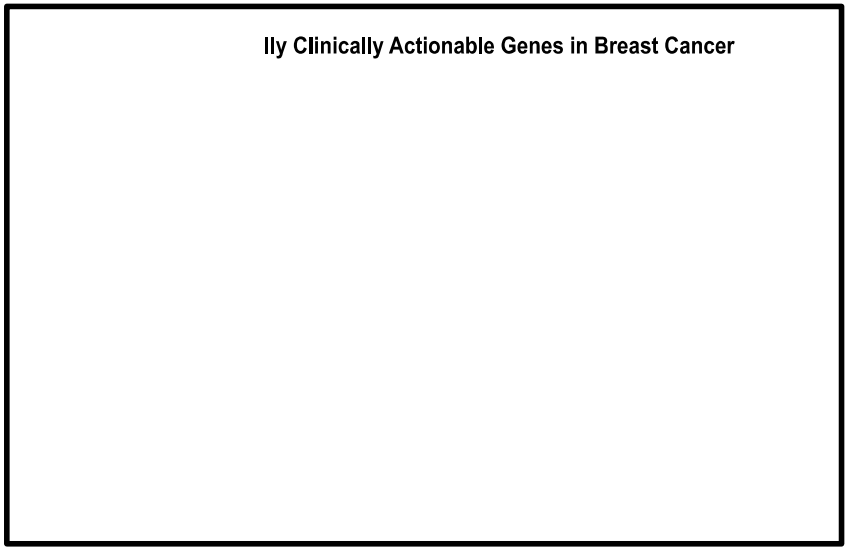
Lang et al, Breast Cancer Research and Treatment 2015



!Hierarchical clustering of 192 differentially expressed genes in CTCs, Mets and PB.

sample	Histology	primary biomarkers	site of met	met biomarkers
79412	IDC	ER/PR+,HER2-	pericardial effusion	ER/PR+,HER2-
79556	IDC	triple negative	pleural effusion	ER-, PR/HER2 unknown
79555	IDC	triple negative	pleural effusion	triple negative
78536	ILC	triple negative	bone	triple negative
38978	ILC	ER+,PR/HER2-	LN	ER+,PR/HER2-
68185	IDC	triple negative	cerebrospinal fluid	unknown
80540	IDC	triple negative	skin	unknown
81103	IDC	ER/HER+,PR-	pleural effusion	unknown

CTCs may be used as a liquid biopsy predicting response to targeted therapies



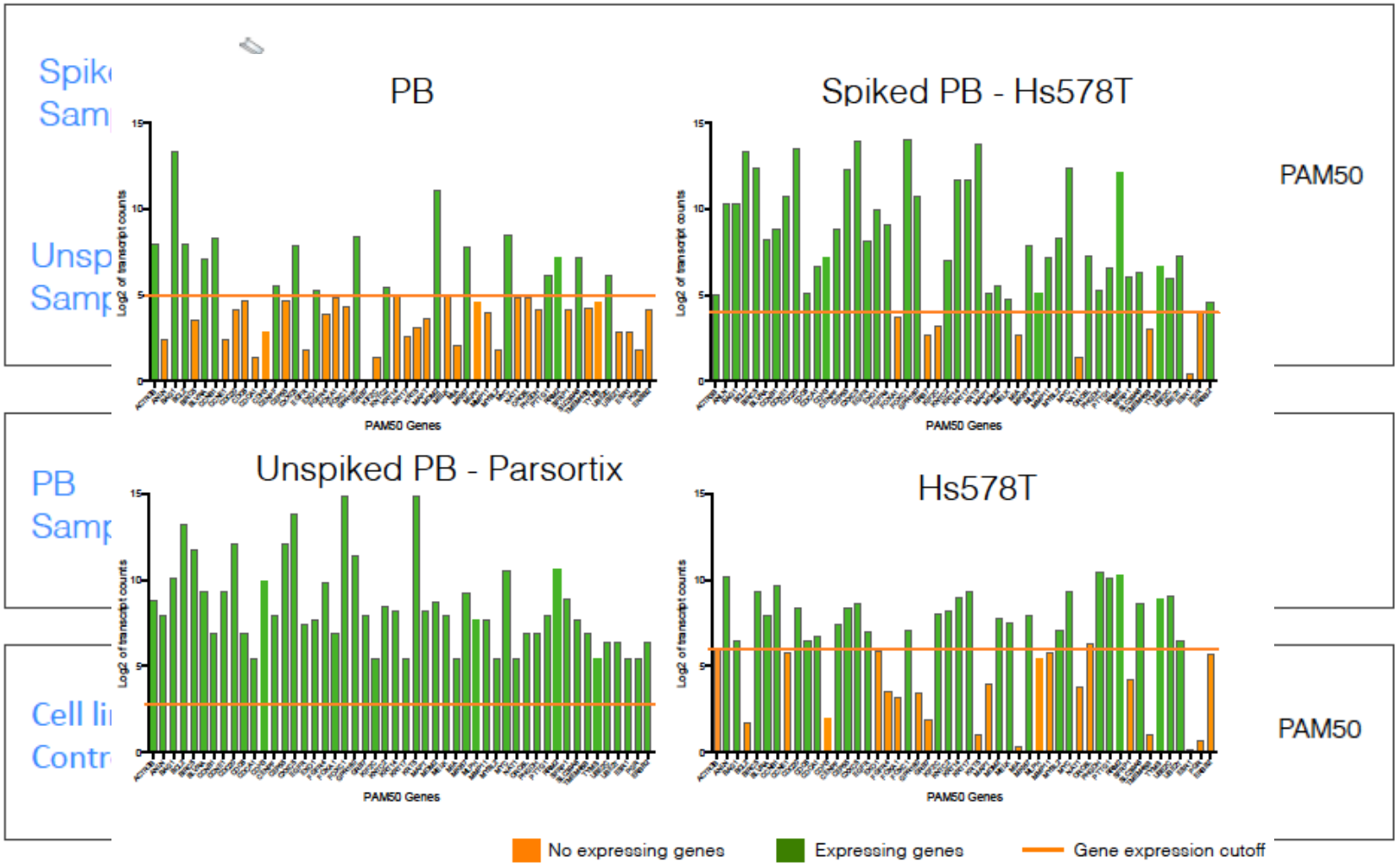
WNT/NOTCH/Hedgehog inhibitors/FGF inhibitors/ MET summary

Ring et al, AACR 2016

Potential Disadvantage of CTCs

- Rare ‘events’ – isolation technically challenging, profiling may be more costly if necessary to also profile blood background
- Sampling bias of captured cells – affinity based, size based selection
- Single-cell/ low cell number sequencing challenging (heterogeneity observed could be biological or technical bias)

A Word of Caution about Amplification Bias for Both CTCs and ctDNA Assays



Optimizing for advantages of both CTCs and ctDNA as companion biomarkers

- CTCs may prove to be better at discovering novel targets and frequency of multiple known targets in multi-institutional cohort studies
- ctDNA of defined targets may be useful in clinical trials, but when resistance emerges it may be necessary to utilize CTCs as a functional assay
- Comparison of both to tissue biopsies in relation to therapeutic response is a critical issue to be addressed to move the field forward

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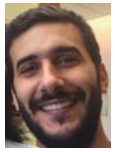

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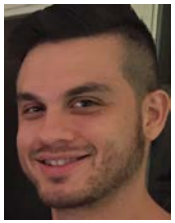


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