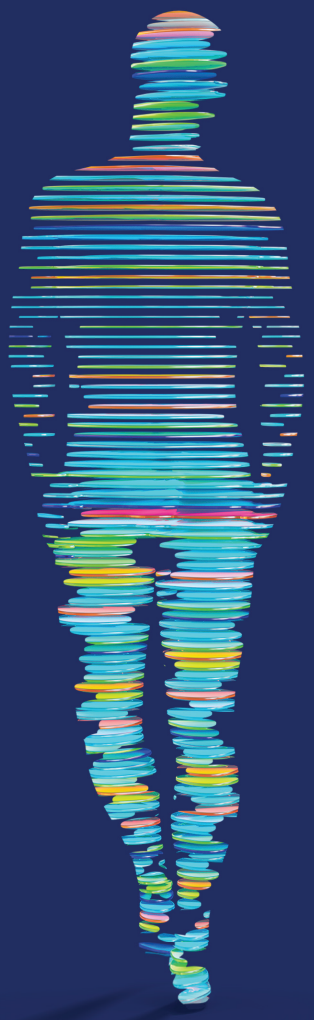
Biomarker Testing in Breast Cancer

An Essential Component of the Treatment Decision Making Process



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BREAST CANCER OVERVIEW

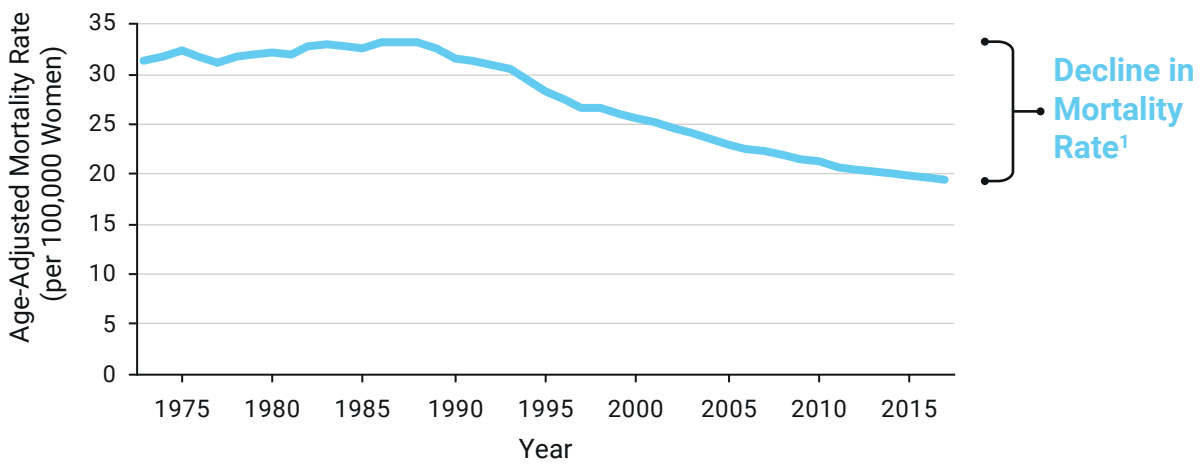
Breast cancer is the **second leading cause** of cancer-related death in women[1]

About **1 in 8** women will **develop breast cancer**[1]

6% of patients will have distant or **metastatic disease by the time of diagnosis**[1]

The expected **5-year survival rate** for women with **metastatic disease** is **29%**[2]

Breast Cancer Mortality Among Women in the United States, 1975-2019[2]



Annual declines in mortality are attributable to earlier diagnosis because of better awareness and mammography screening, as well as to improvements in treatment[1]

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BREAST CANCER SUBTYPES

Surrogate intrinsic subtypes of breast cancer have **key biomarkers**[3]

Surrogate Intrinsic Subtypes[3-5]

	ER	PR	HER2	Ki67	Prognosis	Prevalence
Luminal A-like	+ (high)	+ (high)	–	Low	Good	60-70%
Luminal B-like HER2-negative	+ (low)	+ (low)	–	High	Intermediate	10-20%
HER2-enriched (non-luminal)	–	–	+	High	Intermediate	13-15%
Luminal B-like HER2-positive	+ (low)	+ (low)	+	High	Intermediate	
Triple Negative Breast Cancer (TNBC)	–	–	–	High	Poor	10-15%

- **Prognostic biomarkers** provide information about likely disease course[6]
- In breast cancer, some prognostic biomarkers are also **predictive biomarkers**, which identify patients most likely to benefit from a specific therapy[7-9]

Prognostic and predictive biomarkers continue to evolve, as more are being discovered and several biomarker-specific therapies are under investigation[10,11]

ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; PR, progesterone receptor; TNBC, triple-negative breast cancer.

Based on Literature Review, Prognostic Biomarkers in mBC May Include:

Gene Expression[7,12,a]
(of a defined set of genes)

Expression of specific genes (eg, 21 genes for oncotype) can forecast risk of recurrence, which informs the use of adjuvant chemotherapy

Ki67[13,b]

High Ki67 expression following neoadjuvant therapy correlates with a poor prognosis and may inform the type of adjuvant therapy following surgery

PIK3CA[8,14,15,b]

Prognosis of patients with mBC harboring *PIK3CA* mutations dependent on the breast cancer subtype

- In patients with HR+/HER2- disease, *PIK3CA* mutations are associated with reduced sensitivity to HER2-directed therapy, chemotherapies, and endocrine resistance[15-17]

PD-L1[9,b]

PD-L1 expression may be associated with a poor prognosis

Sites of metastases[18]

Patients with brain metastases or patients with multiple metastatic sites have shorter survival than other patients

Prognostic and/or predictive biomarkers under investigation include:

MYC overexpression / amplification[19]	**CTCs following adjuvant therapy**[20-22]	**HRD**[23]
TIL density in patients with recurrent disease[20-22]	**ctDNA**[23]	**TROP2 expression**[23]
***ESR1* mutations**[20-22]	***PALB2***[23]	***FGFR1* alterations**[24]

CTC, circulating tumor cell; ctDNA, circulating tumor DNA; ESR1, estrogen receptor 1; FGFR1, fibroblast growth factor receptor 1; HR, hormone receptor; HRD, homologous recombination deficiency; mBC, metastatic breast cancer; PALB2, partner and localizer of BRCA2; PD-L1, programmed death-ligand 1; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; TIL, tumor-infiltrating lymphocyte.

[a]Gene expression assays provide prognostic and therapy-predictive information that complements T,N,M and biomarker information. Use of these assays is not required for staging. The 21-gene assay (Oncotype Dx) is preferred by the NCCN Breast Cancer Panel for prognosis and prediction of chemotherapy benefit. Other prognostic gene expression assays can provide prognostic information but the ability to predict chemotherapy benefit is unknown.

[b]The NCCN Breast Cancer Panel does not currently recommend assessment of Ki-67, PIK3CA, or PD-L1 for prognostic purposes.

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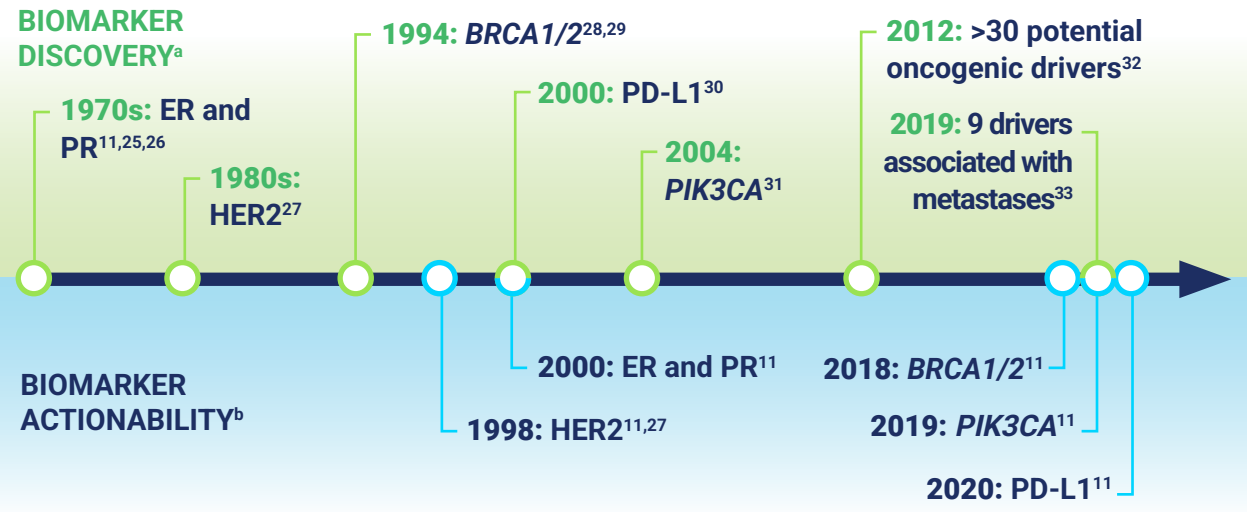
Evolution of Biomarkers in mBC: Discovery and Actionability



Biomarker testing is fundamental to the treatment of mBC and has been for >20 years[11,27]

Categorization of Select Biomarkers in Breast Cancer

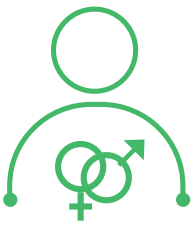
Biomarker	Prevalence (%)	Prognostic	Predictive
ER/PR[4,11,34]	70%[c]	X	X
HER2[4,11,35]	16.6%[d]	—	X
Ki67[13,36]	—	X	—
BRCA1/2[11,37]	5%	—	X
PD-L1[9,11,38]	20%[e]	X	X
PIK3CA[11,15,39]	36%	X	X

BRCA1/2, breast cancer gene 1/2; mBC, metastatic breast cancer.

[a]Discovery refers to the first association with breast cancer.
[b]Actionability is based on the first approval of a therapy for breast cancer defined by this biomarker.
[c]ER/PR positivity defined as >1%.
[d]HER2 negativity defined as IHC0/1+ or 2+ with a FISH amplification ratio of <2.0.
[e]PD-L1 positivity defined as ≥10% tumor cells or immune cells expressing PD-L1.

THE AMERICAN SOCIETY FOR CLINICAL ONCOLOGY (ASCO)[a] AND NATIONAL COMPREHENSIVE CANCER NETWORK® (NCCN®)[b] RECOMMEND TESTING ALL PATIENTS WITH mBC FOR BIOMARKERS[7,23,40]

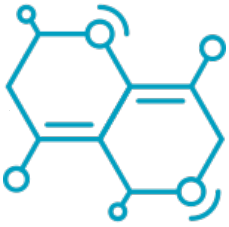
Patients to be tested



Initial diagnosis of stage IV disease

Recurrent breast cancer with stage IV disease

Actionable biomarkers
Category 1 in NCCN Guidelines[b]



Expression
- HR (ER/PR)
- PD-L1
- HER2

Genetic Alterations
- *PIK3CA*
- g*BRCA1/2*

Biomarker defined patient subsets

Subtype	Additional Biomarkers
HR-positive/HER2-negative	No actionable driver alterations
HR-positive/HER2-negative	***PIK3CA* mutation[c]**
HR-positive/HER2-positive	No actionable driver alterations
HR-negative/HER2-positive	No actionable driver alterations
TNBC	No actionable driver alterations
TNBC	**PD-L1 CPS >10**
Any subtype	***BRCA1/2* mutation**

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CPS, combined positivity score.

[a]Includes biomarkers that have a strong recommendation from ASCO only.
[b]Includes biomarkers associated with an NCCN® Category 1 therapy only. NCCN categories of evidence refer to the strength of the recommendation for a therapeutic intervention and are based on the panel vote. Category 1 is based on high-level evidence and represents uniform NCCN consensus that the intervention is appropriate.
[c]*PIK3CA* may be tested following progression.

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GUIDELINES FROM DIFFERENT PROFESSIONAL SOCIETIES IN BREAST CANCER

Guidelines have been issued to improve the use of valid biomarker tests with clinical utility in breast cancer[7,34,41,42]. Incorporating recent guidelines into testing procedures may impact patient care[34,41]

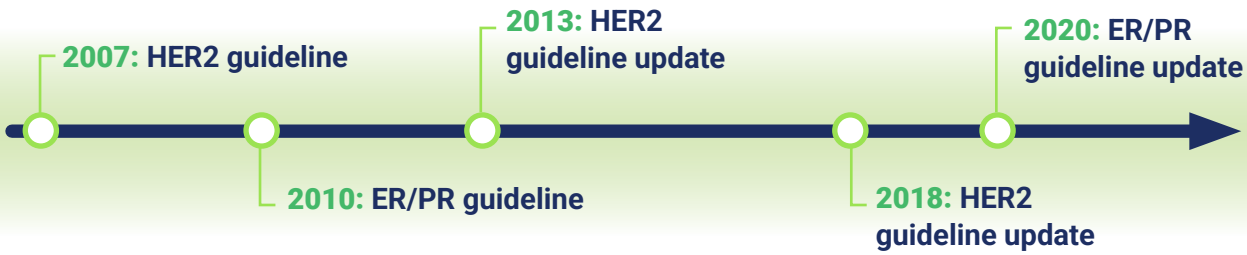
The NCCN issues evidence- and consensus-based guidelines that are updated continually with at least 1 update per year[7,42]

- NCCN Guidelines are consistently updated to include the most recent evidence that informs treatment decisions, including how to test for biomarkers that have recently become actionable[7,42]
  - Patients with mBC are not eligible for some therapies if they are not tested for the appropriate biomarker
- The NCCN recommends ASCO/CAP guidelines on HER2 and ER/PR biomarker testing[7,42]

ASCO and CAP issued evidence-based guidelines for HER2 and ER/PR testing, respectively[34,35,41,43]

- These guidelines were developed with experts in oncology, pathology, epidemiology, and statistics after extensive literature review and are updated periodically
- The introduction of guidelines on ER, PR, and HER2 testing led to increased test consistency among different laboratories[34,41]
  - Inaccurate ER, PR, and HER2 test results decreased by ≥25% after guideline introduction
- ASCO/CAP have not released guidelines on testing for *BRCA1/2*, *PIK3CA*, PD-L1 or Ki67 in breast cancer[44]

History of ASCO/CAP Guideline Release[34,41]



The NCCN and ASCO/CAP guidelines each provide important information on biomarker testing. Each has a place in molecular diagnostics for breast cancer[7,34,41]

Intended to depict biomarker testing methodologies. When testing for therapy selection, please consult product prescribing information and FDA-approved companion diagnostics.

CAP, College of American Pathologists.

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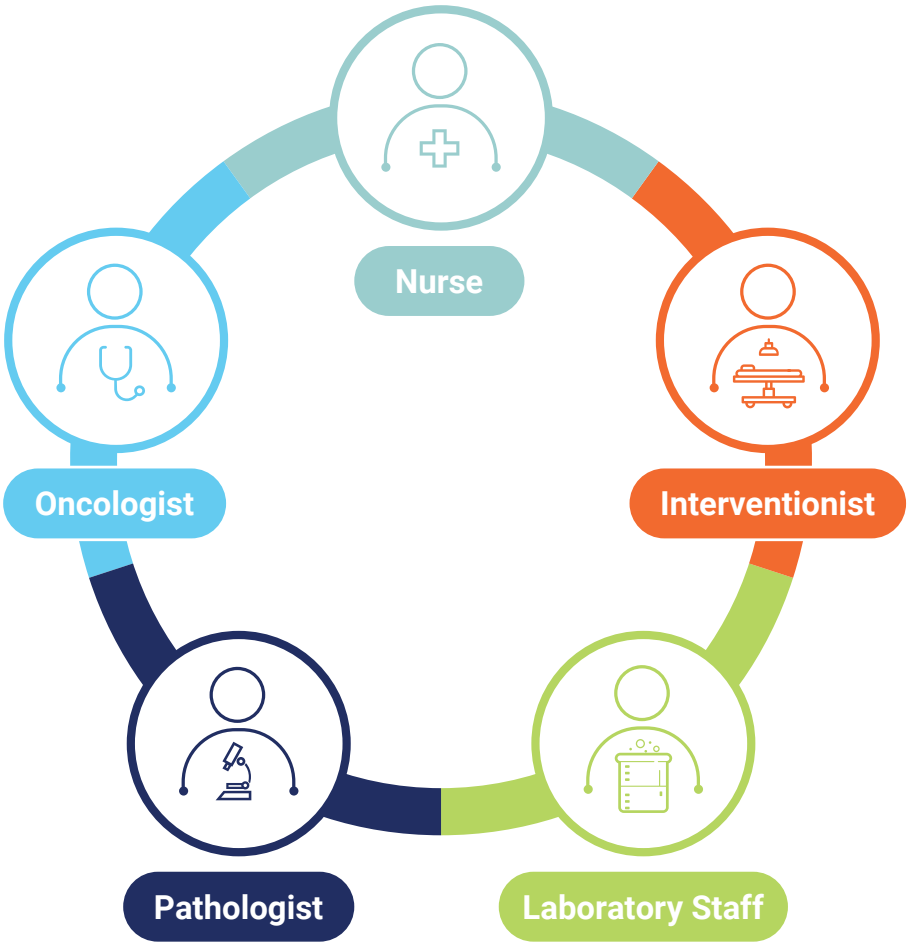
THE BREAST CANCER CARE TEAM

The multidisciplinary team (MDT)

- Molecular diagnostics is a multistep process requiring collaboration among distinct disciplines[45]
- The team is comprised of[45,46]:



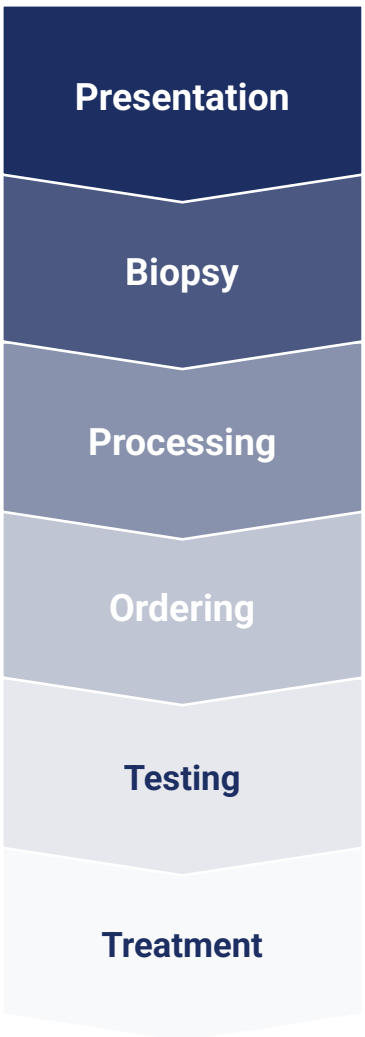
Each member of the MDT plays an important role in breast cancer care[45,46]

- Nurses can be the key point of contact between the patient and MDT or act as a tissue navigator to usher the tissue through the testing process[45,46]

The patient journey and role of each member of the multidisciplinary team

Testing Navigation

Breast cancer nurse
A key point of contact between the patient and the MDT and may facilitate team communication and coordination during testing[45]



Oncologist orders imaging and diagnostic tests after patient presents with suspected mBC[47]

Interventionalist collects tissue with potential input from **pathologist** to confirm sufficiency[45,47]

Laboratory staff prepare sample for testing under **pathologist** supervision[45,47]

The **oncologist**, **interventionalist**, and/or **pathologist** may order testing[45]

Pathologist interprets result(s) and prepares report after performing testing with assistance from **laboratory staff**[45]

Oncologist may use biomarker test results to make treatment decisions. **Pathologist** may be consulted for test interpretation[45,47]

Multidisciplinary teamwork during the patient journey is essential to getting a complete diagnosis for individuals with mBC[45]

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BIOMARKER TESTING MODALITIES IN mBC

Sequencing-based testing

Sequencing-based testing: sequences tumor genetic material

	Sanger Sequencing *Invented in 1977*[48]	Pyrosequencing *Invented in 1988*[48]	Next-Generation Sequencing (NGS) *Invented in the early 2000s*[49]
Detects	Mutations/small indels in the region of interest; read lengths of up to 1000 bases[50]	Point mutations in the region of interest; read lengths of ~100 bases[48]	Dependent on assay design; potential to detect SNVs, indels, CNAs, and fusions[51]
Biomarkers in mBC	*PIK3CA*[52] *BRCA 1 and 2*[53]	*PIK3CA*[54] *BRCA 1 and 2*[55]	*PIK3CA*[3] germline *BRCA*[3]
Sensitivity	Low (>20% VAF)[56]	Variable (LOD >5% VAF)[54]	Dependent on assay; may detect as low as <1% VAF[51]
Turnaround Time	3-4 days (when combined with PCR)[57]	3-4 days (when combined with PCR)[57]	Dependent on assay; targeted assays range from 7-20 days[57]
Contamination/ Bias/Limitations	Some automated Sanger sequencing platforms favor shorter DNA fragments[48]	Short read lengths limit applicability[48]	Bias dependent on specific assay and technology used[49]

PCR-based testing

RT-PCR and dPCR may be used to detect the presence or absence of specific known mutations. Alternatively, amplification products may be sequenced.[57-59]

	Real-Time PCR (RT-PCR)	Digital PCR (dPCR)
Detects[56,57]	Known mutations	Known mutations
Biomarkers	*PIK3CA*[60], *BRCA1/2*[61]	*PIK3CA*[62], *BRCA1/2*[63]
Sensitivity[56,57]	Variable (LOD ~5% VAF)[60]	High (LOD <1% VAF); enrichment may increase sensitivity[62]
Turnaround Time[57]	1-4 days	1-4 days
Contamination/ Bias/Limitations[58,59]	Contamination can be avoided	Low target DNA sample input may require pre-amplification step that may introduce bias

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CNA, copy number alteration; LOD, limit of detection; SNV, single nucleotide variant; VAF, variant allele frequency.

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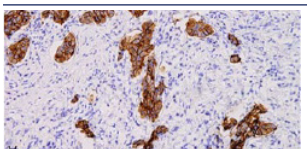
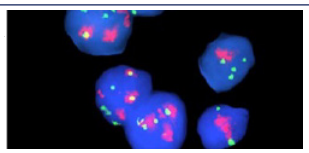
Image-based testing

Imaging-based testing: examines tumor characteristics under the microscope

	IHC	FISH
Method[64]	Assessment of protein expression using antibodies	Assessment of chromosomal aberration using a fluorescent probe
Markers[34,36,41]	ER, PR, HER2, Ki67, PD-L1	HER2
Preparation[65]	Fixation and antibody impact sensitivity and specificity	Time-consuming with standard chemicals, shorter with specific hybridization buffers
Analysis[64]	Qualitative expression level estimation (0, 1+, 2+, 3+)	Quantitative interpretation
Example (HER2)[65]		

Images adapted from D'Alfonso T et al. 2010
Intended to depict biomarker testing methodologies. When testing for therapy selection, please consult product prescribing information and FDA-approved companion diagnostics.

BIOMARKERS MAY CHANGE OVER THE COURSE OF A DISEASE

Meta-analyses and studies examining biomarker status in primary and metastatic tumors (following recurrence) have revealed temporal dynamics in some biomarkers, including:

Receptors That Define Breast Cancer Subtypes[a]

Receptor switching may occur in[66,67,a]:

- 10.2%-19.3% of cases for ER
- 24.8%-30.9% of cases for PR
- 2.9%-10.3% of cases for HER2

Genomic Biomarkers[b]

- *PIK3CA* mutations are generally stable but may change in some patients[68,b]
- *ESR1* mutations occur more frequently in advanced disease and may contribute to resistance[69,b]
- HER2 mutations may arise during treatment and confer resistance to anti-HER2 therapies[70,b]

The NCCN recommends testing a biopsy at first recurrence of disease and to consider rebiopsy upon progression, if feasible[7]

FISH, fluorescent in situ hybridization; IHC, immunohistochemistry.

[a]Data are from a meta-analysis of 39 studies assessing receptor conversion.
[b]Data are from a single retrospective study.

TESTING FOR BIOMARKERS IN mBC

ER/PR

ASCO-CAP guidelines (recommended by the NCCN) for ER/PR testing in breast cancer[7,34]

Large (preferably multiple) **core biopsies** of tumor are preferred for testing if they are representative of the tumor (grade and type) at resection[34]

Samples are fixed in **10% NBF for 6-72 hours**[34]

Samples should be sliced at **5-mm intervals** after appropriate gross inspection and margin designation, and placed in a sufficient volume of NBF to allow adequate tissue penetration[34]

Use of unstained slides cut **more than 6 weeks** before analysis is not r**ecommended**[34]

Standard operating procedures (SOPs) should be used that include routine use of external control materials with each batch of testing and routine evaluation of internal normal epithelial elements or the inclusion of normal breast sections (or other appropriate control) on each tested slide, wherever possible[34]

Validated **IHC is the recommended** standard test[34]

The NCCN recommends using methodologies outlined by ASCO/CAP guidelines[7]

ER and PR may change over the course of disease[66,67]

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HER2

ASCO-CAP guidelines (recommended by the NCCN) for HER2 testing in breast cancer[7,41]

HER2 testing samples are fixed in **10% NBF for 6-72 hours; cytology specimens** must be fixed in **formalin**[41]

Samples should be sliced at **5- to 10-mm intervals** after appropriate gross inspection and margin designation, and placed in a sufficient volume of NBF[41]

Sections should ideally **not be used** for HER2 testing if cut **>6 weeks earlier**; this may vary with primary fixation or storage conditions[41]

Use of **SOPs**, including routine use of control materials, is advised[41]

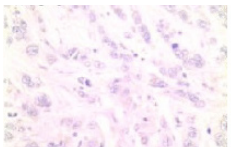
The NCCN recommends using methodologies outlined by ASCO/CAP guidelines[7]

HER2 may change over the course of disease[66,67]

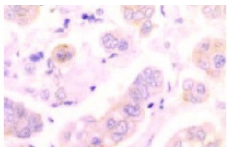
~3% of patients with HER2 positive disease develop brain metastasis at the time of first recurrence, which is associated with a worse prognosis[71]

IHC Detects HER2 Protein Overexpression

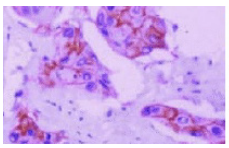
IHC 0



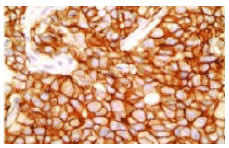
IHC 1+



IHC 2+



IHC 3+



- Membrane staining cutoff value is set at 10% of tumor cells[41]
- For IHC positive (2+) tumors, order a reflex test (same specimen using ISH) or a new test (new specimen if available, using IHC or ISH)[41]

ASCO/CAP guidelines recommend HER2 testing in breast cancer with IHC, then with in situ hybridization (ISH) if IHC results are equivocal[41]

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Ki67[36]

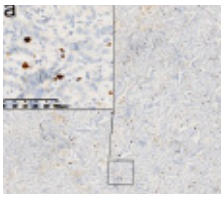
Ki67 is associated with poor prognosis, but analytical validity concerns have prevented adoption

Since 2011, the International Ki67 in Breast Cancer Working Group has crafted and updated guidelines to improve Ki67 reproducibility. Current considerations and recommendations include:

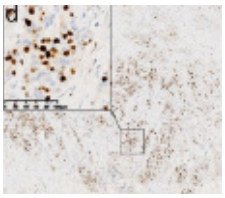
Preanalytical	Avoid: • Prefixation delays to prevent changes in nuclear morphology • Ethanol-fixed or decalcified preparations • Prolonged exposure to air of cut section
Analytical	• Mandatory **high-temperature antigen retrieval** • Counterstain all negative nuclei • **Antibody selection** – MIB1 is the most validated antibody
Scoring	• **Count all positive invasive carcinoma cells within the region in which all nuclei have been stained** – Scoring is the percentage of cells positive among total number of invasive cancer cells • **Report Ki67 as a percentage**

Clinical utility is evident only for prognosis estimation in patients who have anatomically favorable ER-positive/HER2-negative disease when Ki67 expression is ≤5% or ≥30%[36]

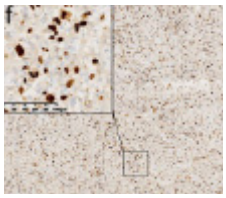
Examples of Ki67 Staining in TNBC Specimens[73]



Ki67 = 5%



Ki67 = 30%



Ki67 = 60%

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PD-L1

- PD-L1 expression may serve as both a prognostic and predictive biomarker[7,9]
- PD-L1 positivity is associated with a worse prognosis in patients with mBC, and eligibility for immunotherapy in patients with TNBC[7,9,74]

PD-L1 expression level may be impacted by[74-76]

PD-L1 differences in expression between the primary tumor and the metastatic sites

Choice of anti–PD-L1 antibody

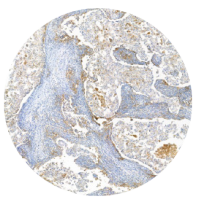
Interobserver agreement

There are different ways to assess PD-L1 positivity. In TNBC, PD-L1 expression CPS ≥10 is clinically informative[77]

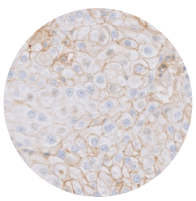
Type of PD-L1 Score	Definition[77]
Tumor proportion score	Ratio of PD-L1–positive tumor cells, relative to all vital tumor cells, multiplied by 100%
Immune cell score	Percentage of the area occupied by all PD-L1–positive immune cells relative to the whole tumor area
Combined positive score	Ratio of PD-L1–positive cells, including tumor and immune cells, to the total number of viable tumor cells, multiplied by 100

Anti–PD-L1 antibodies are not interchangeable when testing tissue from a patient with breast cancer[75]

PD-L1–Positive TNBC Specimens[78,79]



PD-L1 antibodies >90% of TIL



CPS=100

The NCCN recommends testing for PD-L1 expression in cases of metastatic TNBC[7]

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BRCA1/2

Testing for g*BRCA1/2* mutations can:

Identify women with a **greater risk for breast cancer**[80]

- ≈70% of women with either a *BRCA1* or *BRCA2* mutation will develop cancer by age 80
- ~19% of women harboring a *BRCA1* or *BRCA2* mutation will have brain metastases at first distant recurrence, which is associated with a worse prognosis[81]

Identify patients whose **family members may have an increased risk for breast cancer**[80]

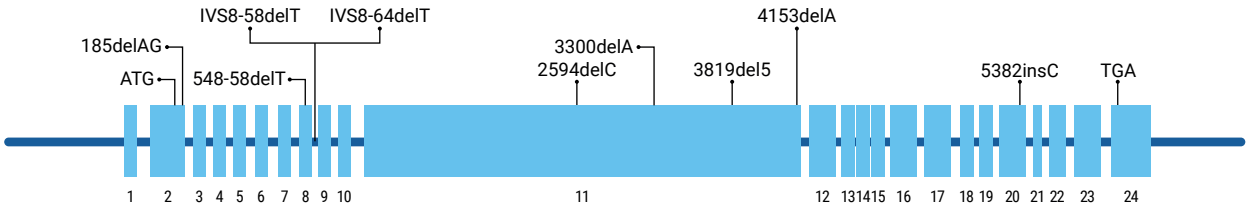
Identify **patients who may be eligible for treatment with a PARP inhibitor**[37,82]

- 5% of patients with breast cancer carry a g*BRCA* mutation

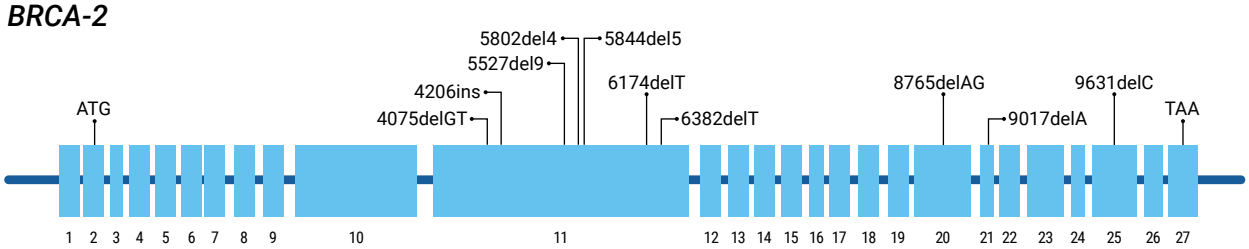
Canonical Mutations in the *BRCA* Genes

Important loss of function mutations include frameshift, nonsense, missense, and splice site mutations[83]

BRCA-1



BRCA-2



***BRCA1/2* alterations function as both susceptibility and predictive biomarkers**[37,80,82]

Intended to depict biomarker testing methodologies. When testing for therapy selection, please consult product prescribing information and FDA-approved companion diagnostics.

Figure adapted with permission from Wang F et al. 2012.

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PIK3CA

PIK3CA is a common mutation[a] in breast cancer, found in[39]:

of all patients with breast cancer

of patients with HR-positive/ HER2-negative disease

of patients with HER2-positive disease

of patients with TNBC

Patients with metastatic breast cancer harboring a *PIK3CA* **mutation have a *poorer* prognosis** than non-mutated[15]

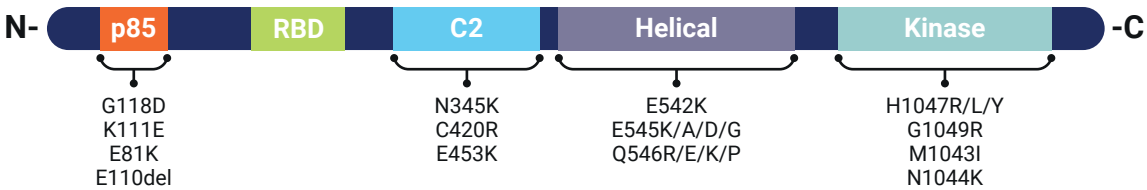
- **~30% of *PIK3CA*+ patients with mBC have brain metastases**, which are associated with a worse prognosis[18,84]

PIK3CA mutations have been associated with **reduced sensitivity to HER2-directed therapies** and cytotoxic therapies as well as **resistance to endocrine therapies**[15-17]

Knowledge of *PIK3CA* mutation status can inform treatment decisions in appropriate HR-positive/HER-2 negative patients[16]

***PIK3CA* mutations are generally stable from initial diagnosis,** but *PIK3CA* mutations may arise or be lost during the course of disease[68, 85]

Canonical Mutations in the *PIK3CA* Gene[39,68,85,86]



The majority of the *PIK3CA* mutations in patients with breast cancer are point mutations at the helical or kinase domain[39,68]

- Most common *PIK3CA* mutations can be detected in **tissue biopsies** and **liquid biopsies**[87]
- *PIK3CA* mutations can be detected with **qPCR** and **NGS**[39]

PIK3CA mutation testing can be done on tumor tissue or in ctDNA (liquid biopsy). If liquid biopsy is negative, NCCN recommends tumor tissue testing[7]

Intended to depict biomarker testing methodologies. When testing for therapy selection, please consult product prescribing information and FDA-approved companion diagnostics.

Figure adapted with permission from Dirican et al. 2016.

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Testing for Key Biomarkers in Breast Cancer Summary

Biomarker	Prevalence	Prognosis	Predictive	Testing Methods[3,7,35-37,39,40,63,64]
ER/PR[4,11,34]	70%[a]	X	X	IHC
HER2[4,11,35]	16.6%[b]	—	X	IHC, FISH
Ki67[13,36]	—	X	—	IHC
BRCA1/2[3,11,37,61,63]	5%	—	X	RT-PCR, dPCR, NGS
PD-L1[9,11,38,75]	20%[c]	X	X	IHC
PIK3CA[3,11,15,39,60,62]	36%	X	X	RT-PCR, dPCR, NGS

[a]ER/PR positivity defined as >1%.
[b]HER2 negativity defined as IHC0/1+ or 2+ with a FISH amplification ratio of <2.0.
[c]PD-L1 positivity defined as ≥10% tumor cells or immune cells expressing PD-L1.

Whatever biopsy sample or testing technology is used, the assay should be able to detect clinically relevant mutations[39]

Biomarker testing is fundamental to the treatment of mBC and has been for >20 years[11,27]

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SUMMARY

Biomarker testing is fundamental to breast cancer care and is essential to guiding therapeutic decisions[85]

A **complete diagnosis** in recurrent/stage IV breast cancer requires testing for all actionable biomarkers, including **ER, PR, HER2, BRCA1/2, PD-L1, and PIK3CA**[7,10,13,23,40,44]

Following **guideline recommendations** may help improve biopsy quality and testing outcomes in mBC[7,23,34,35,45]

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