National Cancer Treatment Alliance™

Best Practices When Choosing Tissue or Liquid Biopsy Specimens For Biomarker Testing in Advanced Breast Cancer

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Biomarker Testing for Early-Stage, Advanced & Recurrent Breast Cancer: Background

Molecular Diagnostics in Clinical Oncology

Hereditary Cancer Risk Testing

Applications

- Identification of patients at risk for developing cancer
- Familial Risk
- May inform therapy decisions (e.g. *BRCA*m)

Assay Highlights

- Germline DNA-based Single Gene Tests, Multi-Gene Panels, WES, or WGS
- Germline RNA: Splice Variants

Screening & Early Detection Testing

Applications

- Early Cancer Detection
- Screening

Assay Highlights

- Liquid Biopsy Tests in Development
- ctDNA-based panels
- CTCs or Circulating Proteins

Testing for Early-Stage Cancer

Applications

- Prognosis & Risk Stratification
- Therapy Selection (e.g. *BRCA*m)
- Minimal Residual Disease

Assay Highlights

- ER / HER2 IHC
- Multi-Gene Expression Panel
- Liquid (ctDNA) Biopsy for MRD or Monitoring under Investigation

Testing for Advanced-Stage Cancer

Applications

- Tumor Genomic Profiling
- Targeted Therapy Selection
- Immunotherapy Selection
- Therapy Monitoring

Assay Highlights

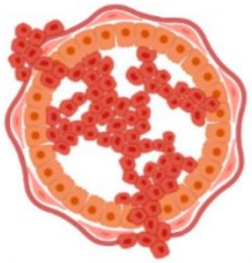
- Somatic Tissue & Liquid (ctDNA) Biopsy
- Testing to evaluate DNA, RNA, Proteins, or CTCs
- Single-gene, Hotspot, CGP

WES=whole-exome sequencing; WGS=whole-genome sequencing; CTCs= circulating tumor cells; ctDNA=circulating tumor DNA; CGP=Comprehensive Genomic Profiling

Adapted from Sokolenko AP, Imyanitov EN.Front Mol Biosci. 2018 Aug 27;5:76

Breast Cancer Biomarkers Vary Across Stage, By Subtype, & Line of Therapy

New Diagnosis: Early Stage 1-3 Invasive Carcinoma



- ER / HER2 testing
- Multi-Gene Expression panels
- Hereditary Cancer Risk Testing (e.g. *BRCA*m)
- Overall treatment pathway determined

Post Neoadjuvant Therapy

- **Complete Response** → Continue with standard therapy
- **Residual Disease** → Additional therapies offered

Metastatic Progression

Repeat ER & HER2 Testing

ER+
- First Line: Endocrine therapy + CDK4/6i
- Second Line: ***PIK3CA***, ***AKT1***, ***PTEN***, ***ESR1*** mutation-driven targeted therapy options

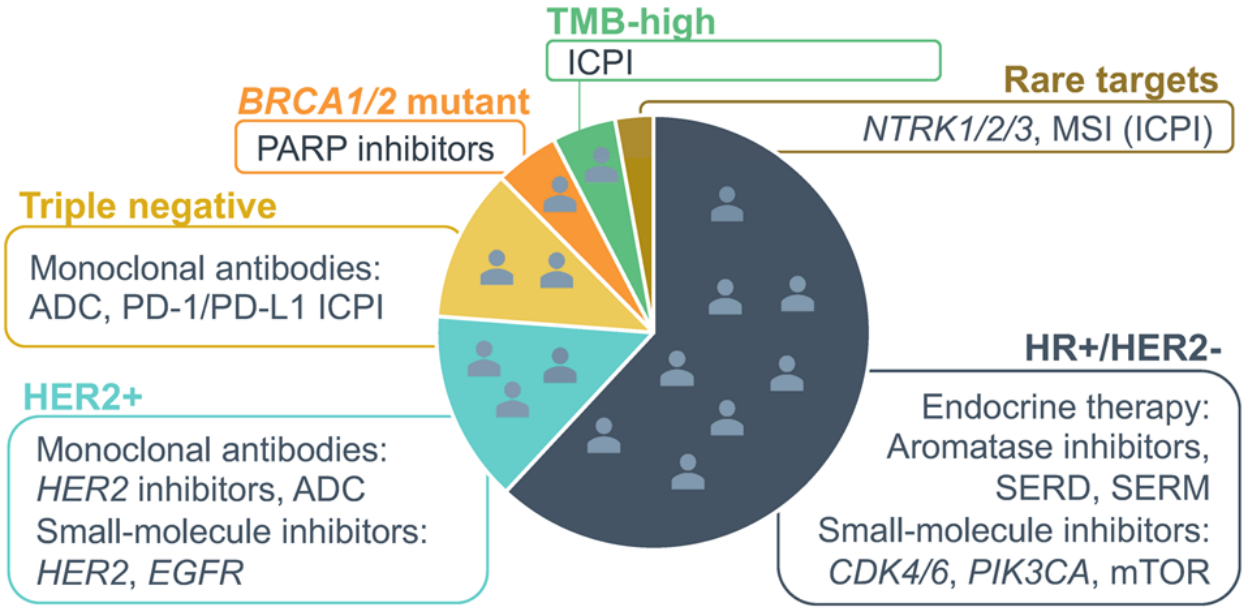
HER2+
- Various HER2 antibody + chemotherapy
- Antibody drug conjugate (ADC) therapy

HR-/HER2-
- First Line: PD-L1 (CPS 10+) status drives Chemo/Immunotherapy decisions
- Second Line: ADCs, chemotherapy, or other targeted agents
- Third Line: Biomarker positive (i.e, MSI, *NTRK*, *RET*, TMB)

ALL SUBTYPES
- ***BRCA1/2***, ***PALB2*** (germline) PARPi
- Rare Fusions ***NTRK*** or ***RET*** inhibitors
- TMB-H or MSI-H Immunotherapy

Najjar, S., Allison, K.H. Virchows Arch 480, 163-176 (2022).
N. Lynn Henry et al. Biomarkers for Systemic Therapy in Metastatic Breast Cancer: ASCO Guideline Update. JCO 40, 3205-3221(2022).

Targeted Therapy Options For Patients Living with Advanced/Metastatic Breast Cancer Continues to Grow



**ESR1* resistance mutations from ET exposure found increasingly over time

Key Biomarkers With Drug Approvals:

- *AKT1*
- *BRCA1/2*
- *FGFR1-3*
- *PALB2*
- PD-L1
- *PIK3CA*
- *PTEN*
- *ESR1**

Rare Biomarkers :

- *NTRK*
- MSI-H
- TMB-H
- *RET*

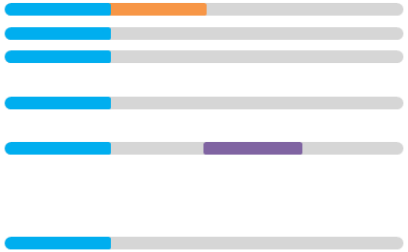
SERD=Selective Estrogen Receptor Degrader, SERM=Selective Estrogen Receptor Modulator, TMB=tumor mutational burden, MSI=microsatellite instability, ICPI=immune checkpoint inhibitor, ADC=antibody-drug conjugate. Sivakumar S, et al. Nat Commun. 2022 Dec 5;13(1):7495.

Broad Range of Molecular Diagnostic Approaches To Determine Biomarker Status & Targeted Therapy Selection

Single Marker Testing

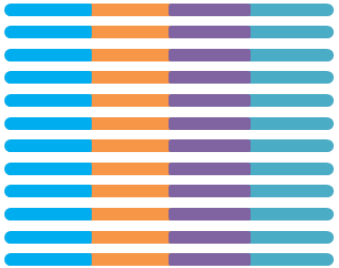
- Identifies mutations in a single gene using conventional approaches like FISH, PCR, or IHC[1]
- Would miss clinically relevant mutations in other genes[2,3]

Hotspot Panel



- Interrogates specific mutations in select genes[4,5]
- Would miss other clinically relevant classes of alterations and mutations in other genes[2,4-7]

Comprehensive Genomic Profiling



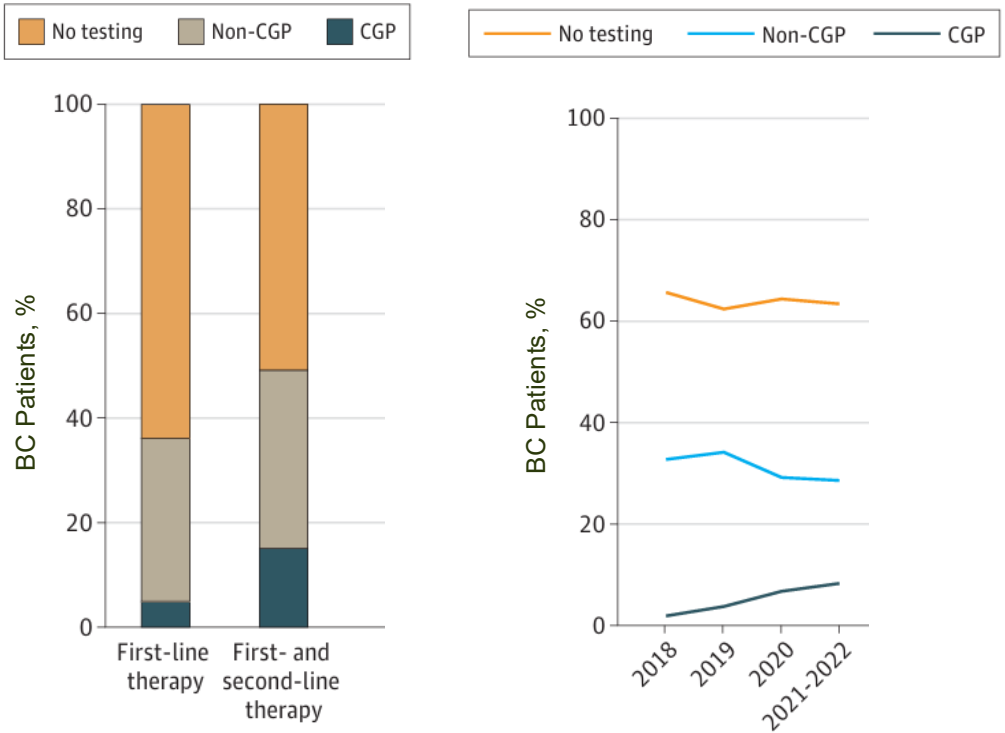
Analyzes all four types of genomic mutations across a large panel of cancer-related genes and genomic signatures (ie, TMB, MSI)[8,9]

Base substitutions | Insertions and deletions | Rearrangements | Copy number alterations | No genomic alterations detected

CGP=comprehensive genomic profiling; FISH=fluorescence in situ hybridization; IHC=immunohistochemistry; MSI=microsatellite instability; PCR=polymerase chain reaction; TMB=tumor mutational burden

1. Malone ER, Oliva M, Sabatini PJB, Stockley TL Siu LL. Genome Med. 2020;12:8; 2. Reitsma M, Fox J, Borre PV, et al. J Manag Care Spec Pharm. 2019;25(5):601-611; 3. Ali SM, Hensing T, Schrock AB, et al. *Oncologist*. 2016;21:762-770; 4. Singh RR, Patel KP, Routbort MJ, et al. J Mol Diagn. 2013;15(5):607-622; 5. Kopetz S, Mills Shaw KR, Lee JJ, et al. JCO Precis Oncol. 2019;3:PO.18.00213; 6. Zehir A, Benayed R, Shah RH, et al. Nat Med. 2017;23(6):703-713; 7. Drilon A, Wang L, Arcila ME, et al. Clin Cancer Res. 2015;21(16):3631-3639; 8. Kroeze LI, de Voer RM, Kamping EJ, et al. J Mol Diagn. 2020;22(6):757-769; 9. Pestinger V, Smith M, Sillo T, et al. Mol Diagn Ther. 2020;24(3):339-349

Real-World Biomarker Testing Rates From Commercial Health Plans and Medicare Advantage Claims Data



What does real-world data tell us about biomarker testing rates in women with advanced/metastatic BC?

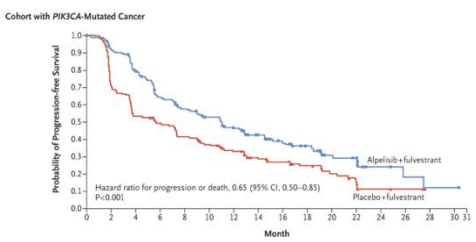
- Biomarker testing rates in mBC patients increase after first-line therapy.
- Rates of CGP testing go up after first-line therapy and has increased in utilization over the past 5 years.
- **Although biomarker testing rates overall are increasing, they remain suboptimal**. For many patients, there was no evidence of molecular testing performed.

CGP=Comprehensive Genomic Profiling

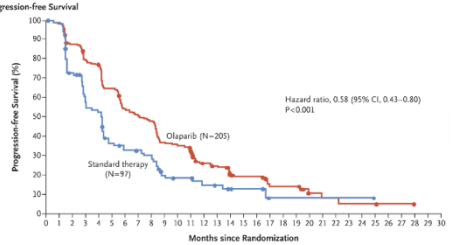
DaCosta Byfield S, et al. JAMA Netw Open. 2025 Jul 1;8(7):e2519963.

Improved Survival in mBC Patients Receiving Genomically-Matched Targeted Therapies

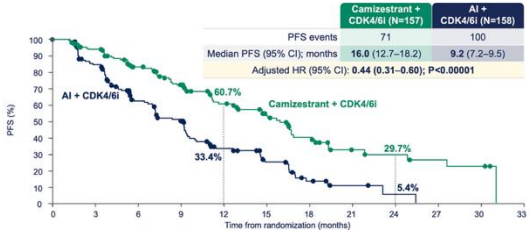
SOLAR-1 — *PIK3CA*



OlympiAD — *gBRCA1/2*



SERENA-6 — *ESR1*



- Three different trials demonstrate the value of biomarker testing and matched targeted therapy
- Three different molecular tests performed: **Tissue Biopsy**, **Germline Testing**, **Liquid Biopsy**
- **Q: What type of molecular test should be performed and when?**

Andre F, et al. N Engl J Med. 2019 May 16;380(20):1929-1940. Robson M, et al. N Engl J Med. 2017 Aug 10;377(6):523-533. Bidard FC, et al. N Engl J Med. 2025 Aug 7;393(6):569-580.

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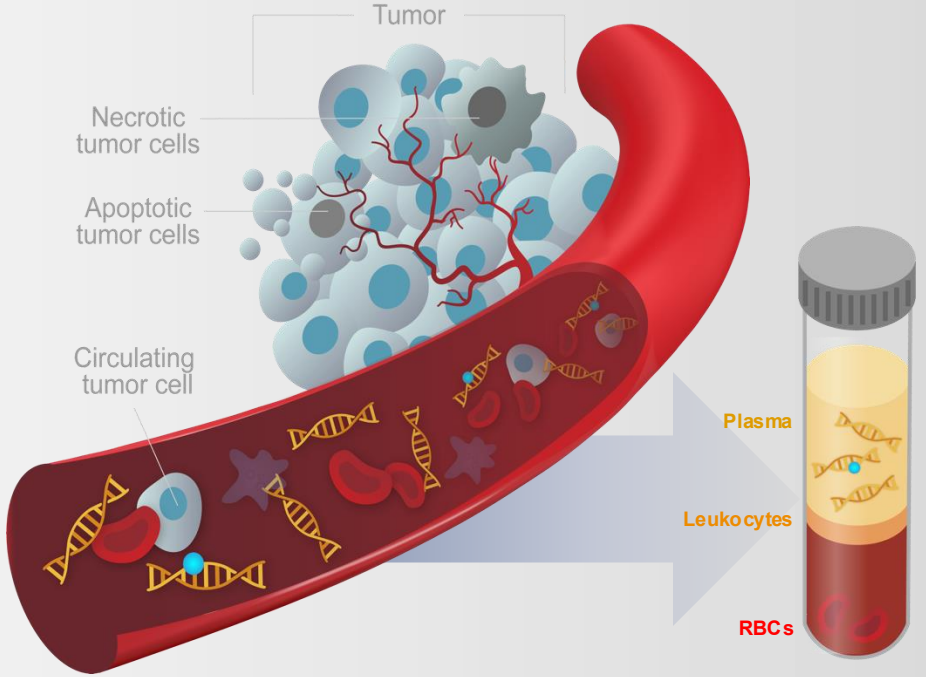
Clinical utility of tissue & liquid biopsy specimens

Biomarker Testing for Advanced/Metastatic Breast Cancer

Background: Circulating Tumor DNA (ctDNA)

Cell-free DNA (cfDNA) are small DNA fragments that are shed into the circulation via necrosis or apoptosis. cfDNA is found in both healthy individuals and those with cancer

Circulating tumor DNA (ctDNA) is the fraction of circulating DNA coming from the tumor

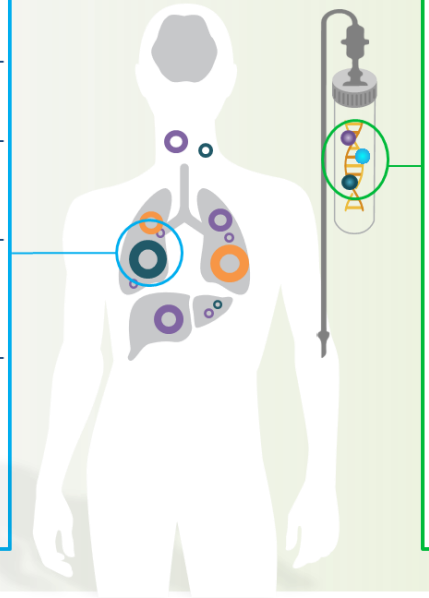


Elazezy M, Joosse SA. Comput Struct Biotechnol J. 2018;16:370-378

Key Factors Influencing ctDNA Levels and Detection

Disease characteristics

- Tumor type
- Tumor burden and staging
- Disease status (stable vs progressive disease)
- Tumor microenvironment (stroma and vascularization)



Other factors

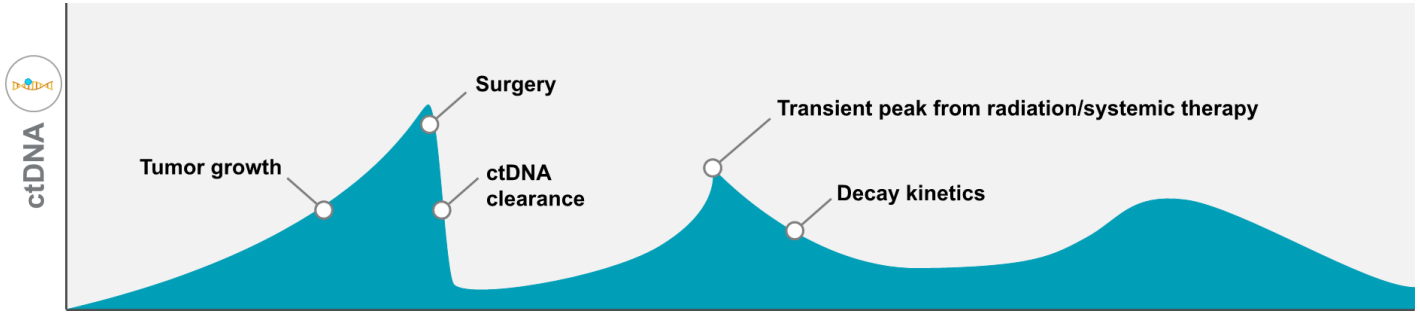
- Sample timing related to treatment or procedures
- Sample acquisition, transport, and processing procedures
- Type of variant
- Assay sensitivity

Not all patients may be candidates for liquid biopsy at a given time.

ctDNA=circulating tumor DNA

Vivancos A, Tabernero J. Nat Med. 2022;28(11):2255-2256

Timing of Blood Sampling & ctDNA Dynamics in Relation to Treatment



Surgery	Radiation	Systemic therapy
Substantial increases in ctDNA have been observed following the clearance of ctDNA	Patients exhibit a transient peak in ctDNA fraction in the days following initiation of radiotherapy, followed by a steady decline over the course of treatment, measurable by weeks 2 and 3	ctDNA fraction typically declines within the first few days of treatment regardless of clinical response

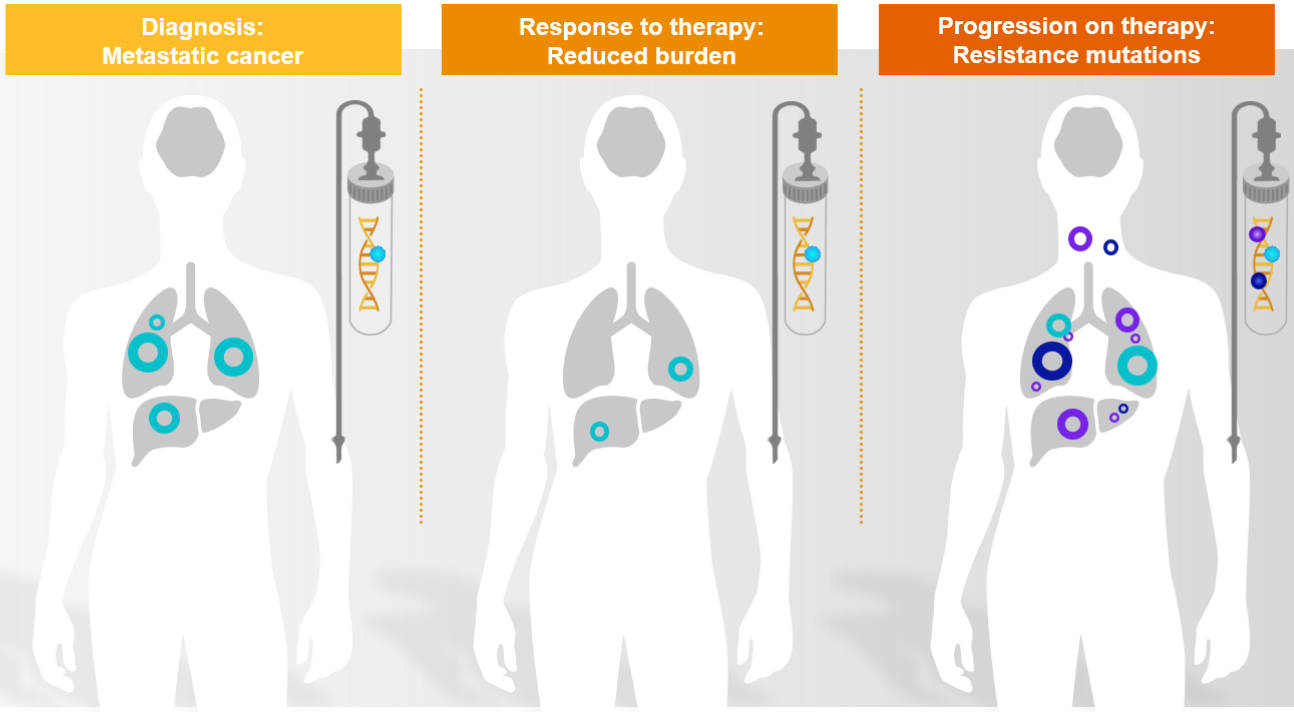
If biomarker testing from liquid biopsy is needed to determine the **next line of therapy**, consider sampling at ***time of progression*** to maximize the amount of ctDNA present in specimen.

ctDNA=circulating tumor DNA

Sanz-Garcia E, Zhao E, Bratman SV, Siu LL. *Sci Adv*. 2022;8(4):eabi8618

Liquid Biopsies Can Capture Intratumoral & Intertumoral Heterogeneity

ctDNA from blood can represent clones from a single primary tumor, as well as multiple metastatic sites



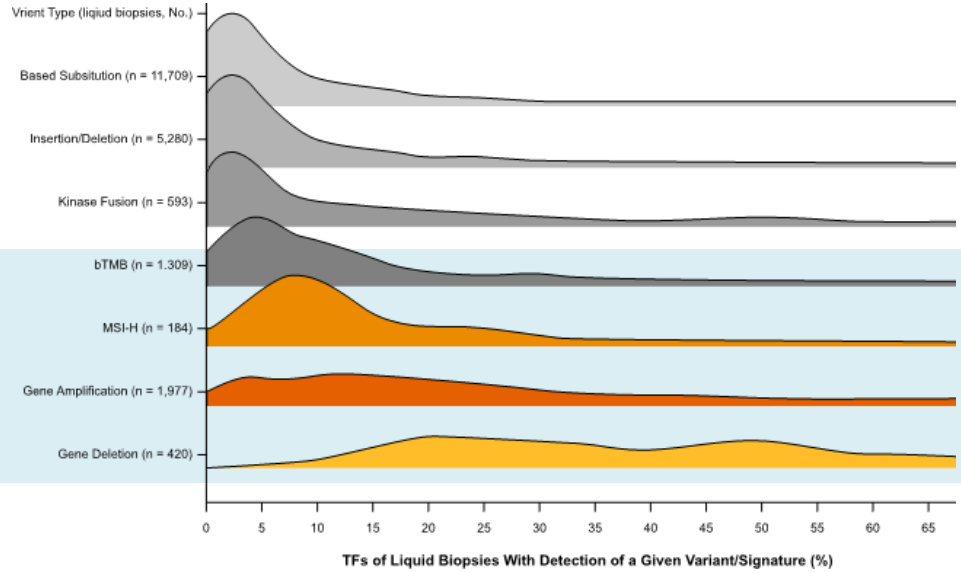
ctDNA=circulating tumor DNA

Rapisuwon S, Vietsch EE, Wellstein A. Comput Struct Biotechnol J. 2016;14:211-222

Biomarker Sensitivity and Specificity are Higher in Samples with a High ctDNA Fraction

Genomic biomarkers, such as MSI-H, TMB-H, and CNVs, require a higher ctDNA fraction for detection.

Making interpretations on CNVs like *PTEN* loss can be challenging in samples with a low ctDNA fraction.

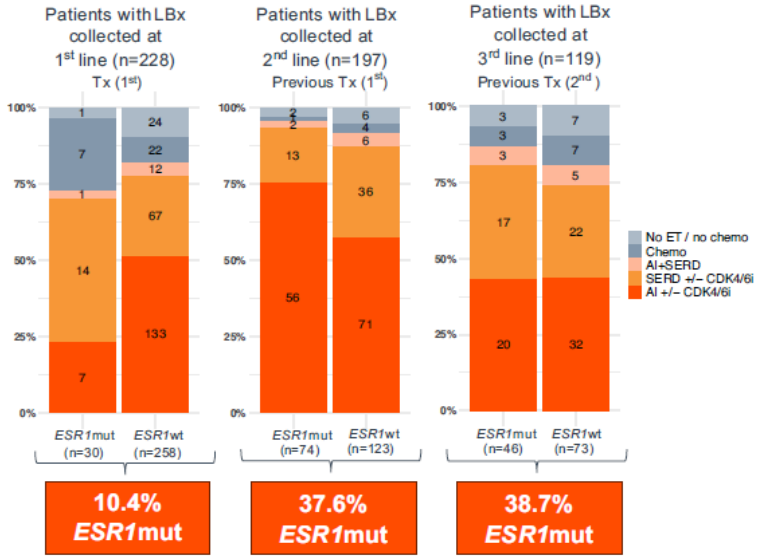


✓ Reflexing to a recent tissue biopsy should be considered to confirm negative findings, especially if the ctDNA fraction is low.

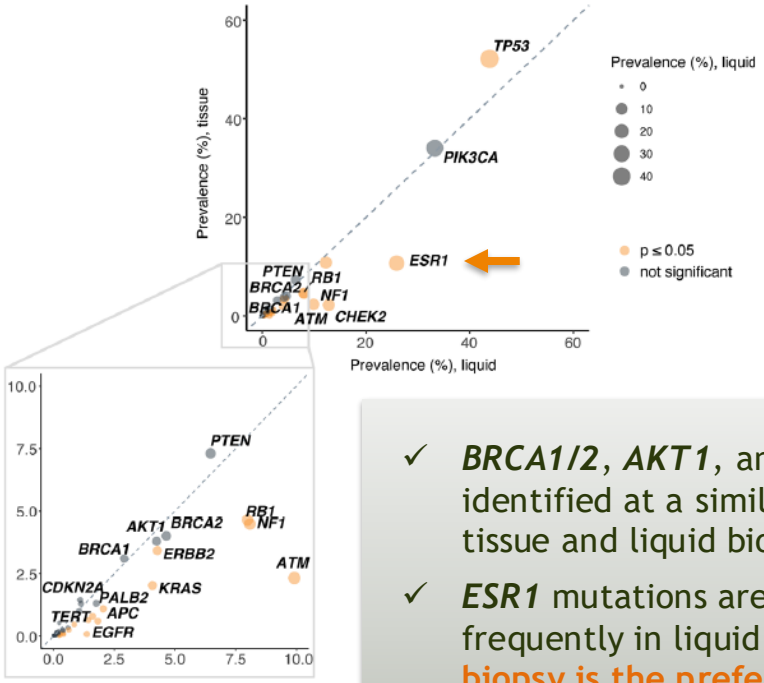
bTMB=blood tumor mutational burden; CNV=copy number variation; ctDNA=circulating tumor DNA; MSI-H=microsatellite instability-high; TF=tumor fraction

Husain H, Pavlick DC, Fendler BJ, et al. JCO Precis Oncol. 2022;6:e2200261

Liquid Biopsy is Uniquely Positioned to Detect ESR1 mutations Related to Therapy Resistance



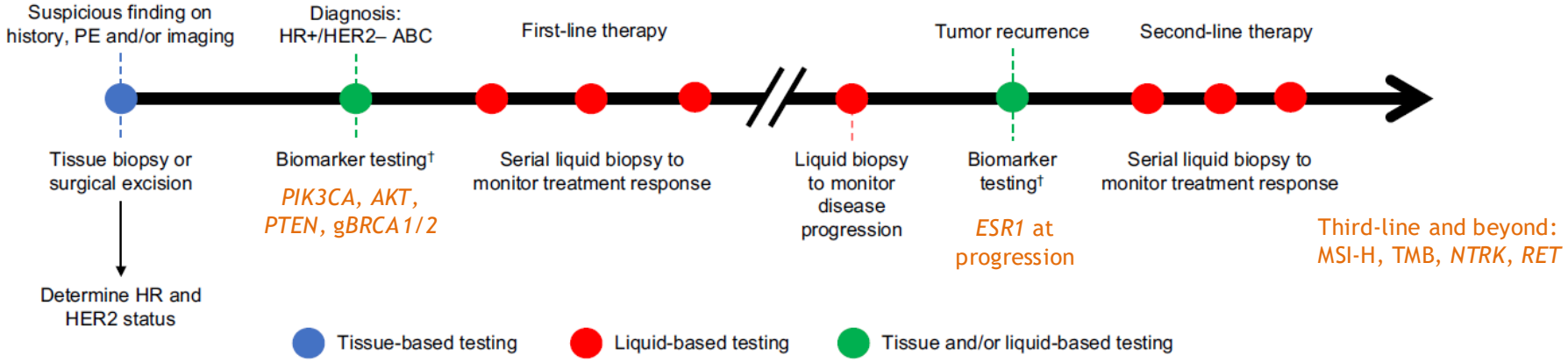
ESR1 resistance mutations are increasingly found in patients treated with second- and third-line therapy.



- ✓ ***BRCA1/2***, ***AKT1***, and ***PIK3CA*** are identified at a similar rate in both tissue and liquid biopsy.
- ✓ ***ESR1*** mutations are found more frequently in liquid biopsy. Liquid biopsy is the preferred method for ESR1 detection.

Sivakumar S, et al. Nat Commun. 2022 Dec 5;13(1):7495.
Bhave MA, et al. Breast Cancer Res Treat. 2024 Oct;207(3):599-609.

Liquid and Tissue Biopsy: Opportunities for Biomarker Testing Along Key Clinical Timepoints in Advanced Breast Cancer



Key Challenges Remain:

- Lack of consensus around optimal biomarker testing methods
- Variation in testing protocols and sampling timepoints
- What do we do with conflicting or ambiguous results?
- Will testing and therapy be covered/reimbursed?

ABC, advanced breast cancer; dMMR, mismatch repair deficient; HER2, human epidermal growth factor receptor 2; HR, hormone receptor; MSI-H, microsatellite instability-high; PE, physical examination; *PIK3CA*, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha. †Other biomarkers: *BRCA1/BRCA2*, *NTRK* fusion, MSI-H/dMMR
Toppmeyer DL, Press MF. Cancer Med. 2020 Sep;9(18):6463-6472.

Summary

- **Biomarker testing is critical to identify patients with advanced breast cancer that may benefit from targeted therapy.**
  - **Key Trials**: SOLAR-1 (*PIK3CA*), CAPItello-291 (*PIK3CA, AKT1, PTEN*), OlympiAD & EMBRACA (*BRCA1/2*), SERENA-6 & EMERALD (*ESR1),* STARTK-1 & TRIDENT-1 *(NTRK),* LIBRETTO-001 *(RET), KEYNOTE-158 (TMB).*
- **Molecular testing rates have increased in the past several years, but rates remain suboptimal for targeted therapy biomarkers beyond ER/HER2.**
- **Molecular profiling from liquid and tissue biopsy come with unique benefits and limitations and can compliment each other by tailoring to the clinical need.**
  - Tissue biopsy is generally more sensitive to detect copy number variants and genomic signatures
  - Liquid biopsy is uniquely positioned to pick up resistance markers such as *ESR1* at a higher rate, but reflex to tissue should be considered when the ctDNA fraction is low.
- **Key challenges remain around optimal testing methods and sampling timepoints, reimbursement for testing and targeted therapy.**

Footnote

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Coverage & Reimbursement

Biomarker Testing for Advanced Solid Tumors

- John Fox, MD, MHA – Senior Medical Director, Illumina

Companion Diagnostics (CDx) Claims

- ☑ are FDA approved by **demonstrating analytical and clinical validity** of the test through retrospective or prospective analyses, to accurately identify patients eligible to receive a targeted therapy for a defined biomarker

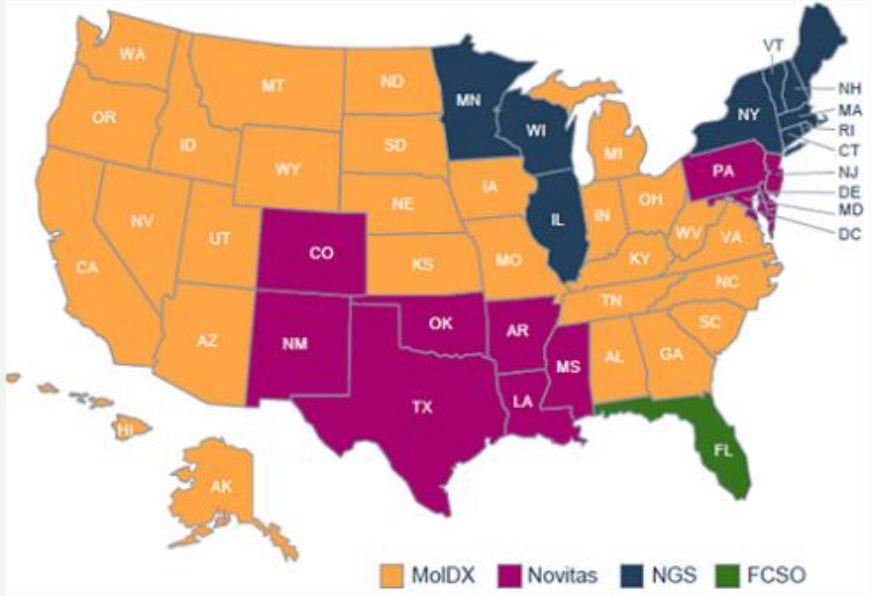
- ☑ are supported by **analytical validity** of the test for each specific biomarker **and a clinical study** establishing either the **link between the result of that specific biomarkers in the test and patient outcomes or clinical concordance to a previously approved CDx test**

National Coverage Determination (NCD) 90.2 in a Nutshell

This National Coverage Determination (NCD) is only applicable to diagnostic lab tests using NGS for somatic (acquired) and germline (inherited) cancer.

For somatic testing:

- Only relapsed, refractory, recurrent, metastatic, advance stage III or IV cancer
- Only panels predictive of drug response—no diagnostic or prognostic applications
- Requires FDA approval or clearance as a CDx
- Exclusive to NGS-based DNA panels—FISH, IHC, PCR excluded
- Is agnostic to liquid versus tissue; allows both since they are different tests
- MACs decide on NGS for RNA-based tests, lab-developed tests, and MRD



Current states participating in the MolDX Program

Payer	Comprehensive genomic profiling coverage for tissue	Coverage for liquid	Allows for concurrent testing	Allows for repeat testing with liquid
United Healthcare	CDx only	CDx only	Yes (lung/breast)	up to 3 times a year
Anthem	CMP for selected tumors	CDx only for NSCLC, Breast, Prostate	No	Yes
Aetna	TMB only	Panels <50 genes for NSCLC	No	No
Cigna	CMP for selected tumors	CDx only	No	No
HCSC (TX, IL, NM, MT, OK)	CMP for selected tumors	CDx only for NSCLC, Breast, Prostate	No	Yes-
BCBSM	CDx only for select tumors	CDx only	silent	silent
Florida Blue	CDx only	CDx only	No	No
Horizon BCBS	CMP for selected tumors	CDx only	No	No
BS of CA	No limitations	covered for certain tumor types	Yes for lung only	silent

Commercial Coverage Policy Examples

FDA Companion Diagnostic Testing medical coverage policy
CDx tests are proven and medically necessary when the oncology indication has a corresponding diagnostic test and biomarker on the US FDA list of cleared or approved Companion diagnostic devices. (Agnostic to tissue or liquid.)

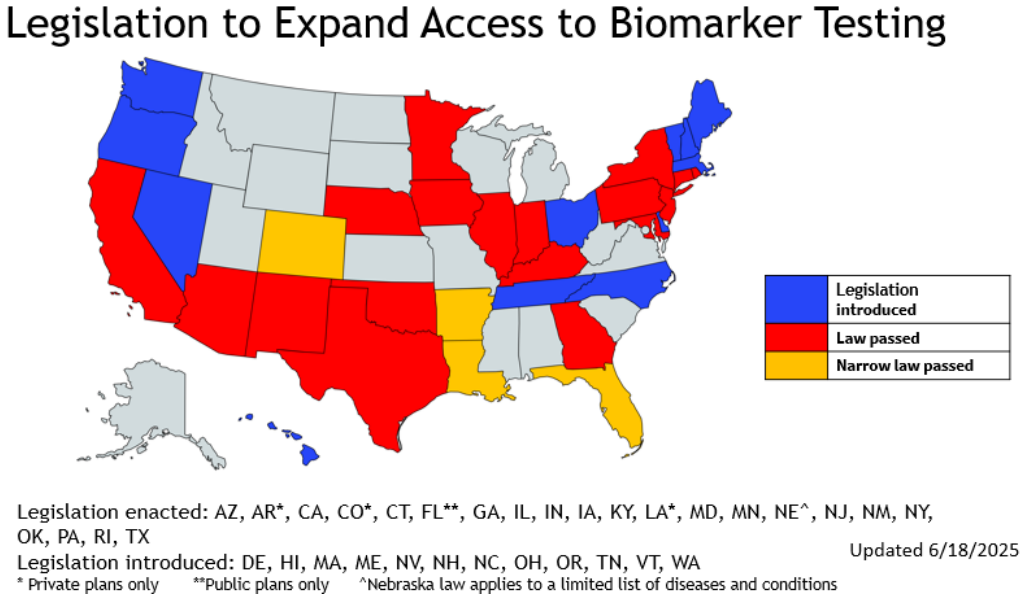
Tumor Markers medical coverage policy
Circulating tumor DNA (ctDNA) (a type of liquid biopsy) for any indication (other than small panels, less than 50 genes, for non-small cell lung cancer), including, but not limited to, colorectal cancer, melanoma, ovarian cancer or prostate cancer are **considered experimental and investigational.**

Footnote

ACS/CAN Biomarker Testing Bill Coverage in the US

Biomarker testing must be covered when the test is supported by medical and scientific evidence, including, but not limited to:

- Labeled indications for an FDA-approved or -cleared test;
- Indicated tests for an FDA-approved drug;
- Warnings and precautions on FDA-approved drug labels;
- CMS National Coverage or Medicare Administrative Contractor (MAC) Local Coverage of a test; or
- Nationally recognized clinical practice guidelines (and consensus statements).



Coverage and Reimbursement Summary

- Coverage is not universal, lots of heterogeneity in coverage of both tissue- and blood-based sequencing
- Ensure test is covered and prior authorized to avoid treatment delays and patient bills
- National efforts to ensure coverage for LDTs and comprehensive liquid and tissue sequencing ongoing

Footnote

National Cancer Treatment Alliance™

Q&A

Thank you for attending!