Precision Medicine Digital Binder

To view each resource, click on the titles below.

Diagnostic Journey *Chapters*

Essential Elements of Biomarker Testing During the Diagnostic Journey

Considerations for Biomarker Testing Reimbursement

Testing 101 *Chapters*

Biomarker Testing in Breast Cancer
An Essential Component of the Treatment Decision Making Process

The Importance of a Molecular Diagnosis in mNSCLC
Understanding the Essential Role of Biomarker Testing in Patient Care

Molecular Diagnostics in Personalized Cancer Care
Best Practices and Overcoming Challenges

Knowledge Checks

Molecular Diagnosis in Lung Cancer
Knowledge Check #1

Molecular Diagnosis in Lung Cancer
Knowledge Check #2

Biomarker Testing in Breast Cancer
Knowledge Check #1

Biomarker Testing in Breast Cancer
Knowledge Check #2

Molecular Diagnostics in Oncology
Knowledge Check #1

Molecular Diagnostics in Oncology
Knowledge Check #2

Resources

Molecular Profiling in Common Cancers

The Ins and Outs of Test Requisition Forms

Essential Elements of Biomarker Testing During the Diagnostic Journey

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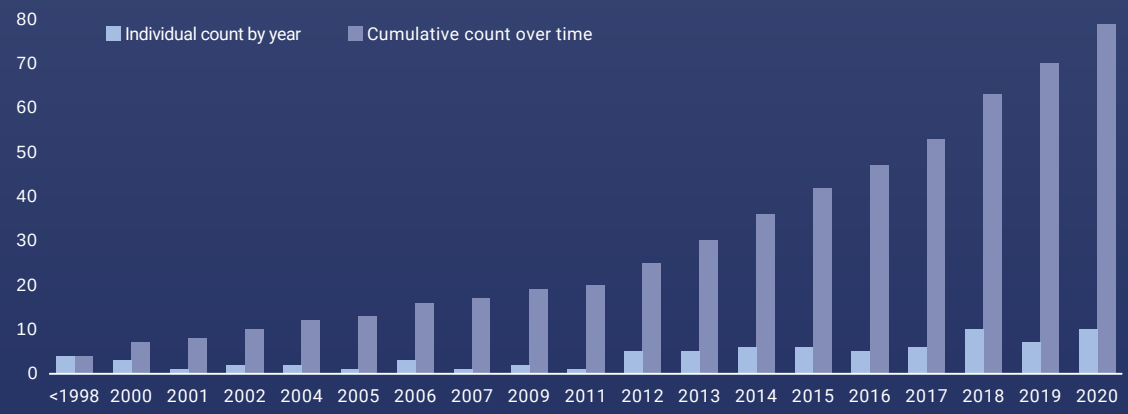
PRECISION ONCOLOGY OVERVIEW

Precision oncology, which aims to pair patients with therapeutic options suited to the biological basis for their cancer, has grown dramatically since the first targeted therapy for a solid tumor in 1998[1-5]

Number of US Oncology Approvals With Required or Recommended Predictive Biomarker Testing[2]



As of June 2022, there are:[1,6]

≥70
FDA-approved biomarker-linked indications

43
actionable genomic alterations

28
cancer types treatable by Precision Oncology

1in3
cancer patients may be candidates for an FDA-approved biomarker-linked therapy[7]

Precision Oncology Requires Molecular Diagnostics[1]

FDA, US Food and Drug Administration.

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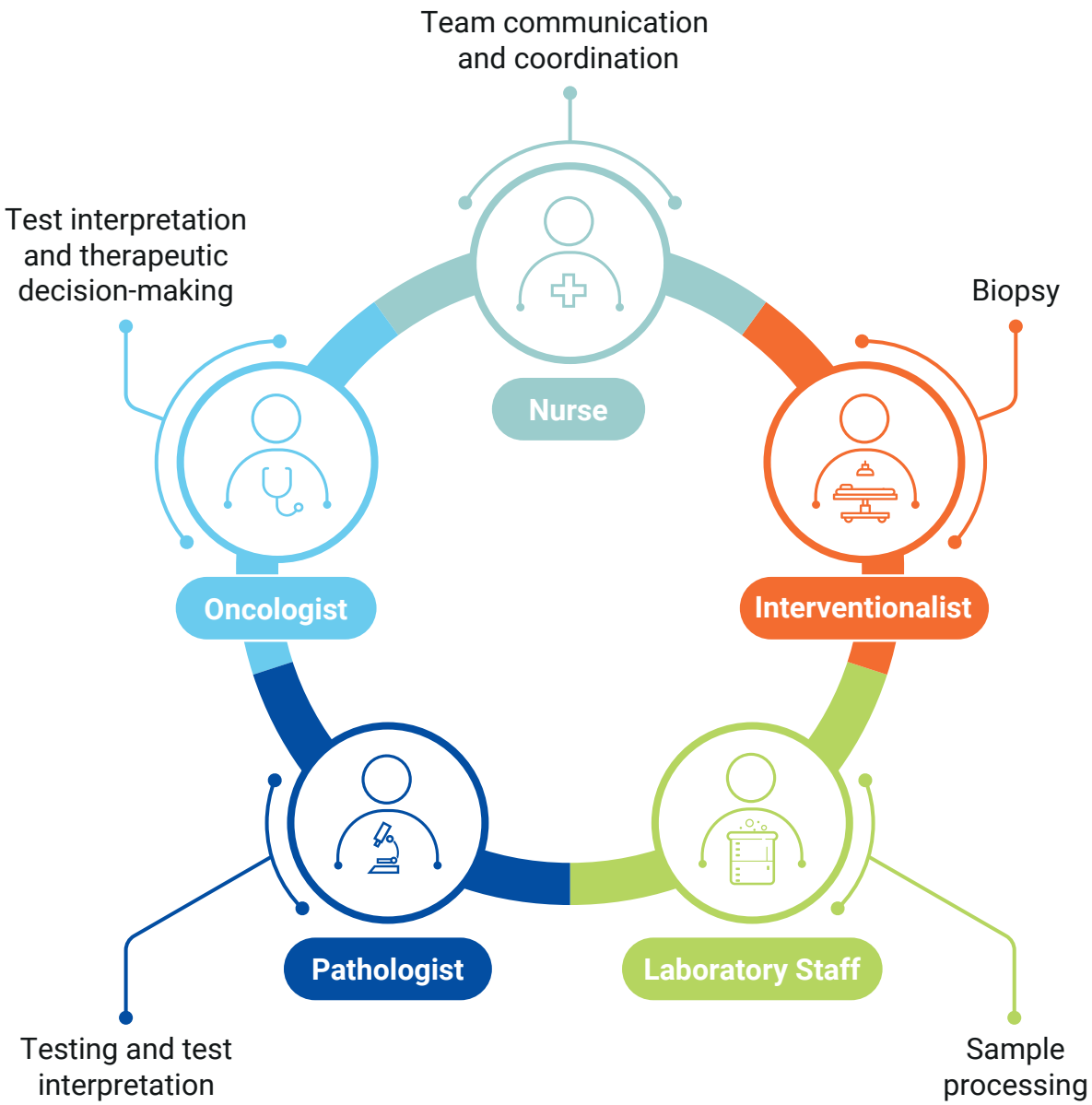
MOLECULAR DIAGNOSTICS OVERVIEW

Molecular diagnostics is a multistep process requiring collaboration among distinct disciplines[8,9]

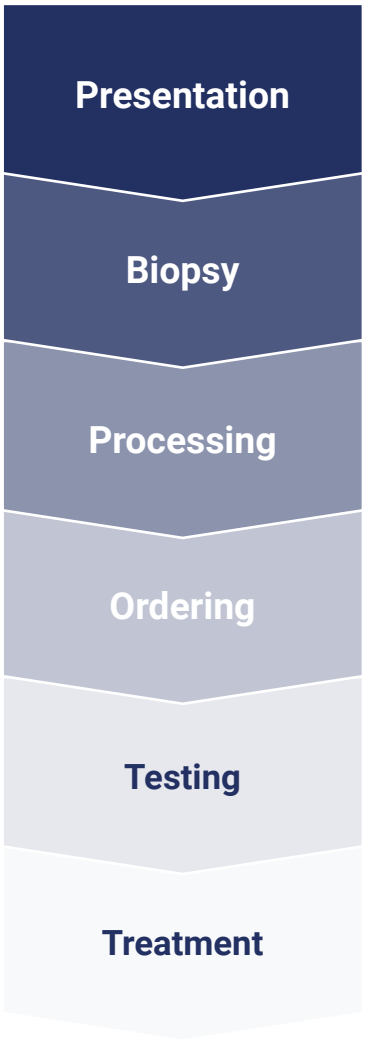
The Multidisciplinary Team (MDT)



MDT Roles in the Diagnostic Journey for Patients With Metastatic Cancer

Testing Navigation

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[8,9]



Oncologist orders imaging and diagnostic tests after patient presents with suspected metastatic cancer[10]

Interventionalist collects tissue with potential input from **pathologist** to confirm sufficiency[8,10]

Laboratory staff prepare sample for evaluation and testing under **pathologist** supervision[8,10]

The **oncologist**, **surgeon/interventionalist,** and/or **pathologist** may order testing[8]

Pathologist interprets result(s) and prepares report after performing testing, with assistance from **laboratory staff**[8]

Oncologist may use biomarker test results to make treatment decisions. **Pathologist** may be consulted for test interpretation

Problems at Any Step in the Diagnostic Process May Negatively Impact Patient Care

Successful Biomarker Testing Depends on Key Factors

Testing tissue of sufficient quantity and quality[11]

Ordering process for actionable biomarkers[1]

Use of appropriate tests[1]

Access to clear and searchable report data[12]

There May Be Solutions for Possible Challenges Associated With Each Key Factor

DIAGNOSTIC JOURNEY FOR PATIENTS WITH METASTATIC CANCER: **TISSUE SUFFICIENCY**

Presentation | **Biopsy** | **Processing**

Failure to obtain sufficient tissue during biopsy[11]

Inappropriate sample processing (eg, fixation, sectioning)[8]

- In a survey, **57%** of oncologists cited **tissue sufficiency** as a barrier to multimarker tumor panel testing[11]
- **Core needle biopsies** may provide inadequate malignant tissue[13]
- Biomarker discordance between the primary tumor and a metastatic site may occur[14-16,a]
- **Bone biopsies** may have increased odds of containing insufficient tumor cells[13]

- **Prolonged ischemic** times may lead to sample degradation[8]
- **Fixation** may influence suitability for downstream testing[8]
- Preparing tissue using only one cassette may contribute to **tissue exhaustion**[8]
- **Necrotic regions** may be incompatible with PCR- and NGS-based sequencing[8]

- Working with the **MDT** to identify lesions to sample and potentially assess tissue adequacy during the procedure may help obtain sufficient tissue[8]
- Consider implementation of **ROSE** to overcome tissue inadequacy in small biopsies[17,18]

- Consider **limiting cold ischemia to <30 min** if performing RNA/proteomic analyses[8]
- Consider **downstream testing** when choosing fixation methods[8]
- Dividing tissues into **>1 cassette** may prevent tissue waste[8]
- **Microdissections** may increase viable tumor fraction[8,19]
  - NGS assays typically require 10-20% tumor nuclei[20]

[a]Based on a meta-analysis from 61 studies including more than 5,700 patients with metastatic colorectal cancer.
NGS, next generation sequencing; PCR, polymerase chain reaction; ROSE, rapid on-site evaluation.

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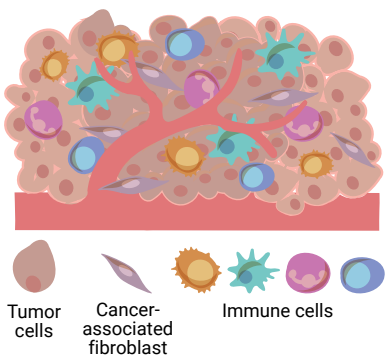
Ordering | Testing | Treatment

Tumor heterogeneity and overestimation of tumor content prior to testing[21]



The tumor microenvironment consists of heterogeneous cellular matrix and extracellular matrix components[13]

Lung Adenocarcinoma Example[21]
Tumor content 30% to 40%

- **Tumor heterogeneity** can affect tissue sufficiency and biomarker testing[22]
  - **False-negatives** may occur in samples with few tumor cells[23]
- Inaccurate estimation of tumor content is a potential challenge – **38%** of samples have overestimated tumor content[23]

Microdissections may increase tumor percentage and detectability of tumor DNA[24]

Successful Biomarker Testing Depends on Maintaining Tumor Tissue Quality[8,25]
Remember: What You Put in Is What You Get Out

Images adapted with permission from Baghban R et al. *Cell Commun Signal* and 2020;18(1):59. Mikubo M et al. *J Thorac Oncol*. 2020;15(1):130-137.

DIAGNOSTIC JOURNEY FOR PATIENTS WITH METASTATIC CANCER: **ORDERING**

Presentation → **Biopsy** → **Processing**

MDT communication[46]

- Common terms like "panel" may have **multiple interpretations**[26,27]
- Variability in **requisition forms** between different institutions may result in confusion among MDT members[28]
- Test requisition form **formatting** may impact test utilization, including under- or overtesting[29,30]

The American Society of Clinical Oncology (ASCO) published a Provisional Clinical Opinion that includes **definitions** for biomarker testing terminology[1]

- **ASCO** defines a multigene panel as an "NGS test with a defined set of genes of at least 50 genes"

*Where are the **NGS** results? I thought we ordered a **panel**.*

*Our **panel** doesn't include **NGS**. Did you want a **CGP** as well?*

Establishing a common language with the MDT may help ensure that patients are not missed because of communication errors

National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines® in Oncology (NCCN Guidelines®) Issues Evidence- And Consensus-Based Guidelines That Are Updated Continually, With At Least 1 Update Per Year[31]

CGP, comprehensive genomic profiling.

Ordering | Testing | Treatment

Multiple testing options[32]

Too many testing options (eg, multiple testing platforms or vendors, each with unique sample requirements), within a hospital system may lead to[32]:

- Confusion among providers
- Disorganized processes within the laboratory
- Potentially longer turnaround times

One community hospital system saw improvements in biomarker testing after **creating standard ordering processes** with **minimal testing platforms** to streamline laboratory processes[35]

Guideline differences[32]

Guidelines may differ based on the **timing** of their **most recent update**[32]

- NCCN Clinical Practice guidelines in Oncology (NCCN Guidelines®) update **at least once per year**[31]
- CAP Guidelines are reviewed and updated every **5 years**[33,34]

Consider reviewing and incorporating recommendations from different guidelines at a cadence that keeps pace with updates[32]

CAP Guidelines (Pathology Guidelines) Are Evidence-Based Guidelines[33,34]

CAP, College of American Pathologists.

DIAGNOSTIC JOURNEY FOR PATIENTS WITH METASTATIC CANCER: **USE OF APPROPRIATE TESTS**

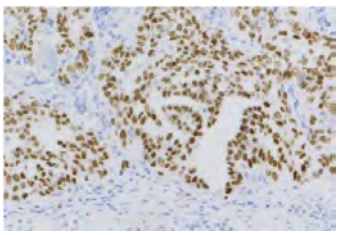
Presentation | **Biopsy** | **Processing**

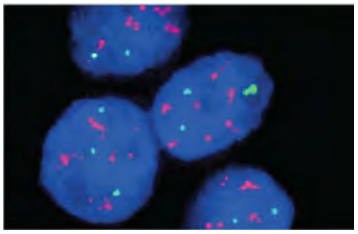
Use of tests that cannot detect the biomarker in question[1,19]

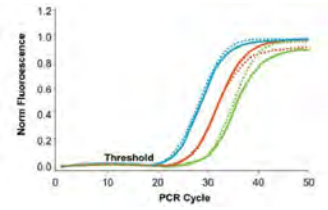
- Some biomarkers may be detected more reliably by **some specific testing techniques** than by others[1,19]
- **Gene rearrangements** can be reliably detected by **FISH** and **RNA**-based NGS; enrichment strategy for a **DNA**-based NGS assay impacts the detection of **fusions**[1,19]

- Understanding **assay limitations** is critical to identifying patients with actionable biomarkers[1]
- **ASCO** recommends being **familiar** with the genomic testing platforms available to ensure **fusion testing** is performed when indicated[1]

IHC[36]	FISH[37]	RT-PCR[38]







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

FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; RT-PCR, real-time polymerase chain reaction.
Images adapted with permission from Yatabe Y et al. *J Thorac Oncol*. 2019;14(3):377-407, Yu J et al. *Sci Rep*. 2019;9(1):7518, and Kipf E et al. *J Mol Diagn*. 2022;24(1):57-68.

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Ordering | **Testing** | **Treatment**

Extensive turnaround time[39]

Multigene panels may have turnaround times of **>10 days**[39]

- Hybrid capture panels typically have longer turnaround times than amplicon panels but may detect more types of alterations

Reflex testing may speed turnaround times by streamlining the ordering process[39,40]

- Having results available for the **first visit** may speed the time to **treatment initiation**[41]

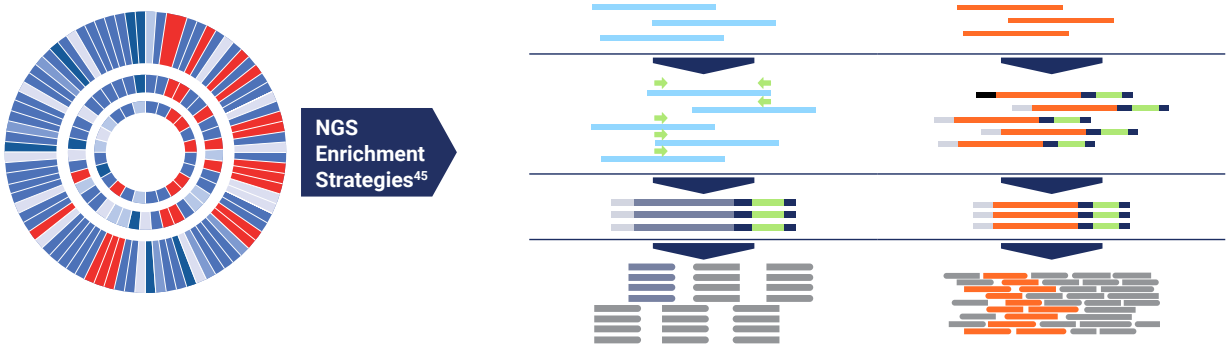
Sequential single-gene testing in some cancers[1,43]

In some cancers with multiple biomarkers, studies suggest **sequential single-gene testing** may contribute to tissue exhaustion, potentially leading to[42,43]:

- Patients **not receiving testing** for all biomarkers
- **Prolonged turnaround time** for all biomarkers (relative to a multigene panel)

For patients with advanced or metastatic cancers, **ASCO recommends multigene panel–based** genomic testing whenever **>1** genomic biomarker is linked to an FDA-approved therapy[1]

NGS[44] | **Amplicon** | **Hybrid capture**



Images adapted with permission from Cheng H et al. *Cell Rep.* 2018;25(5):1332-1345.e5 and Church A. Next-generation sequencing. In: Tafe L, Arcila M, eds. *Genomic Medicine*. Cham, Switzerland: Springer; 2020:25-40.

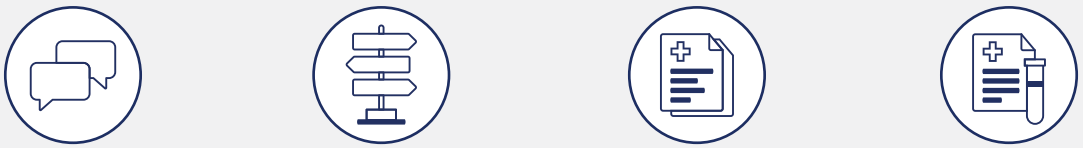
DIAGNOSTIC JOURNEY FOR PATIENTS WITH METASTATIC CANCER: **CLEAR AND SEARCHABLE REPORTS**

Presentation | **Biopsy** | **Processing**

Communication Issues May Arise During Biomarker Testing

In a survey, HCPs cited communication challenges across the MDT as 1 of the top 5 barriers to biomarker testing[46]

Sources of **communication** issues may include:



Use of jargon[12,32]

Guideline differences[32]

Requisition form variability and/or ambiguity[32]

Pathology reports and EHR incompatibility[12,32,47]

Multiple professional societies have developed resources to assist with testing barriers

Speaking the same language[1,48]
- ASCO provided definitions of common terms for clinicians
- Working group created common terms and their definitions for patients

Incorporating multiple guidelines[32]
- Frequently updated guidelines may be the source for updates to internal SOPs

Generating internal standards for testing documentation[49]
- Involving representatives of the MDT may address this issue

ASCO recommends using precision oncology knowledge databases to assess a list of genomic alterations considered clinically actionable[1]
- OncoKB monitors FDA-approved indications

EHR, electronic health record; HCPs, health care professionals; SOPs, standard operating procedures.

Ordering | Testing | Treatment

LIS/EHR incompatibility[12]

Confusing/narrative reports[12]

- Incompatibility between LIS and EHR systems may lead to missing or incomplete reports[12,32,47]
- When added as an addendum report, biomarker test results may be missed if not linked to pathology reports in the EHR[12]
  - Oncologists are not notified when addendum reports are added to the EHR

- Narrative reports may be challenging to interpret quickly[11,12,47,50-52]
- NGS reports may not state actionable genomic alterations, further complicating interpretability[11,12,47,50-52]

ACCC recommends[12]:

- Utilizing CAP electronic Cancer Checklists to facilitate structured data capture and reporting
- Exploring ways to improve report readability and searchability across electronic systems
- Minimizing the use of scanned reports
- Considering using pathology LIS modules built by the inpatient EHR vendor

ASCO recommends using precision oncology knowledge databases to assess a list of genomic alterations considered clinically actionable[1]

EHR, electronic health record; LIS, laboratory information system; NGS, next-generation sequencing.

SUCCESSFUL BIOMARKER TESTING DEPENDS ON KEY FACTORS:

Testing **tissue** of sufficient quantity and quality[11]

Failure to obtain sufficient tissue during biopsy	• Working with the MDT to identify lesions to sample and assess tissue adequacy during the procedure may help obtain sufficient tissue [8] • Consider implementation of ROSE to overcome tissue inadequacy in small biopsies[17,18]
Inappropriate sample processing (eg, fixation, sectioning)	• Consider limiting cold ischemia to <30 min if performing RNA/proteomic analyses[8] • Consider downstream testing when choosing fixation methods[8] • Dividing tissues into >1 cassette may prevent tissue waste[8] • Microdissections may increase viable tumor fraction[8,19]
Overestimation of tumor content prior to testing	

Ordering process for actionable biomarkers[1]

MDT communication	• Consider incorporating definitions for biomarker testing terminology included in an ASCO Provision Clinical Opinion
Multiple testing options	• Consider creating standard ordering processes with minimal testing platforms to streamline laboratory processes [35]
Guideline differences	• Consider reviewing and incorporating recommendations from different guidelines at a cadence that keeps pace with updates[32]

Use of **appropriate tests**[1]

Use of tests that cannot detect the biomarker in question	• Understand assay limitations to identify patients with actionable biomarkers[1] • ASCO recommends being familiar with genomic testing platforms available to ensure fusion testing is performed when indicated[1]
Sequential single-gene testing in some cancers	• For patients with advanced or metastatic cancers, ASCO recommends multigene panel–based genomic testing whenever >1 genomic biomarker is linked to an FDA-approved therapy[1]
Extensive turnaround time	• Consider reflex testing, which may speed turnaround times by streamlining the ordering process[39,40] • Having results available for the first visit may speed the time to treatment initiation[41]

Access to **clear and searchable** report data[12]

LIS/EHR incompatibility	• ACCC recommends utilizing CAP electronic Cancer Checklists to facilitate structured data capture and reporting[12] • ACCC recommends exploring ways to improve report readability and searchability across electronic systems[12] • ACCC recommends minimizing the use of scanned reports[12] • ACCC recommends considering using pathology LIS modules built by the inpatient EHR vendor[12]
Confusing/narrative reports	• ASCO recommends using precision oncology knowledge databases to assess a list of genomic alterations considered clinically actionable[1]

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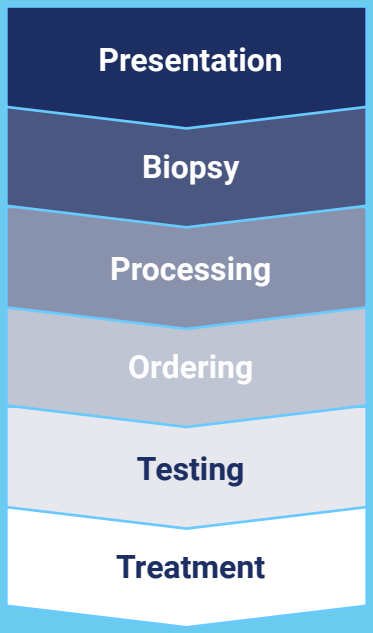
SUMMARY



Molecular diagnostics is a multistep process requiring collaboration among distinct disciplines[8,9]

Multiple professional societies have developed resources to assist with testing barriers[1,32,48,49]

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Considerations for **Biomarker Testing Reimbursement**

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14-DAY RULE EXPLANATION

The goal of this chapter is to provide educational information regarding reimbursement for biomarker testing. It is being provided for informational purposes only. It is the sole responsibility of the health care provider to select the proper codes and to ensure the accuracy of all statements used in seeking coverage and reimbursement for the care of individual patients.

The **14-day rule** dictates who may be billed for diagnostic tests based on a patient's status and the specific biomarker test(s).[1]

Laboratories are required to bill the hospital for some test(s) ordered <14 days after an inpatient discharge or outpatient visit[1]

- In the **outpatient** setting, exempted tests may be billed to Medicare within the 14-day window[1]

Many tests used to identify genes relevant in cancer are exempt from the 14-day rule[1-3]

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14-DAY RULE EXPLANATION (CONTINUED)

Essential definitions

INPATIENT: formally admitted to a hospital with a physician's order[4]

- The last inpatient day is the day before discharge

OUTPATIENT: visits the hospital for services, treatment or tests, but does not have a physician's formal admission order[4]

NONHOSPITAL PATIENT: has sample(s) collected at a private physician's office or commercial laboratory[5]

- There is no hospital visit on the date of collection

Billing by the lab for hospital outpatients depends on whether the testing is exempt from the 14-day rule, unlike hospital inpatients and nonhospital patients[1,6]

Types of biomarker tests that may be exempt[1,7]

Molecular pathology tests*	Advanced diagnostic laboratory tests (ADLTs)	Cancer-related protein-based multi-analyte algorithmic assays (MAAAs)

*IHC, FISH, and immunoassay are not exempt from the 14-day rule and must be billed to the hospital if performed less than 14 days from outpatient discharge

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WHAT ARE THE MOST FREQUENT SCENARIOS OF THE 14-DAY RULE?

	Days from Discharge	For EXEMPT Tests, Lab Bills:	For NON-EXEMPT Tests, Lab Bills
HOSPITAL INPATIENTS have been formally admitted to a hospital with a physician's order[1]	<14	Hospital	Hospital
	≥14	**Medicare**	**Medicare**
HOSPITAL OUTPATIENTS visit the hospital for services, treatment or tests but have not received a physician's formal admission order[1]	<14	Medicare	Hospital
	≥14	**Medicare**	**Medicare**
NONHOSPITAL PATIENTS are patients whose samples are collected at a private physician's office or commercial laboratory with no hospital visit on the date of collection[6]	<14	Medicare	Medicare
	≥14	**Medicare**	**Medicare**

75% of possible scenarios can be billed to Medicare[1,6]

SELECT BIOMARKER TESTS IN ONCOLOGY THAT ARE EXEMPT FROM THE 14-DAY RULE[2,8]

Test Name	CPT / PLA code(s)
FOUNDATIONONE® CDX	0037U
MI PROFILE™	0211U
GUARDANT360® CDX	0242U
TEMPUS XF	81479, 81455
NEOTYPE® PRECISION PROFILE FOR SOLID TUMORS (NGS)	81479
MSK IMPACT™	0048U
PENN PRECISION PANEL 2.0	81479, 81445
MOFFITT STAR™ (TRUSIGHT® TUMOR 170)	81455
HOPESEQ SOLID TUMOR COMPLETE	81479, 81455
JOHNS HOPKINS UNIVERSITY SOLID TUMOR PANEL V6	81455
EMPOWER™ CANCER TEST	81479, 81455
STANFORD SOLID TUMOR ACTIONABLE MUTATION PANEL (STAMP)	81479, 81455
ONCOPANEL (POPV3)	81455
ONCOTYPE MAP™ PAN-CANCER TISSUE TEST	0244U
CLEVELAND CLINIC PAN-SOLID TUMOR NGS PANEL	81445
MYCHOICE® CDX	0172U
PCMP - PERSONALIZED CANCER MUTATION PANEL (ION AMPLISEQ™ CANCER HOTSPOT PANEL V2)	81445
STRATANGS®	81479, 81455
ION AMPLISEQ™ CANCER HOTSPOT PANEL V2	81445, 81450, 81455, 81479
TRUSIGHT™ ONCOLOGY 500	81445, 81479
Oncomine™ Focus Assay	81445

Tests performed by dedicated, external laboratories
Tests that can be ordered as kits by independent laboratories

The select tests listed above represent the top 20 NGS solid tissue cancer tests by market share in Q1 2022. This information is not exhaustive and is not intended to endorse a particular test.
When testing for therapy selection, please consult product prescribing information and FDA-approved companion diagnostics.
It is the sole responsibility of the health care provider to select the proper codes and to ensure the accuracy of all statements used in seeking coverage and reimbursement for the care of individual patients.

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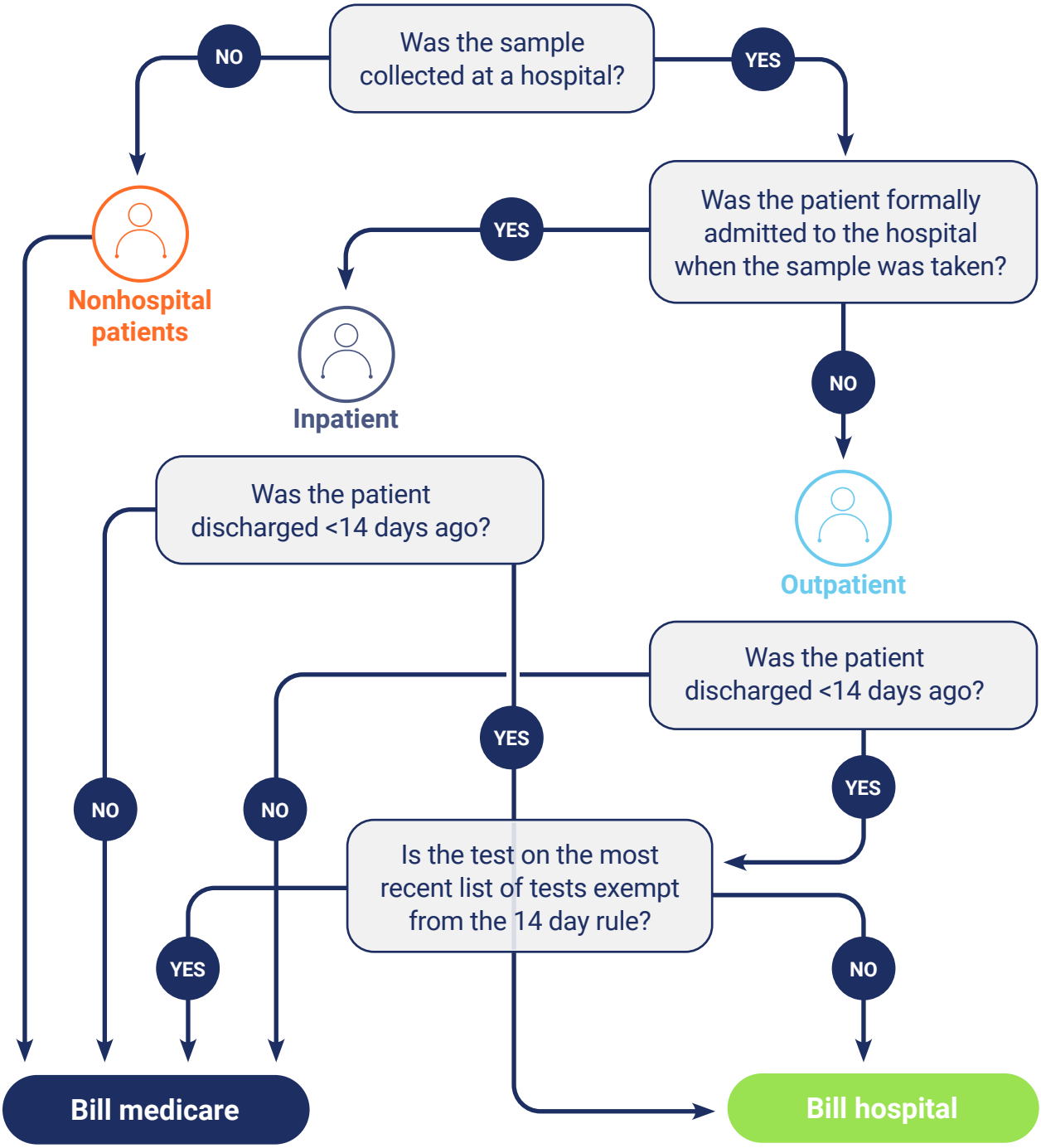
QUESTIONS TO CONSIDER WHEN DETERMINING WHO MAY BE BILLED FOR A TEST:



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1. CMS. Laboratory Date of Service Policy. https://www.cms.gov/Medicare/Medicare-Fee-for-Service-Payment/ClinicalLabFeeSched/Clinical-Lab-DOS-Policy. Accessed May 16, 2022. **2.** Centers for Medicare and Medicaid Services. https://www.cms.gov/Medicare/Medicare-Fee-for-Service-Payment/ClinicalLabFeeSched/Downloads/CLFS-Test-Codes-DOS-Exception.zip. Accessed May 25, 2022. **3.** Colomer R, et al. *EClinicalMedicine*. 2020;25:100487. **4.** Medicare. Are You a Hospital Inpatient or Outpatient. https://www.medicare.gov/sites/default/files/2018-09/11435-Are-You-an-Inpatient-or-Outpatient.pdf. Accessed May 16, 2022. **5.** CMS Manual System. Pub 100-04 Medicare Claims Processing Transmittal 2971. https://www.cms.gov/regulations-and-guidance/guidance/transmittals/downloads/r2971cp.pdf. Accessed May 16, 2022. **6.** CMS. Frequently Asked Questions Revised Laboratory Date of Service Exception Policy. https://www.cms.gov/Medicare/Medicare-Fee-for-Service-Payment/ClinicalLabFeeSched/Downloads/CLFS-DOS-FAQs.pdf. Accessed May 16, 2022. **7.** CMS. Laboratory Date of Service Policy. https://www.govinfo.gov/content/pkg/FR-2020-12-29/pdf/2020-26819.pdf. Accessed August 1, 2022. **8.** Data on File. Novartis Pharmaceuticals Corp; 2022.

SUMMARY

The 14-day rule instructs laboratories to bill the hospital for tests ordered <14 days after an inpatient discharge or outpatient visit[1]

The 14-day rule only applies in certain situations

- In the inpatient setting, the rule always applies[1]
- In the outpatient setting, the rule ***only applies to nonexempt tests***[1]
- In the nonpatient setting, the rule ***does not apply***[6]

In oncology, numerous biomarker tests are exempt from the 14-day rule[1]

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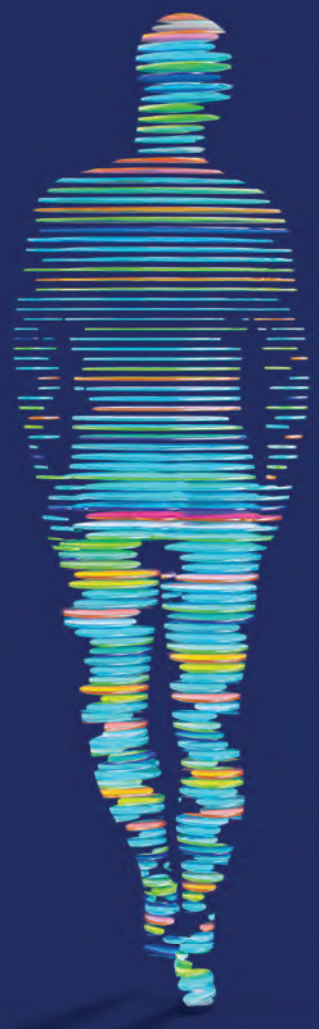
Biomarker Testing in Breast Cancer

An Essential Component of the Treatment Decision Making Process



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BREAST CANCER OVERVIEW

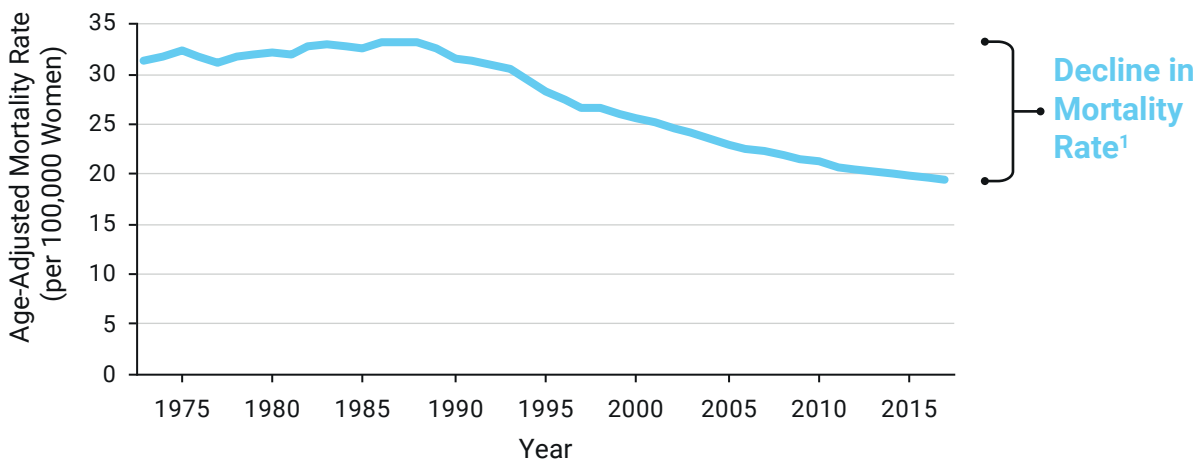
Breast cancer is the **second leading cause** of cancer-related death in women[1]

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]

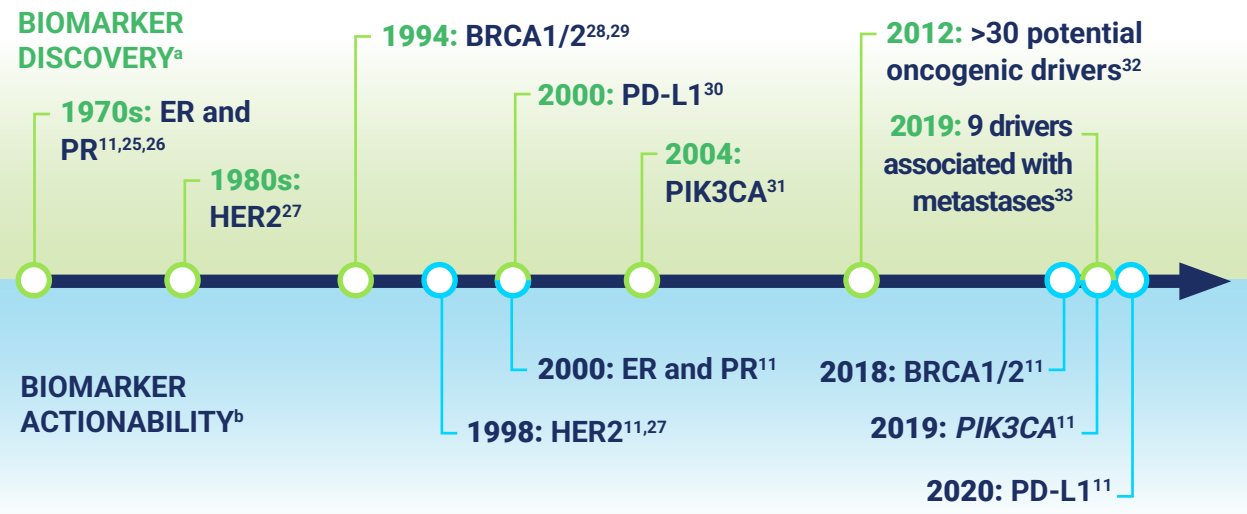
Patients with brain metastases or patients with multiple metastatic sites have shorter survival than other patients

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]

[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.
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.

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

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

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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	Actionable biomarkers Category 1 in NCCN Guidelines[b]
Initial diagnosis of stage IV disease Recurrent breast cancer with stage IV disease	**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**

[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.
CPS, combined positivity score.

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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]

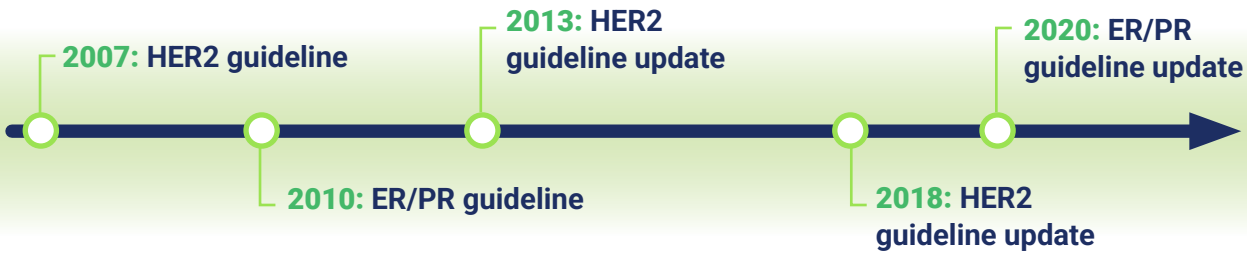
The NCCN issues evidence- and consensus-based guidelines that are updated continually with at least 1 update per year[7,42]

- 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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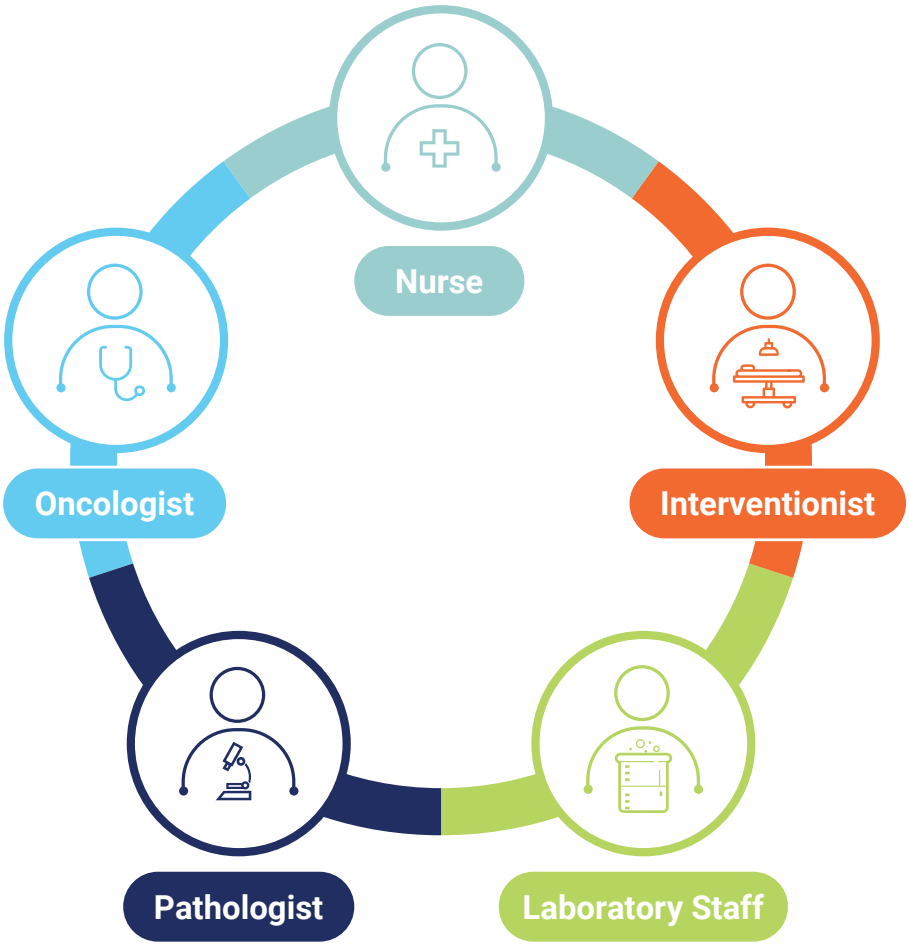
THE BREAST CANCER CARE TEAM

The multidisciplinary team (MDT)

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



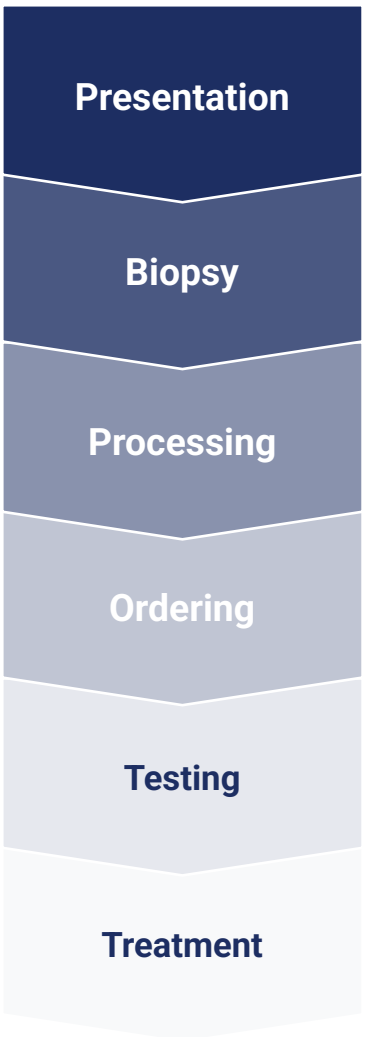
Each member of the MDT plays an important role in breast cancer care[45,46]

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

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

Laboratory staff 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 w(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

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

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

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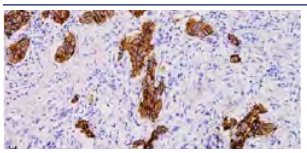
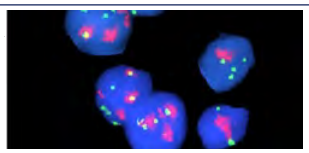
Image-based testing

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]

[a]Data are from a meta-analysis of 39 of studies assessing receptor conversion. [b]Data are from a single retrospective study.
FISH, fluorescent in situ hybridization; IHC, immunohistochemistry.

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]

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]

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

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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]

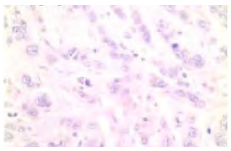
The NCCN recommends using methodologies outlined by ASCO/CAP guidelines[7]

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

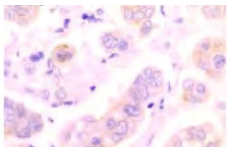
~3% of patients with HER2 positive disease develop brain metastasis at the time of first recurrence, which is associated with a worse prognosis[71]

IHC Detects HER2 Protein Overexpression

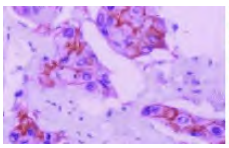
IHC 0



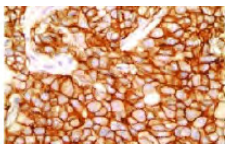
IHC 1+



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]

Intended to depict biomarker testing methodologies. When testing for therapy selection, please consult product prescribing information and FDA-approved companion diagnostics.
Images adapted with permission from Royce et al. 2016.

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Ki67[36]

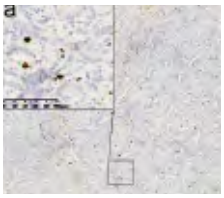
Ki67 is associated with poor prognosis, but analytical validity concerns have prevented adoption

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

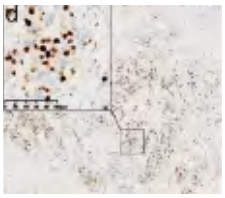
Preanalytical	Avoid: • Prefixation delays to prevent changes in nuclear morphology • Ethanol-fixed or decalcified preparations • Prolonged exposure to air of cut section
Analytical	• Mandatory **high-temperature antigen retrieval** • Counterstain all negative nuclei • **Antibody selection** – MIB1 is 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]

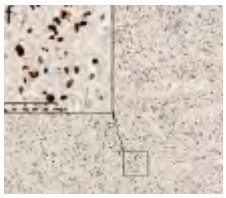
Examples of Ki67 Staining in TNBC Specimens[73]



Ki67 = 5%



Ki67 = 30%



Ki67 = 60%

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

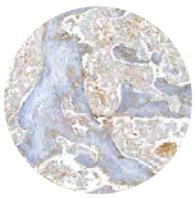
Interobserver agreement

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

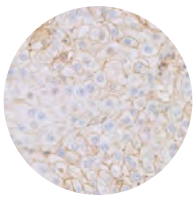
Type of PD-L1 Score	Definition[77]
Tumor proportion score	Ratio of PD-L1–positive tumor cells, relative to all vital tumor cells, multiplied by 100%
Immune cell score	Percentage of the area occupied by all PD-L1–positive immune cells relative to the whole tumor area
Combined positive score	Ratio of PD-L1–positive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

Intended to depict biomarker testing methodologies. When testing for therapy selection, please consult product prescribing information and FDA-approved companion diagnostics.
Images adapted with permission from Cha et al. 2021 and Aligent

Precision Medicine

BRCA1/2

Testing for gBRCA1/2 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]

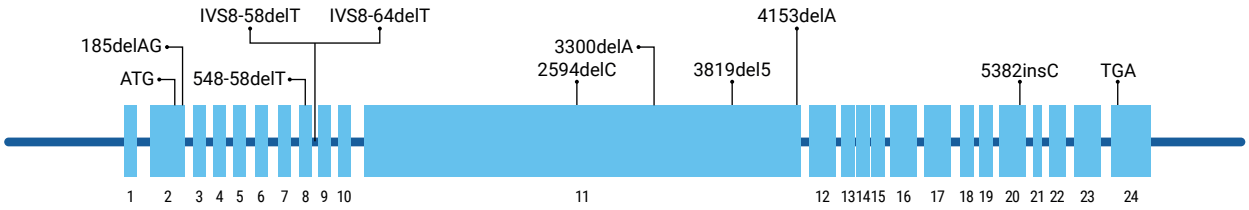
Identify **patients who may be eligible for treatment with a PARP inihibitor**[37,82]

- 5% of patients with breast cancer carry a gBRCA mutation

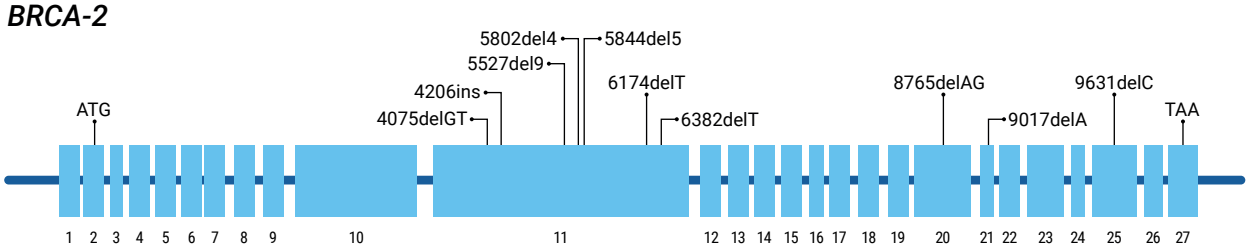
Canonical Mutations in the *BRCA* Genes

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.

Precision Medicine

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]

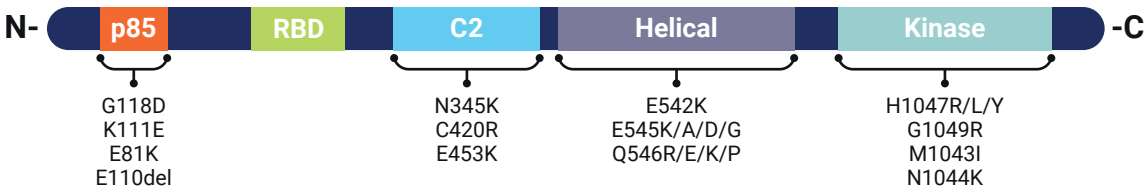
- **~30% of PIK3CA+ patients with mBC have brain metastases**, which are associated with a worse prognosis[18,84]

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.

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Testing for Key Biomarkers in Breast Cancer Summary

Biomarker	Ki67	Prognosis	Prevalence	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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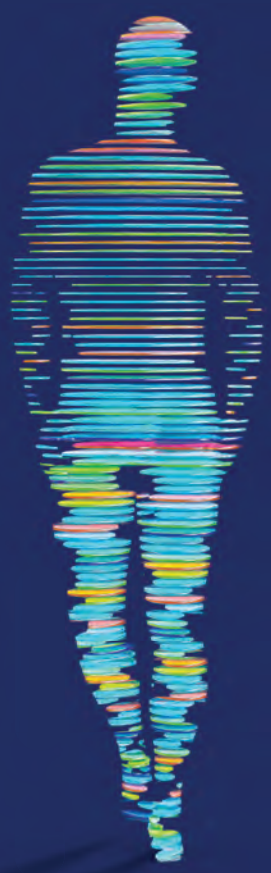
The Importance of a Molecular Diagnosis in mNSCLC

Understanding the essential role of biomarker testing in patient care



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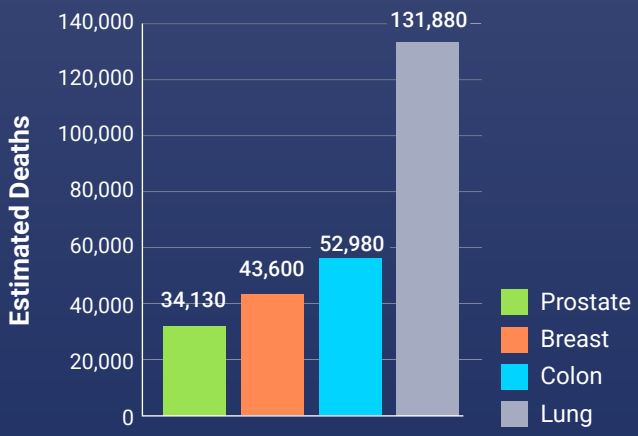
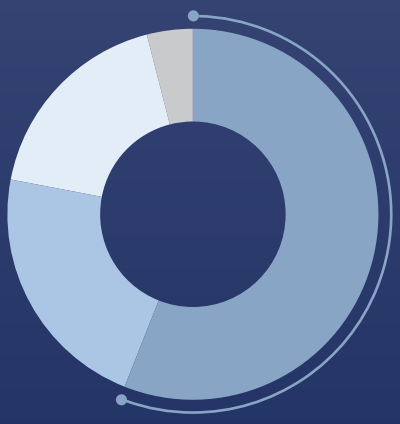
LUNG CANCER OVERVIEW

Lung cancer is the leading cause of cancer-related mortality, and most patients receive a diagnosis of metastatic disease[1]

Estimated Mortality in 2020[1]



Stage at Diagnosis[1]



56%
of patients with lung cancer have **metastatic disease** at diagnosis

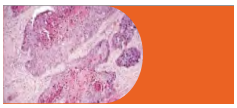
Unknown
Localized
Regional
Metastatic

85% of patients with lung cancer are diagnosed with NSCLC[2]

HISTOLOGIC SUBTYPES OF NSCLC

NSCLC Histologic Subtypes[2,3]

Adenocarcinoma
≈78%



Squamous Cell Carcinoma
≈18%

Other*
≈4% (pictured: large cell carcinoma)

**Includes large cell carcinoma, adenosquamous carcinoma, and others.*

While histology began to guide therapeutic decisions in the early 2000s, molecular subtypes have gained importance in clinical decision-making[2,4-11]

Images reproduced with permission from Beasley MB et al.

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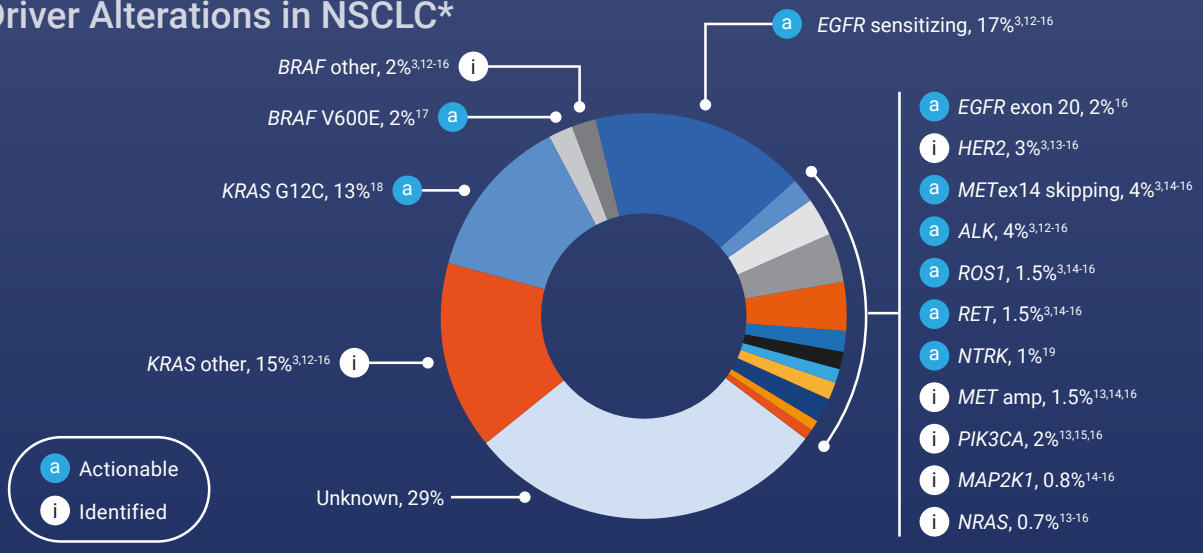
MOLECULAR SUBTYPES OF NSCLC

More than 15 driver alterations have been identified since 2004; these are often mutually exclusive

Driver Alterations in NSCLC*



9 driver alterations have an associated FDA-approved therapy as of June 2021[20]

≈50%
of patients with mNSCLC have an actionable driver[2,3,12-21]

≈20%
of patients with mNSCLC harbor 1 of the 6 less common actionable alterations[2,3,12-21,†]

Per guidelines, the genomic complexity of NSCLC calls for broad molecular profiling to detect actionable biomarkers in eligible patients[22-24]

**Prevalence rates are an average from 6 studies including a total of 8,533 patients and are in accordance with those from The Cancer Genome Atlas (TCGA) Research Network, a joint effort between the National Cancer Institute and the National Human Genome Research Institute. To access the latest TCGA data, please visit: cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga. Please see the appendix to this presentation for the calculations.*
†Less common actionable alterations affect <5% of patients with mNSCLC.

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MOLECULAR DIAGNOSTICS

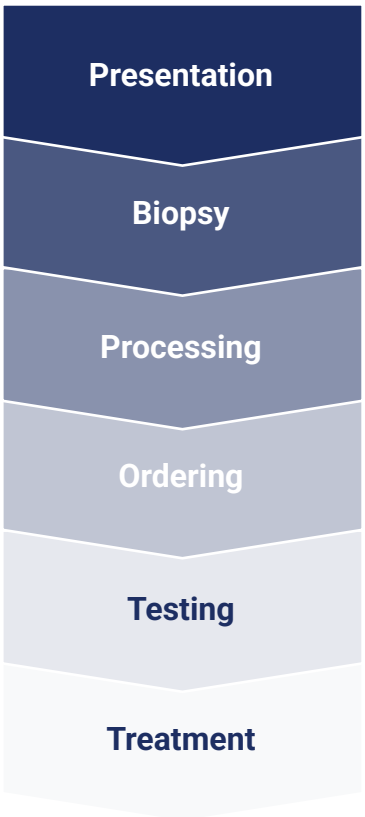
The Multidisciplinary Team (MDT)

Molecular diagnostics is a multistep process requiring collaboration among distinct disciplines[25]

MDT Roles in the Diagnostic Journey for Patients With mNSCLC

Testing Navigation

Monitoring and managing as done by the **nurse and other medical professionals**[25]



Oncologist orders imaging and diagnostic tests after patient presents with suspected mNSCLC[26]

Interventionalist collects tissue or blood sample with potential input from **pathologist** to confirm sufficiency during biopsy via ROSE[25,26]

Laboratory staff prepare sample for testing under **pathologist** supervision who may identify best tissue for subsequent testing[25,26]

The **oncologist**, **pulmonologist/ interventionalist,** and/or **pathologist** may order biomarker testing[25]

Pathologist interprets result(s) and prepares a report after performing testing, with assistance from **laboratory staff**[25]

Oncologist may use biomarker test results to make treatment decisions. **Pathologist** may be consulted for test interpretation[25,26]

Problems at Any Step in the Diagnostic Process May Negatively Impact Patient Care

GUIDELINE RECOMMENDATIONS

Guidelines recommend biomarker testing at initial diagnosis of mNSCLC

2022 NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®)[22,*]

Test all eligible patients up front for:

EGFR	*ALK*
ROS1	*BRAF*
NTRK1/2/3	*RET*
*MET*ex14 skipping	*KRAS*
PD-L1	

NCCN Guidelines (Oncology Guidelines) are evidence- and consensus-based guidelines that are updated continually, with at least 1 update per year[27]

2018 CAP-IASLC-AMP Guidelines[23]

Test all patients for:

EGFR	*ALK*	*ROS1*

Test as part of a broad panel:

BRAF	*RET*	*HER2*
KRAS	*MET*ex14 skipping	

Test for†:

PD-L1

CAP-IASLC-AMP Guidelines (Pathology Guidelines) are evidence-based guidelines[23]

- The next update for the CAP-IASLC-AMP Guidelines is in development and expected in 2023[23,28]

***BRAF*, *NTRK1/2/3*, *RET*, *MET*ex14 skipping, and *KRAS* have all become actionable since the last update of the CAP-IASLC-AMP Guidelines[2,20,23]**

**The NCCN Guidelines for NSCLC provide recommendations for certain individual biomarkers that should be tested and recommend testing techniques but do not endorse any specific commercially available biomarker assays or commercial laboratories.*
†*Opinion; subject of upcoming guideline.*

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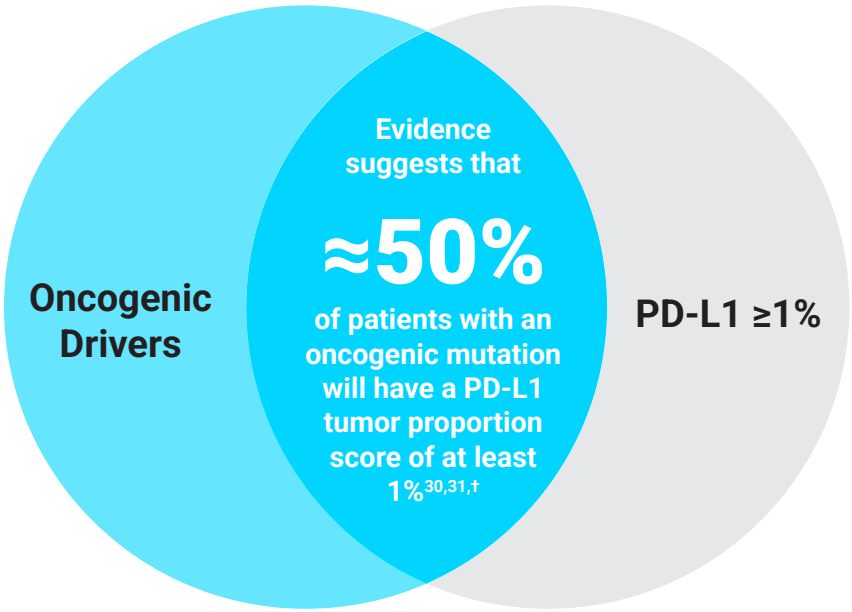
GUIDELINE RECOMMENDATIONS (CONTINUED)

> The NCCN NSCLC Panel strongly advises broader molecular profiling with the goal of identifying rare driver mutations for which effective drugs may already be available"[22,*]

> The NCCN NSCLC Panel recommends that clinicians should obtain molecular testing results for actionable biomarkers before administering first-line ICI therapy, if clinically feasible, including *ALK, BRAF, EGFR*, *MET*ex14 skipping, *NTRK1/2/3*, *RET*, and *ROS1* variants"[22]

An independent retrospective analysis examining the impact of adherence to NCCN Guidelines for testing suggests patients with mNSCLC who receive NCCN Guidelines adherent care had improved outcomes[29]



Importantly, oncogenic drivers are often mutually exclusive, but **the presence of an oncogenic driver is not mutually exclusive with elevated PD-L1 expression**[30-33]

**Broad molecular profiling is defined as molecular testing that identified all (NCCN recommended) biomarkers in either a single assay or a combination of a limited number of assays.*

[†]Based on 2 separate analyses: 1) a prospective analysis conducted in ≈10,000 patients analyzing PD-L1 TPS ≥1% and EGFR, ALK, or KRAS; and 2) a multicenter, registrational study of 214 patients analyzing PD-L1 TPS of 1% and HER2, EGFR, ALK, KRAS, RET, MET, BRAF, or ROS1.

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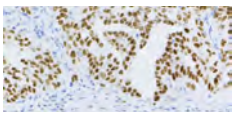
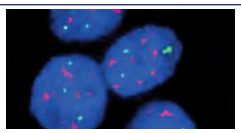
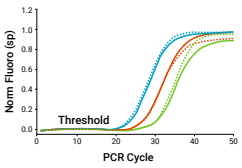
TISSUE REQUIREMENTS FOR BIOMARKER TESTING

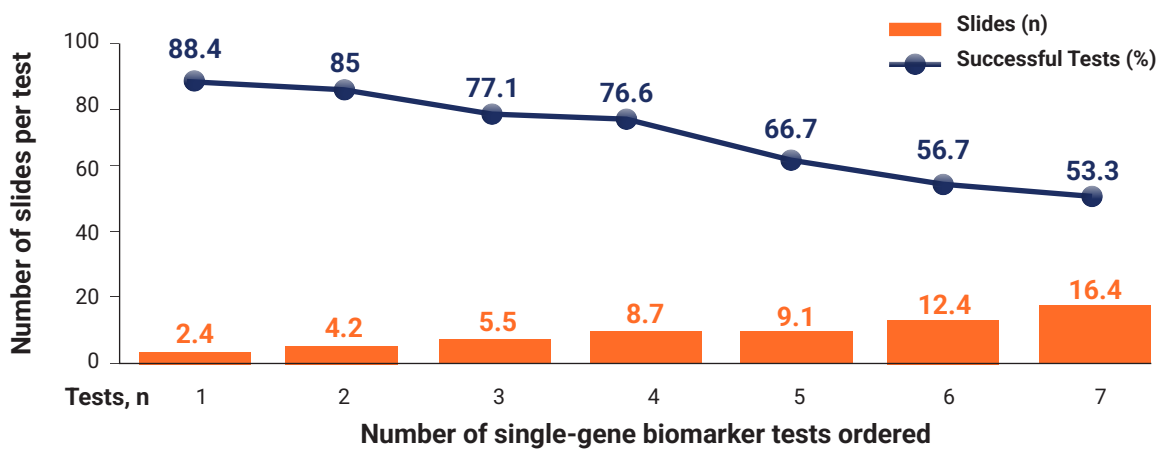
Biopsies may not provide enough tissue to test all biomarkers by single-gene testing approaches

Single-gene testing[20]		Can assess[20]	Biomarkers tested[22,23]	Tissue[20,34-36]
IHC		Protein expression	*ALK, NTRK, PD-L1, ROS1,*	≥100 tumor cells
FISH		Rearrangements, CNVs	*ALK, MET amplification, NTRK, RET, ROS1,*	≥50 tumor cells
RT-PCR		SNVs, indels, known rearrangements	*BRAF V600E, EGFR, KRAS G12C* *While ALK, NTRK, RET, and ROS1 can be detected with targeted RT-PCR assays, these assays are unable to detect novel fusion partners*	≥5% tumor cells

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

Images adapted from Yu J et al. *Sci Rep.* 2019;9(1):7518, Yatabe Y et al. *J Thorac Oncol.* 2019;14(3):377-407, and Kipf E et al. *J Mol Diagn.* 2022;24(1):57-68.

Slide Consumption and Testing Success Rates With Single-Gene Tests[37]



With sequential single-gene testing, ≈50% of patients will not have successful biomarker testing for >7 biomarkers[37]

In a survey, 1 in 3 US oncologists report that inadequate tumor specimens are a barrier to biomarker testing, so obtaining sufficient tissue for biomarker testing during biopsy is critical[38,39]

Precision Medicine

NEXT GENERATION SEQUENCING (NGS)

NGS may overcome some limitations of sequential single-gene testing that may lead to tissue exhaustion

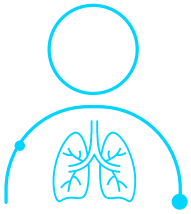
SEQUENTIAL SINGLE-GENE TESTING[37,*]	NGS[37,40,41,†]
8 tests	1 test
8 biomarkers	>50 biomarkers[‡]
17 slides	1-10 slides (≈50-1000 ng DNA)

Intended to depict biomarker testing methodologies. When testing for therapy selection, please consult product prescribing information and FDA-approved companion diagnostics.
*Based on a retrospective study on 1402 samples for single-gene tests done in a large, US-based, Clinical Laboratory Improvement Amendments–certified, commercial reference laboratory from September 2015 to October 2016. †Range is based on the specimen instructions of FoundationOne CDx and a retrospective study on 169 investigational use cases of the Oncomine Dx Target Test done in a large, US-based, Clinical Laboratory Improvement Amendments–certified, commercial reference laboratory from April 2016 to July 2016. Tissue needs vary by assay. ‡Number refers to the number of biomarkers that an NGS assay may be capable of detecting and does not reflect the current number of actionable biomarkers.

NGS assays are not identical[24,37,41-43]

Assays vary by:	The number of biomarkers detected	The types of biomarkers detected	The enrichment method used (specific to targeted assays)	Tissue requirements	Cost

BENEFITS OF NGS



NGS uses
44%-94%
less tissue[37,40,*]

NGS was associated with a
17%-41%
reduction in cost
in a 2017 Medicare study[44,†]

It is important to know what types of alterations your NGS assay can and cannot reliably detect[24]

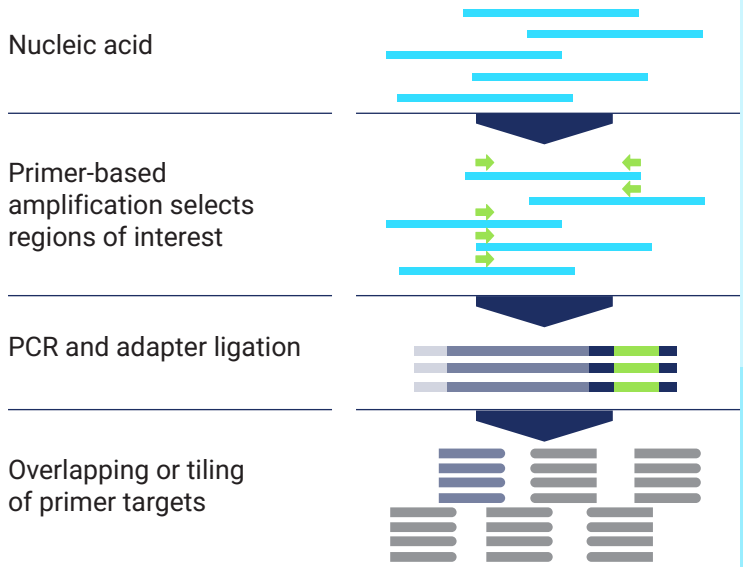
Intended to depict biomarker testing methodologies. When testing for therapy selection, please consult product prescribing information and FDA-approved companion diagnostics.
*Range is based on the specimen instructions of FoundationOne CDx and a retrospective study on 169 investigational use cases of the Oncomine Dx Target Test done in a large, US-based, Clinical Laboratory Improvement Amendments–certified, commercial reference laboratory from April 2016 to July 2016. Tissue needs vary by assay. †Total testing cost for 2066 Medicare-insured patients in 2017.

Precision Medicine

TARGETED NGS ENRICHMENT STRATEGIES

Amplicon-based assays use multiple PCR primers to directly amplify genomic regions of interest[24]

Amplicon-based target enrichment[42]



Pros

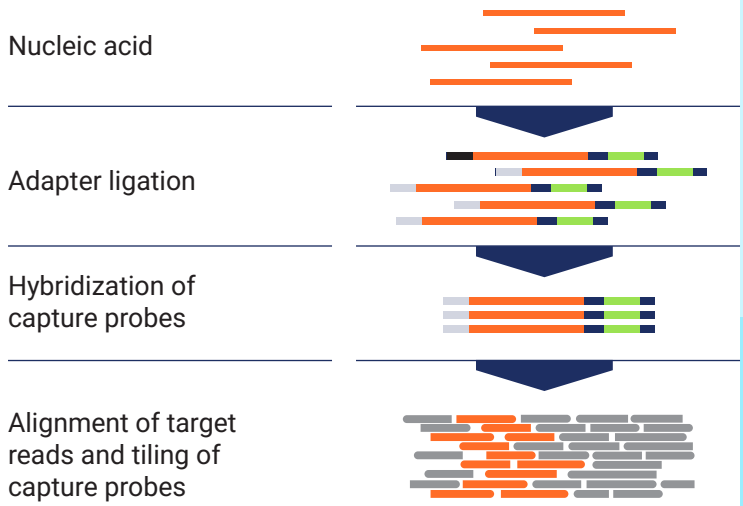
- Efficient for smaller panels[42]
- Can reliably identify point mutations and small indels[24]
- Less nucleic acid required (5-10 ng) and shorter turnaround time (7-10 days) compared to hybrid capture assays[24,42]

Cons

- Cannot reliably detect fusion events or CNVs[24]
- Limitations in the number of genes and regions that can be covered[24]

Hybrid capture-based assays use hybridization to capture large genomic regions and allow a broader assessment of mutations, CNVs, and gene rearrangements incorporated in the panel design[24]

Hybrid capture–based target enrichment[42]



Pros

- Applicable for large assays[40]
- Allow for broader assessment to include CNVs[24]
- Less likely to miss variants[40]
- More uniform and complete coverage[40]

Cons

- Require more nucleic acid (100-200 ng) and longer library preparation and turnaround time (15-20 days) compared with amplicon-based assays[24,42,45]

Image adapted from Church AJ. Next-generation sequencing. In: Tafe L, Arcila M, eds. *Genomic Medicine.* Cham, Switzerland: Springer; 2020:25-40.

Precision Medicine

OPTIMIZING BIOPSY SAMPLE ACQUISITION

Societies* recommend several considerations in optimizing sample acquisition during biopsy[46]

Needle Size

- **EBUS-TBNA: 19- to 22-gauge**
- **Transthoracic FNA:** Up to 25 gauge
- **Transthoracic CNB:** Up to 20 gauge

Passes

- **EBUS-TBNA:** 3-5 passes
- **Transthoracic FNA w/o CNB:** Multiple passes enough for a tissue block

Sample Amount/Volume

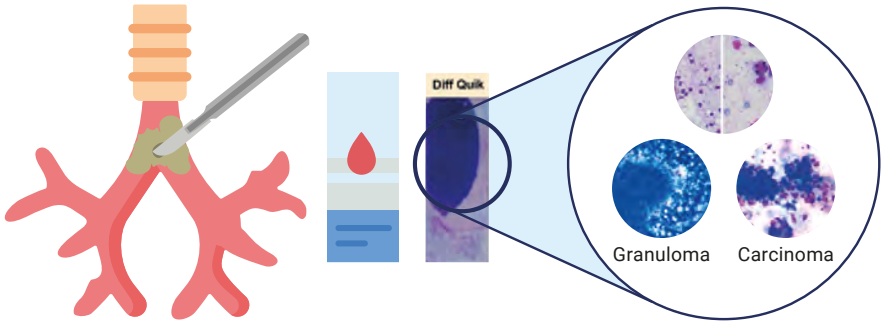
- **Transthoracic CNB:** At least 3 core samples
- As much **pleural fluid** volume as reasonably attainable for cytologic evaluation and ancillary studies

**College of American Pathologists in collaboration with the American College of Chest Physicians, Association for Molecular, American Society of Cytopathology, American Thoracic Society, Pulmonary Pathology Society, Papanicolaou Society of Cytopathology, Society of Interventional Radiology, and Society of Thoracic Radiology.*

RAPID ON-SITE EVALUATION (ROSE)

Multiple societies recommend incorporating ROSE into biopsy procedures[46,*]

ROSE directs the interventionalist in real-time to either acquire more tissue or terminate a sampling procedure once **sufficient material** is acquired[46,47]



An **interventionalist** obtains a tissue specimen, a **cytotechnologist** prepares the slide, and a **cytopathologist** immediately assesses the slide for both adequacy and preliminary diagnosis[46,47]

An MDT is essential in implementing ROSE during tumor biopsy[46]

**Guidelines from the College of American Pathologists in collaboration with the American College of Chest Physicians, Association for Molecular Pathology, American Society of Cytopathology, American Thoracic Society, Pulmonary Pathology Society, Papanicolaou Society of Cytopathology, Society of Interventional Radiology, and Society of Thoracic Radiology.*

Image adapted from Jain D et al. *Arch Pathol Lab Med.* 2018;142:253-262.

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TISSUE INSUFFICIENCY

In some patients, NGS of tissue samples may not be possible because of tissue insufficiency. Tissue insufficiency may occur when:

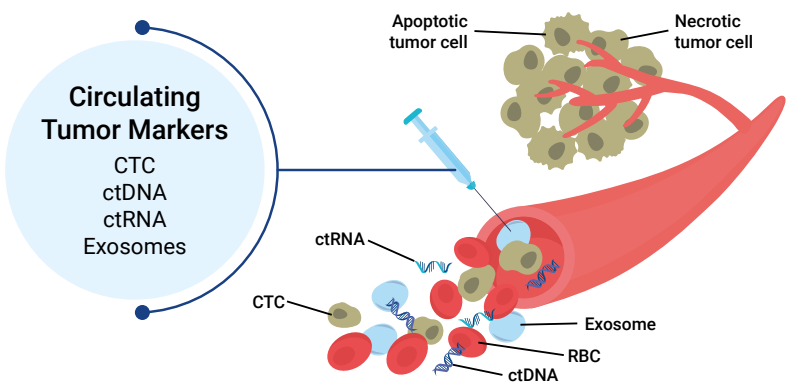
Diagnostic biopsy cannot be obtained[48,49]	Insufficient tissue on initial biopsy[50,51]	Repeat biopsy is not feasible[50]

NCCN recommends that liquid biopsy–based (plasma ctDNA) testing can be considered for eligible patients with mNSCLC in certain specific clinical circumstances[22]

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

LIQUID BIOPSY

Different diagnostic tests performed on biological fluids (eg, blood, saliva, urine), with the aim of investigating the presence of CTCs or ctDNA that can be shed from the tumor[52,53]



Key Characteristics of Liquid Biopsy[52,53]

Advantages

- Is minimally invasive
- Can capture tumor genetic heterogeneity and follow subclonal evolution through serial biopsy
- Potentially represents genetic make-up from entire tumor and metastatic sites
- May have a shorter overall turnaround time than tissue-based NGS relative to the date the test is ordered

Disadvantages

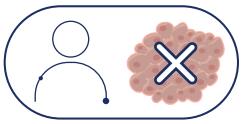
- Cannot directly correlate ctDNA results with histology or cellular phenotype
- Genetic analyses may have biased representation from differential tumor cell turnover
- May be associated with false negatives
- Special processing and handling are required

Image adapted from Qi Z et al. *J Cancer.* 2018;9(18):3417-3426.

IASLC and NCCN Propose 3 Approaches to the Use of Liquid Biopsy (Plasma ctDNA) Testing During Initial Diagnostic Workup in Eligible Patients With mNSCLC

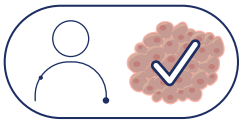
Patients **without tissue** sample for tumor testing

Plasma first approach: Perform liquid biopsy testing first in eligible patients with histologically confirmed mNSCLC. Note that liquid biopsy (plasma ctDNA) testing should not be done in lieu of a histologic tissue diagnosis. Perform rebiopsy for tumor tissue testing in case of a negative result.[22,52] 46% of patients who only received plasma testing had a clinically relevant mutation in one study[54]



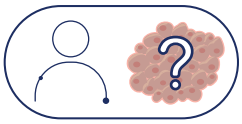
Patients with **adequate tumor** sample

Sequential approach: Test tumor tissue first. Perform liquid biopsy testing in case of incomplete genotyping.[22,52] In one study, the sequential approach increased identification of patients with actionable drivers by 65%[55]



Patients with tumor tissue of **questionable sufficiency**

Complementary approach: Perform liquid and tissue testing simultaneously. The complementary approach may reduce turnaround time and increase the yield of targetable alteration detection[22,52]



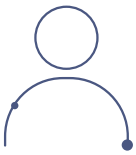
≈30% of samples may be false negative[23]

NCCN recommends that negative plasma ctDNA assay results should be confirmed by tumor tissue testing[22,52]

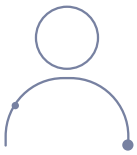
Intended to depict biomarker testing methodologies. When testing for therapy selection, please consult product prescribing information and FDA-approved companion diagnostics.

USE OF LIQUID BIOPSY

Incorporating liquid biopsies into testing algorithms may increase identification of patients with mNSCLC with actionable drivers[52,54,56-58]



13%-19%*
more patients were identified when tissue testing was added to liquid testing



9%-43%*
more patients were identified when plasma testing was added to tissue testing

**Based on 4 studies: The first was a prospective study on 210 patients with aNSCLC enrolled in an IRB-approved plasma NGS genotyping protocol at Memorial Sloan Kettering Cancer Center (New York) and Northern Cancer Institute (Sydney, Australia) from October 21, 2016, to January 1, 2018. The second was a prospective study on 307 patients with mNSCLC undergoing physician discretion SOC tissue genotyping at 1 of 28 North American centers. The third was a prospective study on 186 patients with treatment-naïve aNSCLC who were tested using a well-validated NGS cfDNA panel and SOC tissue testing. The fourth was a prospective study on 323 patients with stage IV NSCLC who underwent routine clinical testing at diagnosis or at disease progression at the Hospital of the University of Pennsylvania from April 1, 2016, to January 2, 2018.*

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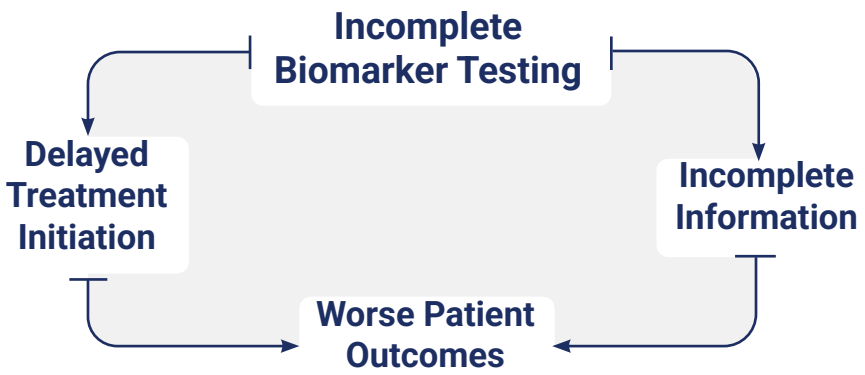
THE PERCEPTION OF BIOMARKER TESTING DOES NOT MATCH REALITY

	Perception[39]		Biomarker Testing Data From EHRs[59]	
Testing is ordered for actionable drivers	**97%**	of HCPs report testing for *EGFR*, *ALK*, *ROS1*, and *BRAF*	**<70%**	Less than 70% of patients were tested for *EGFR*, *ALK*, *ROS1*, *BRAF*, and PD-L1
Testing occurs prior to 1L	**95%**	of HCPs report testing before starting therapy	**<67%**	Fewer than 2/3 of patients had test results available before 1L treatment initiation
NGS is used most of the time	**93%**	of HCPs reported using NGS more than single-gene testing	**<33%**	Fewer than 1/3 of patients received NGS

Incomplete biomarker testing may lead to **delayed treatment initiation** if rebiopsy is needed or if treatment decisions are being made with **incomplete information,** both of which can be associated with **worse patient outcomes**[29,37,44,51,60,61]



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OPPORTUNITIES TO IMPROVE THE DIAGNOSTIC JOURNEY

Diagnostic hurdles

Obtaining sufficient tissue during biopsy

Inability to obtain a tissue biopsy

Tissue exhaustion

Long turnaround time

Potential for improvement

ROSE[46,47]	Allows assessment of tissue adequacy during biopsy	**Liquid biopsy**[52,53]	Minimally invasive procedure that provides tumor material for biomarker testing
NGS[24]	Allows simultaneous testing for multiple oncogenic drivers with less tissue than sequential gene testing for multiple biomarkers	**Reflex testing by pathologists**[24,51,62,63]	Eliminates waiting time for requesting physician to order molecular testing

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SUMMARY

NCCN, AMP, IASLC, and CAP agree: **Biomarker testing is recommended for eligible patients with mNSCLC**[22,23]

Biomarker testing depends on **MDT collaboration and communication**[25,64]

An independent retrospective analysis suggests patients with mNSCLC who received **care consistent with NCCN Guidelines had improved outcomes**[29]

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Molecular Diagnostics in Personalized Cancer Care

Best Practices and Overcoming Challenges

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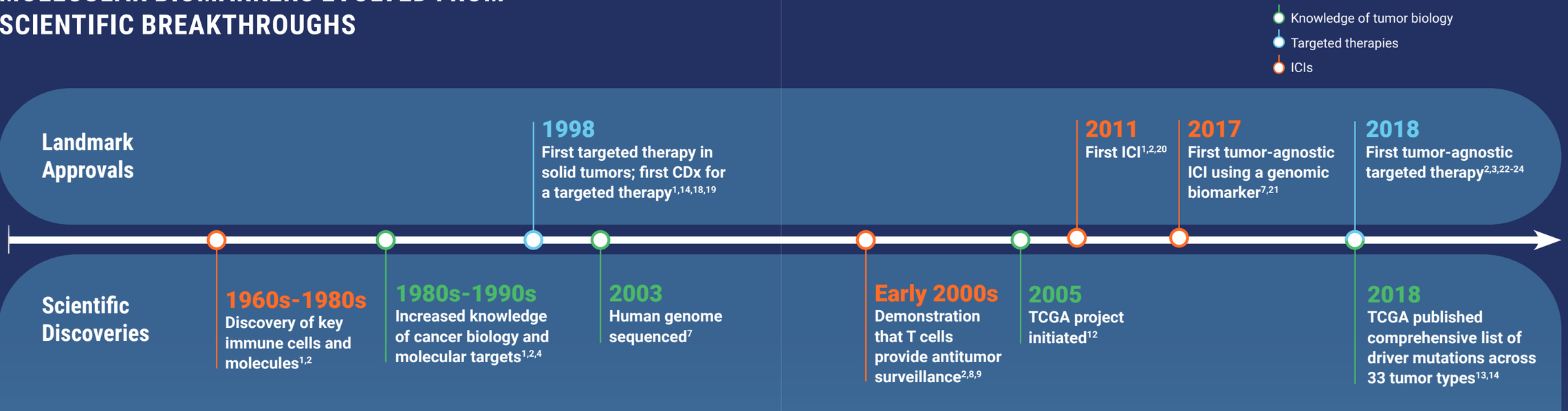
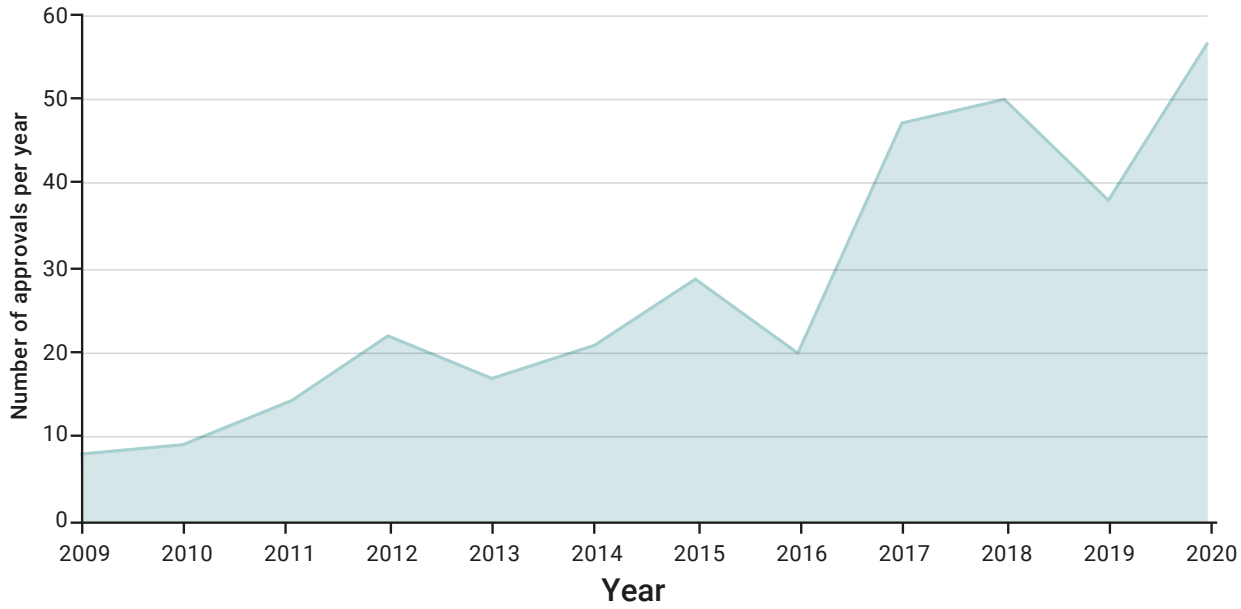
PRECISION ONCOLOGY AND THE USE OF MOLECULAR BIOMARKERS EVOLVED FROM SCIENTIFIC BREAKTHROUGHS



CDx, companion diagnostic; ICI, immune checkpoint inhibitor; TCGA, The Cancer Genome Atlas.

INCREASE OF THERAPEUTIC OPTIONS IN ONCOLOGY

Total Number of Anticancer Therapies Approved by the FDA Between 2009 and 2020[25]



Between 2009 and 2020, there were 332 new anticancer therapy approvals, some of which require biomarker testing[25]

As of June 2022, there are[3,26]:

≥70

FDA-approved biomarker-linked indications

43

actionable genomic alterations

28

cancer types treatable by Precision Oncology

Biomarker testing is a fundamental component of precision oncology[3]

FDA, US Food and Drug Administration.

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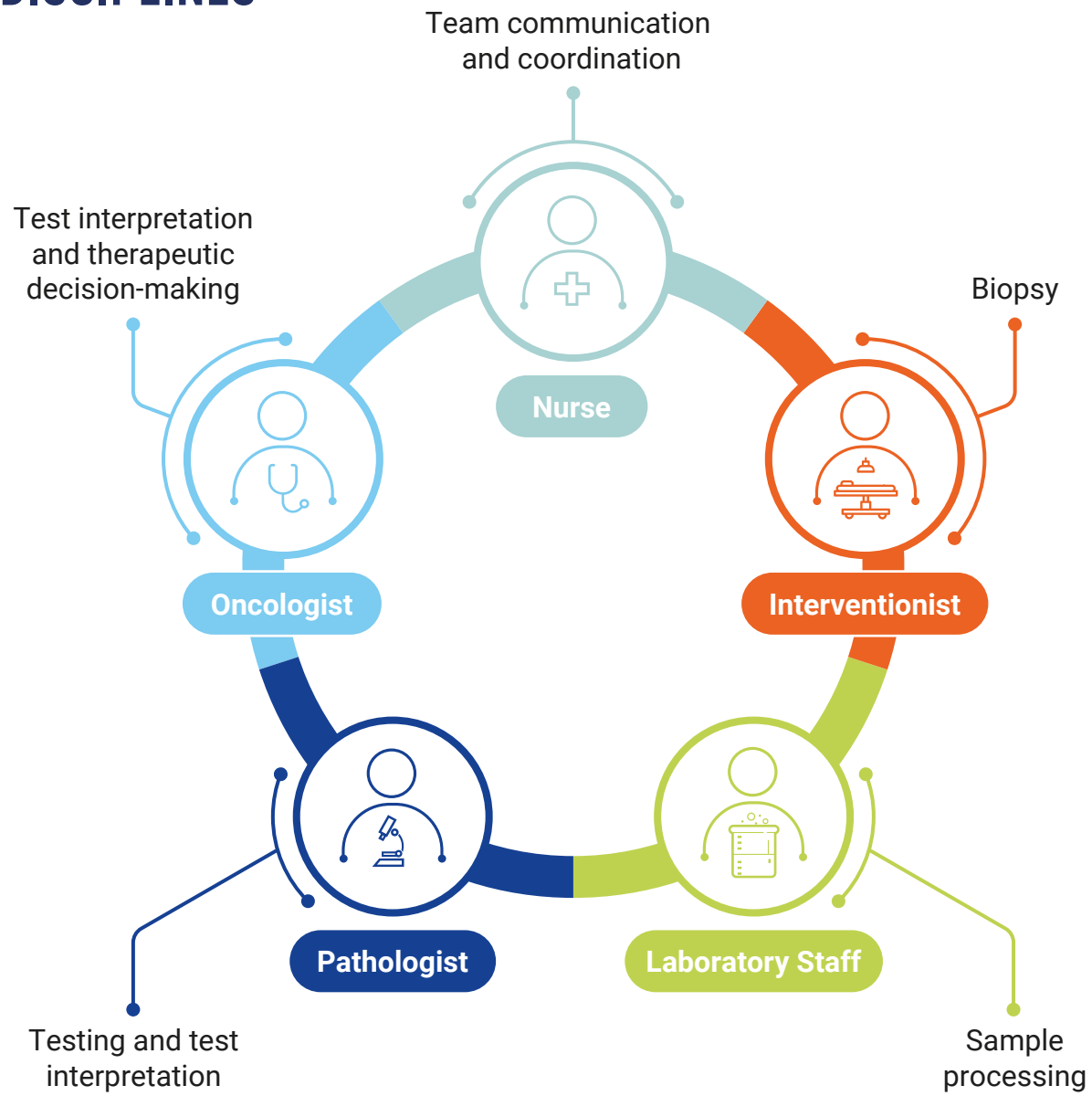
MOLECULAR DIAGNOSTICS IS A MULTISTEP PROCESS REQUIRING COLLABORATION AMONG DISTINCT DISCIPLINES[27,28]

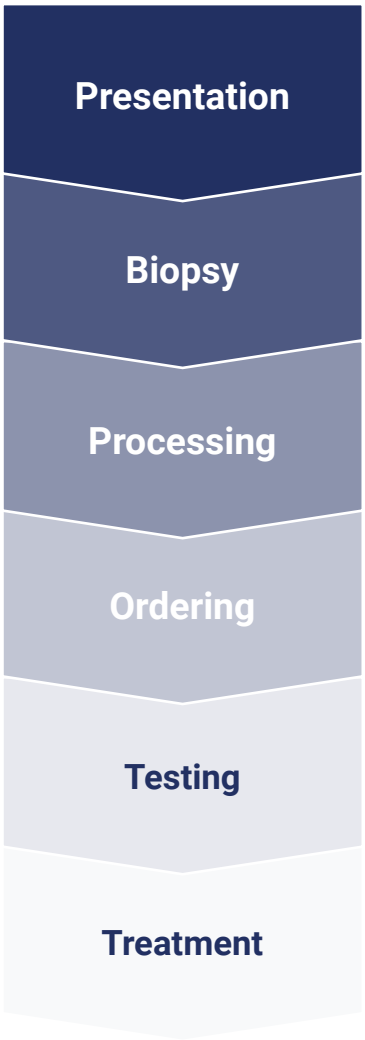


Communication and coordination between members of the core and expanded multidisciplinary team (MDT) are important to the implementation of precision oncology[27,29,30]

DIAGNOSTIC JOURNEY IN PATIENTS WITH METASTATIC CANCER

Testing Navigation

The **Oncology nurse navigator** is a key point of contact between the patient and the MDT and aims to facilitate team communication and coordination during testing[27]



The **oncologist** orders imaging and diagnostic tests after patient presents with suspected metastatic cancer[31]

The **interventionalist** collects tissue with potential input from the **pathologist** to confirm sufficiency[27,31]

The **laboratory staff** prepares a sample for evaluation and testing under **pathologist** supervision[27,31]

The **oncologist**, **surgeon/interventionalist,** and/or **pathologist** may order testing[27]

The **pathologist** interprets result(s) and prepares a report after performing testing, with assistance from **laboratory staff**[27]

The **oncologist** may use biomarker test results to make treatment decisions. The **pathologist** may be consulted for test interpretation[27,31]

SUCCESSFUL BIOMARKER TESTING DEPENDS ON KEY FACTORS

Testing **tissue** of sufficient quantity and quality[32]

Use of appropriate **tests**[3]

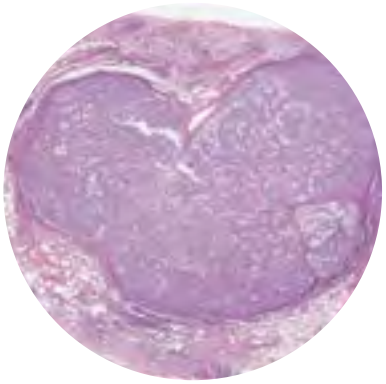
Ordering process for actionable biomarkers[3]

Access to clear and searchable **report** data[33]

TESTING FOR BIOMARKERS GENERALLY REQUIRES 20% OF TUMOR NUCLEI IN SAMPLES[34,35]

Lung Adenocarcinoma Example

- Accurate detection of biomarkers may be difficult in samples with low numbers of tumor cells[36]
- Interobserver variability and misestimation of tumor content are potential challenges[34,36]
  - A study demonstrated that 38% of samples have overestimated tumor content[36]
- Training may help lower discrepancies in estimating tumor content[34]



Lung Adenocarcinoma Example[34]

Tumor content

30%–40%

Figure used with permission from Mikubo M et al. *J Thorac Oncol*. 2020;15(1):130-137.

Biopsy Choice May Impact Testing Outcomes

Biopsy Site

- Biomarker discordance between the primary tumor and a metastatic site may occur[37,38]
- Additional/different drivers/ mutations may occur through clonal evolution over the course of the disease[39-41]

Bone Biopsy

- Bone biopsy requires decalcification, which may impair sample yield and integrity, potentially negatively impacting biomarker testing outcomes[42]

Rebiopsy

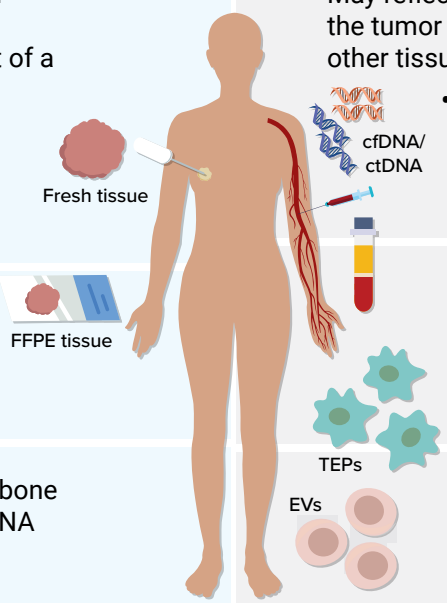
- Rebiopsy after disease progression may provide important and/or new information[43]
- In certain cancers, receptor status may change over the course of the disease[44-46]

LIQUID BIOPSY OVERVIEW

Key Characteristics of Liquid Biopsy[47,48]

ADVANTAGES	DISADVANTAGES
✓ Is minimally invasive	✗ Cannot directly correlate ctDNA results with histology or cellular phenotype
✓ Can capture tumor genetic heterogeneity and follow subclonal evolution through serial biopsy	✗ Genetic analyses may have biased representation from differential tumor cell turnover
✓ Potentially represents genetic make-up from entire tumor and metastatic sites	✗ May be associated with false negatives
✓ May have a shorter overall TAT than tissue-based NGS relative to the date the test is ordered	✗ Special processing and handling required

Tissue Biopsy Testing	Liquid Biopsy Testing
May provide a snapshot of the cellular and molecular characteristics of one part of a single tumor[49] • *Does not provide information from all cancer cells*	May reflect overall genomic landscape of the tumor and all metastatic sites (bone or other tissues)[51,52] • *Does not provide information on TME[53]*
May miss an alteration if it is not present in the tested sample[50]	May miss an alteration if ctDNA concentration is below the LOD, leading to a false negative • *ctDNA levels may vary significantly[52,54,55]*
Processing of biopsies of bone metastases may lead to DNA degradation[50]	CTCs and ctDNA levels may be impacted by the number and sites of metastases, including bone[52,54-56]



cfDNA, cell-free DNA; CTCs, circulating tumor cells; ctDNA, circulating tumor DNA; EVs, extracellular vesicles; FFPE, formalin-fixed, paraffin-embedded; LOD, limit of detection; TEPs, tumor-educated platelets; TME, tumor microenvironment.

Image adapted from Alba-Bernal A et al. *EbioMedicine*. 2020;62:103100.

CHARACTERISTICS OF A GOOD BIOMARKER TEST

Clinical Guidelines and Expert Opinions[57-63]

- Is **actionable, prognostic, and/or predictive**[57,58]
- Provides **reproducible results** (>95%)[59,60]
- Is supported by the highest level of evidence[57]
- Has **tightly controlled specimen** collection, handling, and processing[57]
- Has **predetermined cutoff** points/categories[57]
- Delivers **timely** results that impact treatment decisions[61-63]
- Possesses sufficient **sensitivity, specificity, accuracy, and precision (<1% to 5% LOD) to detect actionable biomarkers**[57-60]

ESSENTIAL QUESTIONS ABOUT A BIOMARKER TEST

	Analytical Validity	Clinical Validity	Clinical Utility
Definition[64]	The test is able to accurately and reliably measure the presence or absence of a biomarker in the appropriate specimen	The test can accurately and reliably identify a biologically defined disorder or separate into two or more groups with distinct clinical or biological outcomes or differences	The test has high levels of evidence that use of the biomarker can result in guiding clinical decisions that result in improved clinical outcomes compared with those if the biomarker test results were not applied
Essential question[48]	*Is the test for the biomarker sensitive, accurate, and reliable?*	*Does the test accurately identify a disorder with distinct clinical or biological outcomes?*	*Is the test predictive of clinical outcomes?*

Analytical and clinical validity is the foundation of *all* biomarker testing.
In addition, to gain FDA approval, a CDx must be evaluated in a clinical study[48,64-66]

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

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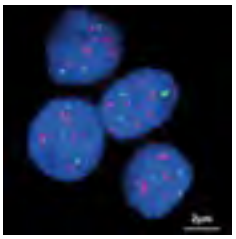
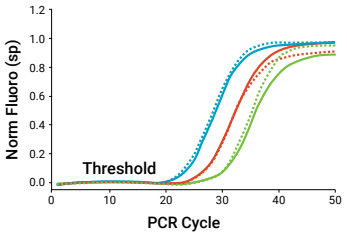
BIOMARKER TESTING METHODS[3]

IHC

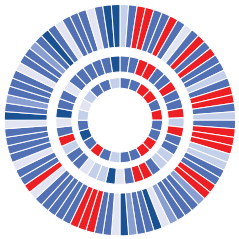
FISH



RT-PCR



NGS



Images adapted with permission from Yu J et al. *Sci Rep*. 2019;9(1):7518, Yatabe Y et al. *J Thorac Oncol*. 2019;14(3):377-407, Kipf E et al. *J Mol Diagn*. 2022;24(1):57-68, and Goldbio. https://www.goldbio.com/articles/article/how-to-fragment-DNA-for-NGS. Accessed April 28, 2022.

USE OF APPROPRIATE TESTS

- Some biomarkers may be detected more reliably by **some specific testing technologies** than by others[3,60]
- **Gene rearrangements** can be reliably detected by **FISH** and **RNA**-based NGS; enrichment strategy for a **DNA**-based NGS assay impacts the detection of **fusions**[3,60]

- Understanding **assay limitations** is critical to identifying patients with actionable biomarkers[3]
- **The American Society of Clinical Oncology (ASCO)** recommends being **familiar** with the genomic testing platforms available to ensure **fusion testing** is performed when indicated[3]

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

NGS MAY BE USED TO IDENTIFY THERAPEUTICALLY ACTIONABLE ALTERATIONS[3]

ASCO recommends multigene panel-based genomic testing or NGS for:

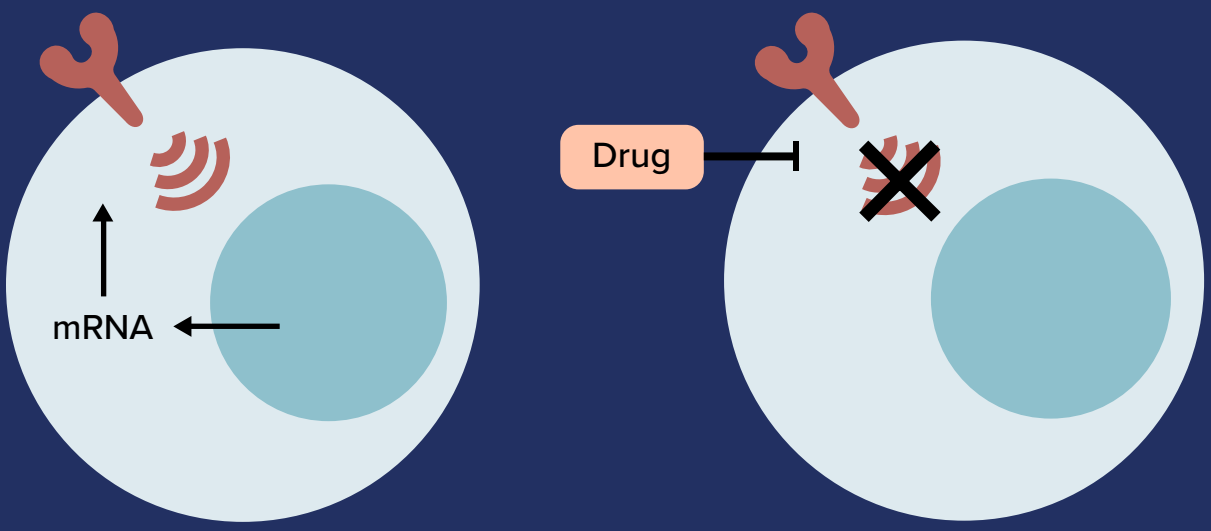
Patients eligible for an approved genomic biomarker–linked therapy	Patients potentially eligible for more than 1 approved genomic biomarker–linked therapy
To detect tumor-agnostic actionable biomarkers like dMMR and/or MSI-H, TMB-H, and *NTRK* fusions, which may not be detected by single-gene tests	To provide the most efficient use of limited tumor biopsy tissue

ASCO recommends using NGS for the most efficient utilization of limited biopsy tissue; it may allow simultaneous testing for multiple approved targeted therapies

dMMR, deficient mismatch repair; FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; MSI-H, microsatellite instability-high; NTRK, neurotrophic tyrosine receptor kinase; RT-PCR, real-time polymerase chain reaction; TMB-H, tumor mutation burden-high.

BIOMARKERS FOR TARGETED THERAPIES ARE DIFFERENT THAN BIOMARKERS FOR ICIS

Targeted Therapy Biomarkers



Images adapted with permission from Camidge DR et al. *Nat Rev Clin Oncol*. 2019;16(6):341-355.

Targeted therapies inhibit cells harboring a specific genomic alteration or protein[3]

Responses to targeted therapies may be primarily influenced by the presence of a driver alteration assumed to be present in most tumor cells[67-70]

Biomarkers for targeted therapies[67]:

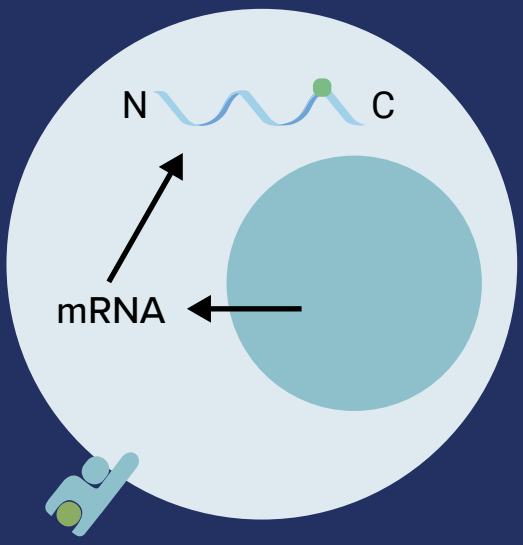
- May be categorial or continuous depending on the alteration (eg, mutation or amplification)[68,71,a]
- Are assumed to be present in most tumor cells[68]

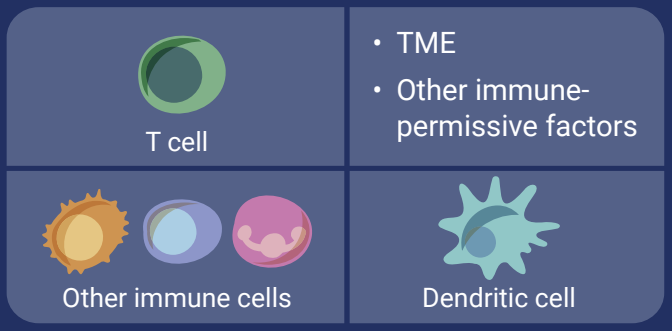
[a]Except for gene amplifications, which are continuous.
mRNA, messenger RNA.

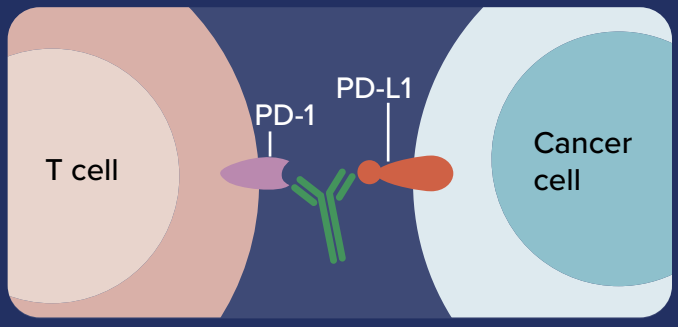
Precision Medicine

BIOMARKERS FOR TARGETED THERAPIES ARE DIFFERENT THAN BIOMARKERS FOR ICIS

ICI Biomarkers







ICIs reduce T-cell exhaustion by disrupting the immune checkpoint[72-75]

Responses to ICIs may be influenced by complex interactions between multiple different cell types[67,76]

Biomarkers for ICIs are[67]:

- Continuous with arbitrary cutoffs[77-79]
- Spatially and temporally variable[79,80]

PD-1, programmed cell death protein 1; PD-L1, programmed death ligand 1.

OVERVIEW OF SELECT BIOMARKER TESTING CHALLENGES

Failure to obtain **sufficient tissue** during biopsy

- Core needle biopsies (CNBs) may provide inadequate malignant tissue[81]
- Biomarker discordance between the primary tumor and a metastatic site may occur[82-84,a]
- Bone biopsies may have increased odds of containing insufficient tumor cells[81]

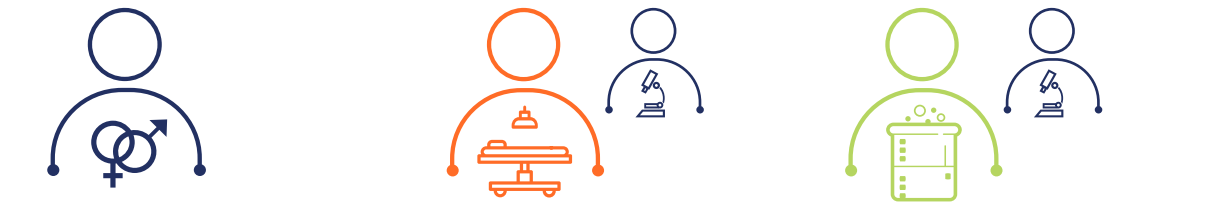
In a survey:

of oncologists cited **tissue sufficiency** as a barrier to multimarker tumor panel testing[32,b]

[a]Based on a meta-analysis of 61 studies including more than 5,700 patients with metastatic colorectal cancer.[32]
[b]Based on the 2017 National Survey of Precision Medicine in Cancer Treatment by the National Cancer Institute. A total of 1,281 medical oncologists participated in this survey.[32]

Overall complexities associated with biomarker testing

- Failure to obtain sufficient tissue during biopsy[32]
- MDT communication[85]
- Multiple testing options[86]
- Guideline differences[86]



OVERVIEW OF SELECT BIOMARKER TESTING CHALLENGES

Extensive TAT

In some cancers with multiple biomarkers, studies suggest sequential single-gene testing may contribute to tissue exhaustion, potentially leading to:

- Patients *not* receiving testing for all biomarkers
- Prolonged TAT for all biomarkers (relative to a multigene panel)[87,88]

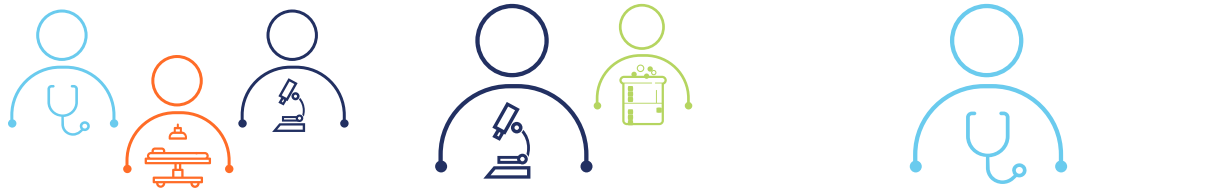
Multigene panels may have TATs of

>10 days[88]

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

Overall complexities associated with biomarker testing

- Confusing/ narrative reports[88]
- NGS report interpretation[32,33,89-92]



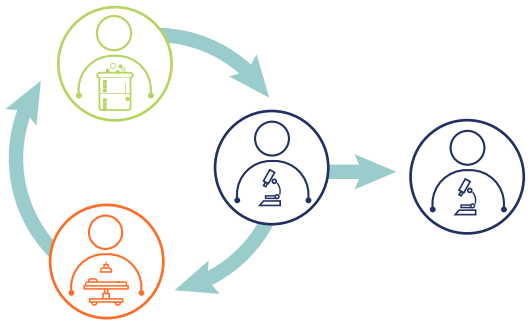
Ordering[27] → Testing[27] → Treatment[27,31]

POTENTIAL SOLUTIONS TO OVERCOME CHALLENGES

Failure to obtain **sufficient tissue** during biopsy

ROSE May Improve Biopsy Yield

Implementation of ROSE has been associated with an **increase in diagnostic yield**



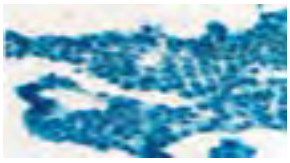
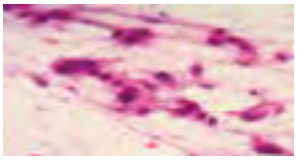
Up to

10% in cytology procedures[93,a]

≈6% in endobronchial ultrasound–guided transbronchial needle aspiration (EBUS-TBNA) procedures[94,b]

- With ROSE at a single center, presence of tumor material was confirmed in 86% of biopsies, 96% of which were sufficient for molecular testing[95,c]
- In one study with ROSE, ≈98% of samples obtained were deemed adequate[96,d]
  - Some studies report that the diagnostic yield and accuracy were comparable in procedures done with and without ROSE[97,98]

ROSE Stains Show Different Cytologic Details

Diff-Quik	Rapid Papanicolaou	H&E	Toluidine Blue
			
• Stain of choice for ROSE	• Most commonly used stain in cytopathology • Very reliable and may be applied to various cytologic preparations	• Standard stain for histopathologic evaluation; rapid H&E stain for cytologic specimens • Widely used for intraoperative frozen service	• A basic thiazine metachromatic dye with high affinity for acidic tissue components

Images adapted with permission from Cai G. Facility, equipment, specimen preparation, and stains. In: Cai G, Adeniran AJ, eds. *Rapid On-site Evaluation (ROSE)*. Cham, Switzerland: Springer Cham; 2019:13-27 and Kim K et al. *J Med Dent Sci*. 2005;52:163-170

[a]Based on a retrospective study of 144 fine needle aspiration (FNA) and CNB specimens.

[b]Based on a prospective study of 348 patients.

[c]Based on a prospective study of 79 cases from 56 patients who underwent FNA or CNB at the University of Pittsburgh Medical Center Hillman Cancer Center.

[d]Based on a retrospective study of 12 patients who underwent EBUS-TBNA thyroid biopsy from February 2010 to February 2013 at the Michael E. DeBakey Veterans Affairs Medical Center and the New York Methodist Hospital.

H&E, hematoxylin and eosin; ROSE, rapid on-site evaluation.

Extensive **TAT**

Reflex Testing Can Help Streamline Biomarker Testing

Reflex testing is the automatic addition of tests in the SOPs by pathologists[99] in specific situations, such as:

An equivocal HER2 IHC result in breast cancer[43]

Reflex testing may be integrated into the electronic health record[100]

Reflex testing is dependent on the cancer type, staging, and institution protocol[101,102]

Reflex Testing May Reduce TAT[102,103]

A retrospective review of 166 patients diagnosed with **lung adenocarcinoma between 2016 and 2018** at a community center assessed biomarker testing rates and TATs for molecular testing[103]

Reflex ordered testing was implemented in February 2017[103]

TATs were compared before and after reflex testing implementation[103]

TAT was defined as the date of the anatomic pathology report confirming lung adenocarcinoma diagnosis to the date of the final molecular diagnostics report[103]

TAT before reflex testing[103]

52.6 days

TAT with reflex testing[103]

15.6 days

Reduced TAT after reflex testing has been observed in other tumor types as well[102]

HER2, human epidermal growth factor receptor 2; SOPs, standard operating procedures.

POTENTIAL SOLUTIONS TO OVERCOME CHALLENGES (CONTINUED)

Overall complexities associated with biomarker testing

MTBs May Help Navigate the Complexities of Precision Oncology

Cancer treatment recommendations from MTBs may be based on many factors, including[104]:

- Tumor type
- Molecular alterations
- Performance status
- Comorbidities

Many specialties may be part of an MTB to help foster discussion[104]

- Clinical oncologists
- Pathologists
- Geneticists — *to facilitate discussions on germline mutations*
- Bioinformaticians and molecular biologists — *to aid in interpretation of big data sets*
- Pharmacists

Real-world evidence from a retrospective review of 782 patients with solid tumors tested with NGS in a tertiary care center suggests MTBs may help in appropriate and actionable clinical decision-making[105]

MTB, molecular tumor board.

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All rights reserved. Accessed June 21, 2022. To view the most recent and complete version of the guideline, go online to NCCN.org. NCCN makes no warranties of any kind whatsoever regarding their content, use or application and disclaims any responsibility for their application or use in any way. **44.** Shachar SS et al. *Clin Breast Cancer*. 2016;16(3):e43-e48. **45.** Swanton C. *Cancer Res*. 2012;72(19):4875-4882. **46.** Gerlinger M et al. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. *N Engl J Med*. 2012;366(10):883-892. **47.** Rolfo C et al. *J Thorac Oncol.* 2021;16(10):1647-1662. **48.** Merker JD et al. *J Clin Oncol*. 2018;36(16):1631-1641. **49.** Tay TKY, Tan PH. *Arch Pathol Lab Med*. 2021;145(6):678-686. **50**. Yeung C et al. *Cancer Metastasis Rev*. 2016;35(3):427-437. **51.** Arneth B. *BMC Cancer*. 2018;18(1):527. **52**. 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SUMMARY

Precision oncology has contributed to improved care for patients[3]

Each member of the MDT is important to biomarker testing **collaboration and communication**[27]

Improving MDT communication and collaboration may increase the number of patients receiving biomarker-informed care[27]

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MOLECULAR DIAGNOSIS IN **LUNG CANCER**

Knowledge Check 1

1 **In 2022, ~50% of patients with mNSCLC have an actionable alteration in 1 of 9 driver genes for which NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) recommends testing. What are the driver genes?**

_____, _____, _____, _____, _____,

_____, _____, _____, _____

2 **At least one of the actionable driver alterations in lung cancer may be classified as:**

a) Rearrangements, SNVs, indels, or exon skipping
b) SNVs, indels, or rearrangements
c) Rearrangements, SNVs, or CNAs
d) None of the above

3 **Which type of NGS assay cannot reliably detect fusion events or CNVs?**

a) DNA-based amplicon assays
b) Hybrid capture-based assays
c) FISH
d) RNA-based amplicon assays

4 **What percentage of patients with mNSCLC who are positive for an oncogenic driver will also have a PD-L1 tumor proportion score of at least 1%?**

a) 10%
b) 20%
c) 50%
d) 80%

5 **True or False: The National Comprehensive Cancer Network® (NCCN®) NSCLC Panel recommends that clinicians should obtain molecular testing results for actionable biomarkers before administering first-line ICI therapy, if clinically feasible, including *ALK*, *BRAF*, *EGFR*, *MET*ex14 skipping, *NTRK1/2/3*, *RET*, and *ROS1* variants**

a) True
b) False

ANSWERS

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1 In 2022, NCCN Guidelines recommends testing for *ALK, BRAF, EGFR, ERBB2 (HER2), KRAS, MET*ex14 skipping, *NTRK*, *RET,* and *ROS1* (page 5)[1]

2 **A.** Actionable driver alterations in NSCLC include rearrangements (ALK, ROS1, RET, NTRK), SNVs (EGFR sensitizing, KRAS, BRAF, ERBB2 (HER2)), indels (EGFR sensitizing, EGFR exon 20, ERBB2 (HER2)), and exon skipping events (METex14 skipping) (page 3, page 7)[2]

3 **A.** Amplicon-based target enrichment is efficient for smaller panels and can reliably identify point mutations and small indels, with less nucleic acid required (5-10 ng) and a shorter turnaround time (7-10 days). However, this technique cannot reliably detect fusion events or copy number variants when using DNA. In addition, these assays have limitations in the number of genes and regions that can be covered (page 9)[3,4]

4 **C.** Oncogenic drivers are often mutually exclusive, but the presence of an oncogenic driver is not mutually exclusive with elevated PD-L1 expression. Evidence suggests that approximately 50% of patients with an oncogenic mutation will have a PD-L1 tumor proportion score of at least 1% (page 6)[5-8]

5 **True.** NCCN NSCLC Panel recommends that clinicians should obtain molecular testing results for actionable biomarkers before administering first-line ICI therapy, if clinically feasible, including *ALK*, *BRAF*, *EGFR*, *MET*ex14 skipping, *NTRK1/2/3*, *RET*, and *ROS1* variants (page 5)[1]

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ALK; anaplastic lymphoma kinase; BRAF, v-raf murine sarcoma viral oncogene homolog B1; CNA, copy number alteration; CNV, copy number variation; DNA, deoxyribonucleic acid; EGFR, epidermal growth factor receptor; ERBB2, erb-b2 receptor tyrosine kinase 2FISH, fluorescence in situ hybridization HER2, human epidermal growth factor receptor 2; ICI, immune checkpoint inhibitor; KRAS, Kirsten rat sarcoma viral oncogene homolog; METex14, MET exon 14; mNSCLC, metastatic non-small cell lung cancer; NGS, next-generation sequencing; NCCN, National Comprehensive Cancer Network® (NCCN®); NTRK, neurotrophic tropomyosin receptor kinase; PD-L1, programmed death-ligand 1; RET, ret proto-oncogene; ROS1, receptor tyrosine kinase; SNV, single-nucleotide variant. NCCN makes no warranties of any kind whatsoever regarding their content, use or application and disclaims any responsibility for their application or use in any way. To view the most recent and complete version of the guideline, go online to NCCN.org.

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MOLECULAR DIAGNOSIS IN **LUNG CANCER**

Knowledge Check 2

1 **What proportion of US oncologists reported that inadequate tumor specimens were a barrier to biomarker testing?**

a 1 in 2
b 1 in 3
c 1 in 7
d 1 in 10

2 **What is the clinical utility of performing rapid on-site evaluation (ROSE) during biopsy procedures for patients with suspected NSCLC?**

a Provide preliminary diagnosis
b Provide a molecular diagnosis
c Ensure sample adequacy
d A and C
e All of the above

3 **True or False: Next-generation sequencing (NGS) uses 17%-41% less tissue than sequential single-gene testing in patients with metastatic NSCLC.**

a True
b False

4 **Under what specific clinical circumstances do NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) recommend considering liquid biopsy–based (plasma ctDNA) testing?**

a Diagnostic biopsy cannot be obtained
b Insufficient tissue on initial biopsy
c Repeat biopsy is not feasible
d All of the above

5 **Adding plasma testing to tissue testing may:**

a Increase turnaround time
b Increase identification of patients with actionable driver alterations
c None of the above

ANSWERS

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1 **B.** In a study, 1 in 3 US oncologists reported that inadequate tumor specimens are a barrier to biomarker testing, so obtaining sufficient tissue for biomarker testing during biopsy is critical (page 7)[1,2]

2 **A and C.** ROSE is an ancillary procedure done during a biopsy, wherein small biopsy samples are rapidly stained and immediately assessed for diagnostic material (page 10)[3,4]

3 **False.** NGS uses less tissue and may cost less than sequential single-gene testing. Specifically, NGS uses 44%-94% less tissue than sequential single-gene testing. NGS was associated with a 17%-41% reduction in cost based on a 2017 Medicare study (page 8)[5-7]

4 **All of the above.** NCCN Guidelines® recommend considering liquid biopsy-based (plasma ctDNA) testing in patients with mNSCLC if a diagnostic biopsy cannot be obtained, there is insufficient tissue on initial biopsy, and/or a repeat biopsy is not feasible (page 11)[8-12]

5 **B.** In one study, performing plasma testing in patients with incomplete genotyping increased identification of patients with an actionable driver by 65%. Additionally, performing plasma testing and tissue testing simultaneously in patients with tumor tissue of questionable sufficiency may reduce turnaround time and increase the yield of targetable alteration detection (page 12)[8,13,14]

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ctDNA, circulating tumor deoxyribonucleic acid; NCCN, National Comprehensive Cancer Network® (NCCN®); NSCLC, non-small cell lung cancer.
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BIOMARKER TESTING IN **BREAST CANCER**

Knowledge Check 1

1 **Categorization of Select Biomarkers in Breast Cancer**

Biomarker	Prognostic	Predictive	Susceptibility
ER/PR			
HER2			
Ki67			
BRCA1/2			
PD-L1			
PIK3CA			

2 **Which of the following statements are true about PD-L1 expression in mBC? (Choose all that apply)**

a Choice of PD-L1 antibody can affect the results
b PD-L1 can inform treatment decisions for all patients with mBC
c Results are observer dependent
d A CPS score of ≥15% is considered informative

3 **PIK3CA mutation is found in what percentage of patients with breast cancer?**

a 5%
b 70%
c 36%
d 25%

4 **True or False: HER2 and PIK3CA mutations are generally stable during the course of the disease.**

a True
b False

5 **NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) recommend testing all patients with recurrent/stage IV breast cancer for which of the following biomarkers? (Select all that apply)**

a ER/PR
b HER2
c BRCA1/2
d PD-L1
e ESR1
f PIK3CA

ANSWERS

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1

Biomarker	Prognostic	Predictive	Susceptibility
ER/PR[1]	---	X	---
HER2[2]	---	X	---
Ki67 [3,4]	X	---	---
BRCA1/2[5]	---	X	X
PD-L1[6,7]	X	X	---
PIK3CA [7-11]	X	X	---

(pages 5, 18)

2

A and C. Anti–PD-L1 antibodies are not interchangeable when testing tissue from a patient with breast cancer. PD-L1 expression level may be impacted by interobserver agreement.[12-14] PD-L1 positivity is associated with eligibility for a treatment with an immunotherapy in patients with TNBC. There are different ways to assess PD-L1 positivity. In TNBC, PD-L1 expression CPS ≥ 10 is clinically informative.[6,14-16] (page 15)

3

C. PIK3CA is a common mutation in breast cancer, found in 36% of all patients with breast cancer and 42% of patients HR-positive/HER2-negative disease.[17] (page 17)

4

False. PIK3CA mutations are generally stable but may change in some patients.[18] Receptor switching may occur in 2.9%-10.3% of cases for HER2.[19,20] HER2 mutations may arise during treatment and confer resistance to anti-HER2 therapies.[21] (page 11)

5

A, B, C, D, F. NCCN Guidelines® recommend testing all patients with mBC for ER, PR, HER2, BRCA1/2, PIK3CA, and PD-L1.[15] (page 6)

This knowledge check is connected to the chapter "Biomarker Testing in Breast Cancer: An Essential Component of the Treatment Decision Making Process." To get a copy of this and other chapters, please visit: **https://www.hcp.novartis.com/precision-medicine**

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BRCA1/2, breast cancer gene 1/2; CPS, combined positive score; ER, estrogen receptor; ESR1, estrogen receptor 1; HER2, human epidermal growth factor receptor 2; HR, hormone receptor; mBC, metastatic breast cancer; PD-L1, programmed death-ligand 1; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; PR, progesterone receptor; TNBC, triple-negative breast cancer.

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BIOMARKER TESTING IN **BREAST CANCER**

Knowledge Check 2

1 **True or False:** NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) recommend verifying negative liquid biopsy results with tumor tissue testing.

a True
b False

2 The collaboration of and clear communication within the entire team taking care of patients with metastatic breast cancer are paramount. Which of the following HCPs are part of the multidisciplinary team and are essential to obtaining a molecular diagnosis for metastatic breast cancer patients?

a Oncologist
b Nurse navigator
c Pathologist
d Interventionalist/surgeon
e Laboratory staff
f All of the above

3 Which of the following institutions provide important guidelines on the clinical utility of valid biomarker tests, as well as recommendations on testing standardization in order to improve testing consistency.

a ASCO-CAP
b NCCN Guidelines®
c International Ki-67 Working Group
d All of the above
e None of the above
f A and B only

4 **True or False:** FISH is recommended for most biomarkers.

a True
b False

5 Match the testing modality with the appropriate statement from the right column.

a Sanger sequencing	i. May require a pre-amplification step in situations with low target DNA sample input
b Pyrosequencing	ii. Can potentially detect SNVs, indels, CNAs, and fusions
c NGS	iii. Has low sensitivity (LOD >20% VAF)
d RT-PCR	iv. Read length capacity is low (~100 bases)
e dPCR	v. Detects known mutations with a variable sensitivity (LOD ~5% VAF)

ANSWERS

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1 **True.** NCCN Guidelines recommend reflex testing to tissue biopsy in case of a negative result with liquid biopsy.[1] (page 17)

2 **F. All of the above.** The oncologist, interventionalist/breast cancer surgeon, nurse navigator, pathologist, and laboratory staff all play an important role in molecular diagnostics and breast cancer care.[2,3] (page 8)

3 **D. All of the above.** ASCO-CAP, NCCN Guidelines, and the International Ki-67 Working Group have all released guidelines that have information pertinent to biomarker testing in breast cancer.[4-9] (pages 7,14)

4 **False.** FISH is recommended for HER2 testing only.[4,10-16] (page 11)

5

A.iii. Sanger sequencing has a low sensitivity (>20% VAF)[17]

B.iv. Pyrosequencing has read lengths of ~100 bases.[18]

C.ii. NGS can potentially detect SNVs, indels, CNAs, and fusions, dependent on assay design.[19]

D.v. RT-PCR detects known mutations with a variable sensitivity (LOD ~5% VAF).[17,20,21]

E.i. dPCR may require a pre-amplification step in situations with low target DNA sample input[22,23] (page 10)

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ASCO, American Society of Clinical Oncology; CAP, College of American Pathologists; CNA, copy number alteration; DNA, deoxyribonucleic acid; dPCR; digital polymerase chain reaction; FISH, fluorescence in situ hybridization; HCP, healthcare professional; HER2, human epidermal growth factor receptor 2; LOD; limit of detection; NGS; next generation sequencing; RT-PCR, real time-polymerase chain reaction; SNV, single nucleotide variant; VAF, variant allele frequency.
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Knowledge Check 1

1 **Fill in the blanks:** Biomarker testing in oncology is complex, as of June 2022, there were: _____ FDA-approved biomarker-linked interaction, _____ actionable genomic alterations, and _____ cancer types:

a ≥70, 43, and 28
b ≥75, 47, and 36
c ≥85, 50, and 40
d ≥100, 62, and 53

2 How does biopsy choice and site impact testing outcomes? **Select all that apply:**

a The decalcification process can risk impairing the sample yield and integrity with bone biopsies
b Receptor status can change over the course of the disease
c Variability between the primary tumor and metastatic site can occur
d Rebiopsy after disease progression does not provide clinically meaningful data

3 **Which of the following statements is true for liquid biopsy test results?**

a Provide a snapshot of the cellular and molecular characteristics of 1 part of a single tumor
b Can be linked with histology
c All of the above
d None of the above

4 **Testing for biomarkers generally requires _____% of tumor nuclei in collected samples to be above the LOD:**

a 10%
b 15%
c 18%
d 20%

5 **True or false**, ROSE can be performed without a cytopathologist present?

a True
b False

ANSWERS

Precision Medicine

1 **A.** As of June 2022, there were more than 70 US Food and Drug Administration (FDA)-approved biomarker-linked indications and 43 actionable genomic alterations.[1,2]

2 **A, B, and C.**

A: Bone biopsy requires decalcification, which may impair sample yield and integrity, potentially negatively impacting biomarker testing outcomes.[3]

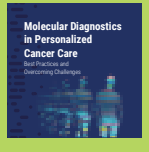
B: The receptor status may change over the course of the disease in certain cancers. Rebiopsy after disease progression may provide important and/or new information.[4-7]

C: Biomarker discordance between the primary tumor and a metastatic site may occur. Additional/different drivers/mutations may occur through clonal evolution over the course of the disease.[8-12]

3 **C.** Liquid biopsy test results may reflect the overall genomic landscape of the tumor and all metastatic sites[13,14]. It cannot directly correlate ctDNA results with histology or cellular phenotype and it may miss an alteration if ctDNA concentration is below the LOD, leading to a false negative.[14-17]

4 **D.** Testing for biomarkers generally requires 20% of tumor nuclei in samples.[18,19] Testing samples with a lower tumor proportion may result in false negatives, depending on the LOD.[20-23] Training may help lower discrepancies in estimating tumor content.[18]

5 **TRUE:** Telecytology allows ROSE to be done with an off-site cytopathologist; in telecytology-performed ROSE, the cytopathologist reviews images of the slides sent via a secured network.[24-26]



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ctDNA, circulating tumor deoxyribonucleic acid; LOD, limit of detection; ROSE, rapid on-site evaluation.

References: **1**. Chakravarty D et al. *J Clin Oncol.* 2022;40(11):1231-1258. **2**. OncoKB. https://www.oncokb.org/actionableGenes#levels=1&sections=Tx. Accessed June 13, 2022. **3**. Singh VM et al. *Ann Diagn Pathol.* 2013;17(4):322-326. **4**. Shachar SS et al. *Clin Breast Cancer.* 2016;16(3):e43-e48. **5**. Swanton C. *Cancer Res.* 2012;72(19):4875-82. **6**. Gerlinger M et al. *N Engl J Med.* 2012;366(10):883–892. **7**. Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Breast Cancer V.4.2022. © National Comprehensive Cancer Network, Inc. 2022. All rights reserved. Accessed June 21, 2022. To view the most recent and complete version of the guideline, go online to NCCN.org. **8**. Stefanovic S et al. *Oncotarget.* 2017;8(31):51416-51428. **9**. Bhullar DS et al. *EBioMedicine.* 2019;40:363-374. **10**. Bardelli A et al. *Proc Natl Acad Sci U S A.* 2001;98(10):5770-5775. **11**. Sidransky D et al. *Nature.* 1992;355(6363):846-847. **12**. Yachida S et al. *Nature.* 2010;467(7319):1114-1117. **13**. Arneth B. *BMC Cancer.* 2018;18(1):527. **14**. Iuliani M et al. *Front Oncol.* 2020;10:789. **15**. Merker JD et al. *J Clin Oncol.* 2018;36(16):1631-1641. **16**. Davis AA et al. *EBioMedicine.* 2020;58:102914. **17**. Gerratana L et al. *Eur J Cancer.* 2021;143:147-157. **18**. Mikubo M et al. *J Thorac Oncol.* 2020;15(1):130-137. **19**. Frampton GM et al. *Nat Biotechnol.* 2013;31(11):1023-1031. **20**. Liam CK et al. *Respirology.* 2020;25(9):933-943. **21**. Grafen M et al. *Lab Invest.* 2017;97(7):863-872. **22**. Kim L, Tsao MS. *Eur Respir J.* 2014;44(4):1011-1022. **23**. Dietel M et al. *Thorax.* 2016;71(2):177-184. **24**. Kraft AO. *Cancer Cytopathol.* 2017;125(S6):449-454. **25**. Sirintrapun SJ et al. *J Pathol Inform.* 2017;8:33. **26**. Thrall M et al. *J Pathol Inform.* 2011;2:51.

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Knowledge Check 2

1 **How is biomarker testing utilized in precision oncology?**

- a Diagnosis purposes
- b Treating cancers
- c Aiding prognosis
- d All of the above

2 **A good biomarker test should have which of the following characteristics? Select all that apply:**

- a Actionable, prognostic and/or predictive
- b Sufficient sensitivity, specificity, accuracy, and precision to detect actionable biomarkers
- c Tightly controlled specimen collection, handling, and processing
- d Highly regulated process to returning results to inform clinical decisions

3 **Which statement(s) best describes clinical utility?**

- a Accurately and reliably measures the presence or absence of a biomarker
- b Accurately and reliably identifies a biologically defined disorder
- c Evidence to support the use of a biomarker that can guide clinical decisions
- d B & C
- e All of the above

4 **Which testing method(s) can reliably detect fusions? Select all that apply:**

- a IHC
- b FISH
- c RT-PCR
- d RNA-based NGS
- e DNA-based NGS

5 **Which of these statements are true about reflex testing?**

- a Can reduce turnaround time
- b Can be integrated into patients EHR
- c Tests are automatically added in specific situations
- d All of the above

ANSWERS

1 **D.** Precision oncology uses molecular biomarkers to aid in the diagnosis, prognosis, or treatment of cancer.[1]

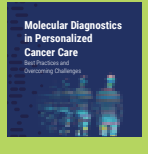
2 **A, B, and C.** According to clinical guidelines and expert opinions, the 7 characteristics of a good biomarker test are as follows:

- It is actionable, prognostic, and/or predictive[2,3]
- It is supported by the highest level of evidence[2]
- It provides reproducible results (>95%)[4,5]
- It possesses sufficient sensitivity, specificity, accuracy, and precision (<1% to 5% LOD) to detect actionable biomarkers[2-5]
- It has tightly controlled specimen collection, handling, and processing[2]
- It delivers timely results which impact treatment decisions[6-8]
- It has predetermined cutoff points/categories[2]

3 **C.** Clinical utility is when a biomarker test has high levels of evidence that use of the biomarker can result in guiding clinical decisions that result in improved clinical outcomes compared with those if the biomarker test results were not applied.[9]

4 **B, C, & D.** Gene rearrangements can be reliably detected by FISH, RT-PCR (known rearrangements) and RNA-based NGS. Some, but not all, DNA-based NGS assays can detect fusions, as the enrichment strategy will impact the ability to detect fusions in these assays. IHC assesses protein expression; therefore, it cannot differentiate between protein overexpression and a bona fide fusion event.[1]

5 **D.** Reflex testing is the automatic addition of tests in the SOPs by pathologists, and it may be integrated into the electronic health record. Studies suggest reflex testing may reduce the turnaround times for molecular testing results.[10-13]



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DNA, deoxyribonucleic acid; FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; LOD, limit of detection; NGS, next-generation sequencing; RNA, ribonucleic acid; RT-PCR, real time-polymerase chain reaction; SOPs, standard operating procedures.

References: 1. Chakravarty D et al. *J Clin Oncol*. 2022;40(11):1231-1258. **2.** Hayes DF. *J Clin Oncol*. 2021;39(3):238-248. **3.** Vidwans SJ et al. *Oncoscience*. 2014;1(10):614-623. **4.** Pepe MS et al. *J Natl Cancer Inst*. 2001;93(14):1054-1061. **5.** Jennings LJ et al. *J Mol Diagn*. 2017;19(3):341-365. **6.** European Society for Medical Oncology. https://oncologypro.esmo.org/education-library/factsheets-on-biomarkers/multigene-sequencing-in-breast-cancer#page. Accessed April 26, 2022. **7.** Veljovic M et al. ASCO 2015. Abstract e17698. **8.** Lim C et al. *Ann Oncol*. 2015;26(7):1415-1421. **9.** Hayes DF. *Mol Oncol*. 2015;9(5):960-966. **10.** Murphy MJ. *Ann Clin Biochem*. 2021;58(2):75-77. **11.** Lau-Min KS et al. *JCO Precis Oncol*. 2021;5:PO.20.00418. **12.** Anand K et al. *Clin Lung Cancer*. 2020;21(5):437-442. **13.** Seidman AD et al. *Popul Health Manag*. 2017;20(4):252-254.

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Molecular Profiling
in Common Cancers

Precision Medicine

PREDICTIVE BIOMARKER TESTING IN CARE FOR PATIENTS WITH METASTATIC CANCER

- Many patients with advanced or metastatic cancer may benefit from biomarker testing and/or genomic sequencing. In fact, professional societies like ASCO recommend biomarker testing to identify appropriate treatment options for patients with metastatic cancer.[1]
- Pan-tumor markers are used to find options for patients who may benefit from tissue- and site-agnostic treatments that are FDA-approved across solid tumor types[1]
- Molecular panel-based approaches that use NGS enable testing for multiple markers simultaneously, allowing for the most efficient use of limited tissues.[1]
- There are several commercially available panel-based biomarker tests.

The NCCN Guidelines for NSCLC provide recommendations for individual biomarkers that should be tested and recommend testing techniques but do not endorse any specific commercially available biomarker assays or commercial laboratories.

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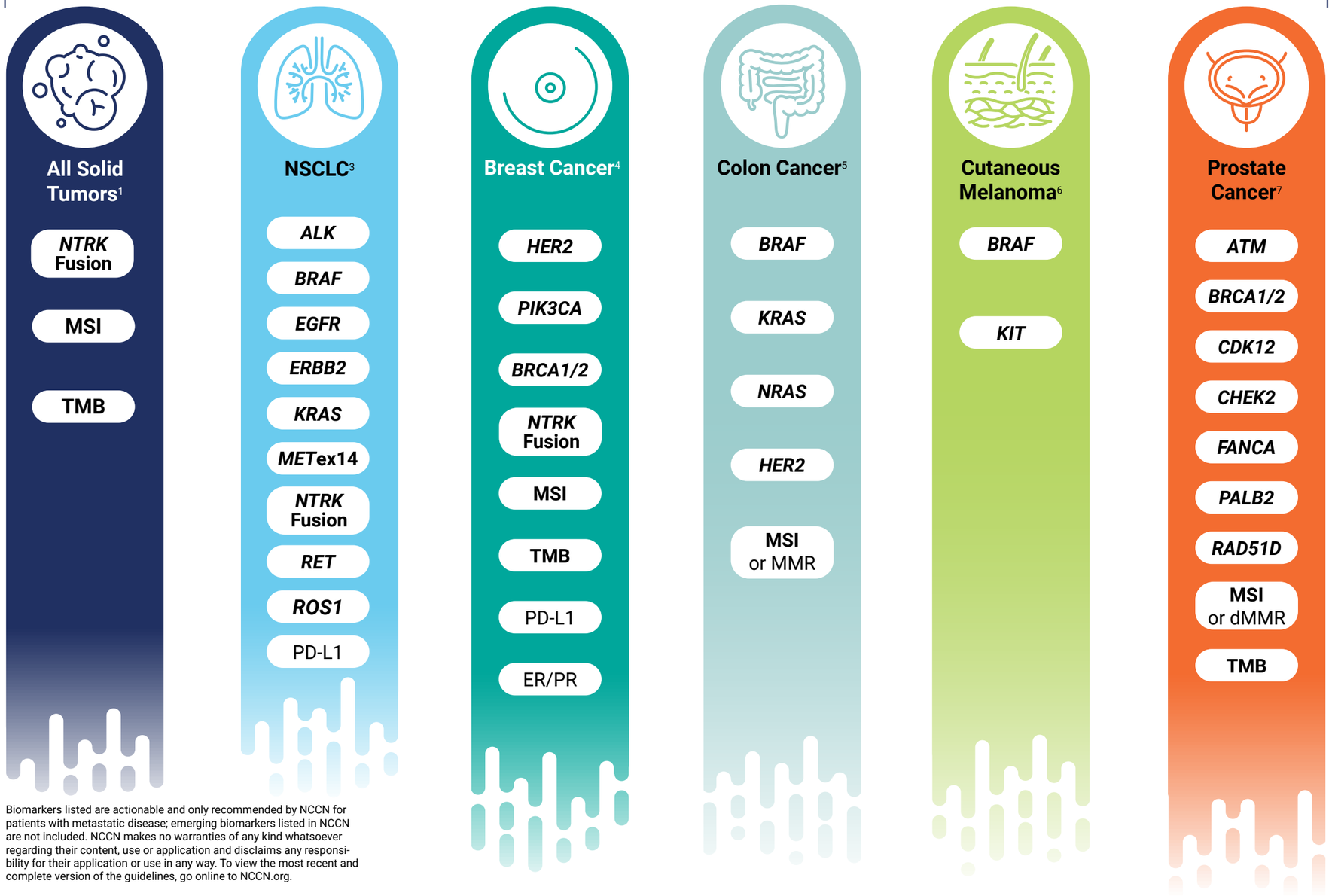
ASCO-Recommended Pan-Tumor Markers and National Comprehensive Cancer Network® (NCCN®)-Recommended Predictive Biomarkers for the 5 Most Common Cancers[1-7]



Biomarkers listed are actionable and only recommended by NCCN for patients with metastatic disease; emerging biomarkers listed in NCCN are not included. NCCN makes no warranties of any kind whatsoever regarding their content, use or application and disclaims any responsibility for their application or use in any way. To view the most recent and complete version of the guidelines, go online to NCCN.org.

MULTI-GENE PANELS AND PROFILES FOR TESTING SOLID TUMORS

Tissue biopsy

Manufacturer Test Name	Testing Method	Genes	TAT	Number of NCCN-Recommended Biomarkers Tested						Sample Requirement
Foundation Medicine FoundationOne® CDx[1,3-7,8-10]	Hybrid capture NGS	324	≤12 days	3/3	9/10	7/10	5/5	2/2	10/10	FFPE block + 1 H&E slide **OR** 10 unstained slides + 1 H&E slide
Caris Life Sciences MI Profile™[1,3-7,11,12]	NGS + IHC IHC tests vary by tumor type	22,000	8-14 days	3/3	10/10	10/10	5/5	2/2	10/10	FFPE block **OR** 10 unstained slides (≥20% tumor cells for DNA; ≥10% for RNA) Additional tissue required for IHC tests; varies by tumor type
NeoGenomics NeoTYPE® Precision Profile for Solid Tumors[1,3-7,13]	NGS + IHC IHC includes PD-L1 and Pan-TRK	79	14 days	3/3	9/10	8/10	5/5	2/2	10/10	FFPE solid tumor tissue (paraffin block preferred; please use positively-charged slides and 10% NBF fixative; do not use zinc fixatives)
MSK IMPACT™[1,3-7,14]	DNA-based NGS	505	Information not publicly available	–	–	–	–	–	–	Information not publicly available
PENN PRECISION PANEL 2.0[1,3-7,15,16]	DNA-based NGS	59	Information not publicly available	0/3	4/10	1/10	4/5	2/2	1/10	FFPE tissue, isolated Genomic DNA, or tissue or fluid in PreservCyt
MOFFITT STAR™ (TRUSIGHT® TUMOR 170)[1,3-7,17]	DNA- and RNA-based NGS	170	Information not publicly available	–	–	–	–	–	–	Information not publicly available

The select tests listed above represent the top 20 NGS solid tissue cancer tests by market share in Q1 2022. Tests are ordered by biopsy type, then by market share. This information is not exhaustive and is not intended to endorse a particular test. When testing for therapy selection, please consult product prescribing information and FDA-approved companion diagnostics.

For the most up-to-date information, please the website for the specific manufacturer. These websites are independently operated and not managed by Novartis Pharmaceuticals Corporation. Novartis assumes no responsibility for the content on the sites. Websites for the specific manufacturers are provided on page 14.

MULTI-GENE PANELS FOR TESTING SOLID TUMORS WITH TISSUE SAMPLES (CONTINUED)

	Manufacturer Test Name	Testing Method	Genes	TAT	Number of NCCN-Recommended Biomarkers Tested						Sample Requirement
Tissue biopsy	HopeSeq Solid Tumors[1,3-7,18-20]	DNA- and RNA-based NGS	523	Information not publicly available	3/3	9/10	7/10	5/5	2/2	10/10	FFPE with highest tumor content, not exposed to decalcifying solutions OR 1 H&E and 15 USS
	Johns Hopkins Solid Tumor Panel V6[1,3-7,21,22]	DNA-based NGS	400+	14-21 days	0/3	4/10	4/10	4/5	2/2	7/10	Information not publicly available
	Natera Empower™ Comprehensive Panel[1,3-7,23]	DNA- and RNA-based NGS	81	≤14 days	–	–	–	–	–	–	Information not publicly available
	Stanford Actionable Mutation Panel for Solid Tumors (STAMP)[1,3-7,24,25,*]	DNA-based NGS	138	21 days	1/3	8/10	4/10	4/5	2/2	6/10	FFPE tissue blocks sent at room temperature, avoiding extreme heat or cold
	OncoPanel (POPV3)[1,3-7,26]	Hybrid capture, DNA-based NGS	447	Information not publicly available	1/3	9/10	5/10	4/5	2/2	8/10	Fresh, frozen or formalin-fixed paraffin-embedded samples
	Exact Sciences Oncotype MAP™ Pan-Cancer Tissue Test[1,3-7,27]	NGS + IHC IHC tests vary by tumor type	257	<7 days	3/3	10/10	8/10	5/5	2/2	10/10	3 mm^2 of tissue with ≥15% tumor content
	Cleveland Clinic CC-SIGN™ Pan-Solid Tumor NGS Panel[1,3-7,28]	RNA-based NGS	59	14 days	1/3	4/10	1/10	0/5	0/2	0/10	FFPE tissue: 10 unstained, 4 µM sections of FFPE on charged, unbaked slides; one H&E stained slide with best tumor area circled by pathologist (minimum of 20% tumor content)
	Myriad Genetics MyChoice® CDx[1,3-7,29]	DNA-based NGS	2	≤14 days	0/3	0/10	2/10	0/5	0/2	2/10	FFPE tumor blocks or slides that contain at least 40 microns of tumor (20% tumor by cellularity)

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*Test only detects fusions in *NTRK1*.

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MULTI-GENE PANELS FOR TESTING SOLID TUMORS WITH TISSUE SAMPLES (CONTINUED)

	Manufacturer Test Name	Testing Method	Genes	TAT	Number of NCCN-Recommended Biomarkers Tested						Sample Requirement
Tissue biopsy	**ThermoFisher Scientific** Ion AmpliSeq™ Cancer Hotspot Panel v2[1,3-7,30]	Multiplex PCR	50	1 day	0/3	4/10	1/10	4/5	2/2	1/10	10ng of DNA for FFPE
	Strata Oncology StrataNGS®[1,3-7,31,32]	DNA and RNA-based NGS	437	7 days (median)	3/3	9/10	7/10	5/5	2/2	9/10	FFPE (minimum >0.5mm² surface area), 10 x 5µm unstained air-dried slides with ≥20% tumor
	Illumina TruSight™ Oncology 500[1,3-7,33]	Hybrid capture NGS (DNA & RNA)	523	4-5 days	3/3	9/10	7/10	5/5	2/2	10/10	FFPE (DNA 40 ng, RNA 40 ng)
	ThermoFisher Scientific Oncomine™ Focus Assay[1,3-7,34-36]	DNA and RNA-based NGS	52	3 days	1/3	9/10	3/10	4/5	2/2	0/10	300-30,000 copies of DNA (10 ng of mammalian gDNA) from normal or FFPE tissue
Liquid biopsy	**Guardant Health, Inc.** Guardant360® CDx[1,3-7,37,38,*]	Hybrid capture NGS	55	7 days	1/3	9/10	5/10	4/5	2/2	4/10	Plasma (Streck cell-free DNA blood collection tubes)
	TEMPUS TEMPUS xF Gene Panel[1,3-7,39,40,*]	DNA sequencing	105	~7-10 days	2/3	9/10	6/10	5/5	2/2	5/10	Peripheral blood (2 Streck tubes, 8.5 mL each)

The select tests listed above represent the top 20 NGS solid tissue cancer tests by market share in Q1 2022. Tests are ordered by biopsy type, then by market share. This information is not exhaustive and is not intended to endorse a particular test. When testing for therapy selection, please consult product prescribing information and FDA-approved companion diagnostics.
*Test only detects fusions in *NTRK1*.

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MULTI-GENE PANELS FOR TESTING LUNG CANCER

Manufacturer Test Name	Testing Method	Biomarkers	Number of NCCN-Recommended Biomarkers Tested	TAT	Sample Requirement
NeoGenomics Lung NGS Fusion Panel (Complete or Limited)[3,41]	NGS (RNA)	8	5/10	21 days	FFPE block **OR** 1 H&E slide + 5-10 unstained slides cut at ≥5 microns (Please use positively-charged slides and 10% NBF fixative; do not use zinc fixatives)
NeoGenomics NeoTYPE® Lung Tumor Profile[3,42]	NGS + 9 single gene assays (Single gene assays include MET exon 14 deletion analysis, FISH, and IHC)	49	10/10	14 days	FFPE block (please use 10% buffered formalin fixative; do not use zinc fixatives.)
Quest Diagnostics™ Lung Cancer Mutation Panel (EGFR, KRAS, ALK)[3,43]	3 single gene assays (Single gene assays include mutation analysis + FISH)	3	3/10	NRPT	FFPE
Thermo Fisher Scientific Ion Torrent™ Oncomine™ Dx Target Test[3,44]	Amplicon NGS	23	7/10	≤4 days	FFPE (DNA/RNA 10 ng)

Ion Torrent™ Oncomine™ Dx Target Test is an in house kit that can be ordered[48]

The select tests listed above represent the top 20 NGS solid tissue cancer tests by market share in Q1 2022. This information is not exhaustive and is not intended to endorse a particular test. When testing for therapy selection, please consult product prescribing information and FDA-approved companion diagnostics.

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MULTI-GENE PANELS FOR TESTING BREAST CANCER

Manufacturer Test Name	Testing Method	Biomarkers	Number of NCCN-Recommended Biomarkers Tested	TAT	Sample Requirement
NeoGenomics NeoTYPE® Breast Tumor Profile[4,46]	NGS, FISH, + IHC	60	8/10	14-17 days	FFPE block (Please use 10% buffered formalin fixative; do not use zinc fixatives)

MULTI-GENE PANELS FOR TESTING COLORECTAL CANCER

Manufacturer Test Name	Testing Method	Biomarkers	Number of NCCN-Recommended Biomarkers Tested	TAT	Sample Requirement
NeoGenomics NeoTYPE® Colorectal Tumor Profile[5,46]	NGS + 7 single gene assays (Single gene assays include MLH1 promoter methylation analysis, FISH, and IHC)	44	5/5	14-17 days	FFPE block (Please use 10% buffered formalin fixative; do not use zinc fixatives)

MULTI-GENE PANELS FOR TESTING MELANOMA

Manufacturer Test Name	Testing Method	Biomarkers	Number of NCCN-Recommended Biomarkers Tested	TAT	Sample Requirement
NeoGenomics NeoTYPE® Melanoma Profile[6,47]	NGS + 3 single gene assays (Single gene assays include FISH and IHC)	28	2/2	14 days	FFPE block (Please use 10% buffered formalin fixative; do not use zinc fixatives)

Test availability and other factors may impact test selection. The list of tests above is not a comprehensive list of all testing options and their inclusion in this chart does not imply that these are FDA approved. This information is not exhaustive and is not intended to endorse a particular test. When testing for therapy selection, please consult product prescribing information and FDA-approved companion diagnostics.

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Manufacturer	Website
Caris Life Sciences	https://www.carismolecularintelligence.com
Exact Sciences	https://precisiononcology.exactsciences.com
Foundation Medicine	https://www.foundationmedicine.com
Guardant Health, Inc.	https://guardant360cdx.com/
Illumina	https://www.illumina.com
Myriad Genetics	https://myriad.com
Natera	https://www.natera.com
NeoGenomics	https://neogenomics.com
Quest Diagnostics™	https://www.questdiagnostics.com
STRATA Oncology	https://strataoncology.com
TEMPUS	https://www.tempus.com
Thermo Fisher Scientific	https://corporate.thermofisher.com/us/en/index.html

ALK, anaplastic lymphoma kinase; ASCO, American Society of Clinical Oncology; *ATM*, ATM serine/threonine kinase; *BARD1*, BRCA1-associated RING domain 1; *BRAF*, v-Raf murine sarcoma viral oncogene; *BRCA1/2*, breast cancer gene 1/2; *BRIP1*, BRCA1-interatcting helicase 1; *CDK12*, cyclin-dependent kinase 12; CDx, companion diagnostic; *CHEK2*, checkpoint kinase 2; dMMR, deficient mismatch repair; EDTA, ethylenediaminetetraacetic acid; *EGFR*, epidermal growth factor receptor; ER, estrogen receptor; *ERBB2*, erythroblastic oncogene B 2; *FANCA*, FA Complementation Group A; FDA, US Food and Drug Administration; FFPE, formalin-fixed paraffin-embedded; FISH, fluorescence in situ hybridization; H&E, hematoxylin and eosin; *HER2*, human epidermal growth factor receptor 2; *Her2/Neu*, human epidermal growth factor receptor 2; IHC, immunohistochemistry; *KIT*, KIT proto-oncogene, receptor tyrosine kinase; *KRAS*, Kirsten ras oncogene homolog; *MET*, MET proto-oncogene, receptor tyrosine kinase; *MLH1*, mutL homolog 1; MMR, mismatch repair; *MSH2/6*, MutS homolog 2/6; MSI, microsatellite instability; N/A, not applicable; NBF, neutral buffered formalin; NCCN, National Comprehensive Cancer Network; NGS, next-generation sequencing; *NRAS*, neuroblastoma RAS viral oncogene homolog; NRPT, not reported; NSCLC, non-small cell lung cancer; *NTRK1/2/3*, neurotrophic receptor tyrosine kinase 1/2/3; *PALB2*, partner and localizer of BRCA2; PD-L1, programmed death-ligand 1; *PIK3CA*, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; *PMS2,* PMS1 homolog 2; PR, progesterone receptor; *PTEN*, phosphatase and tensin homolog; *RAD51*D, RAD51 paralog D; *RAD54L*, RAD54 like; *RET*, ret proto-oncogene; TAT, turnaround time; TMB, tumor mutation burden; *TRK*, tropomyosin receptor kinase.

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SUMMARY

Many patients with advanced or metastatic cancer may benefit from biomarker testing and/or genomic sequencing

Professional societies like ASCO recommend testing for pan-tumor markers and/or tumor-specific predictive biomarkers to identify appropriate treatment options for patients with metastatic cancer

Molecular panel-based approaches that use NGS enable testing for multiple biomarkers simultaneously, allowing for the most efficient use of limited tissues

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The Ins and Outs
of Test Requisition Forms

Precision Medicine

PRECISION MEDICINE ESSENTIALS

Oncogenic Drivers[1,2]

Driver mutations are genomic alterations that directly or indirectly provide a selective advantage to cancer cells by promoting cancer growth, development, and/or survival

Driver Alteration	Driver Gene
A mutation that directly or indirectly confers a selective growth advantage to the cell in which it occurs	**A gene that contains driver gene mutations or is expressed aberrantly in a fashion that confers a selective growth advantage**

Not every mutation in an oncogene or tumor suppressor gene is a driver mutation.

Passenger mutations have no direct or indirect effect on the selective growth advantage of the cell in which it occurred.

Drivers Arise From Specific Genomic Alterations[1,2]

Mutations

Single-Nucleotide Variation	The substitution of one DNA nucleotide for another nucleotide (may be somatic/germline and synonymous/nonsynonymous) Includes **missense mutations**, which result in the substitution of the wild-type amino acid for an alternate amino acid and **silent mutations**, which do not alter the encoded amino acid
Indel/Deletion-Insertion	The replacement of more than one nucleotide by other nucleotides May be "**in-frame**" if the deletion/insertion occurs in multiples of three nucleotides or "**frameshift**" if the deletion/insertion shifts the reading frame, resulting in novel amino acids
Splice Site	A mutation involving the conserved nucleotides at the exon-intron boundary that may disrupt RNA splicing May result in exon skipping, intron retention, frameshift, and premature protein truncation
Extension	The normal stop codon is lost, allowing translation to continue
Truncating/Nonsense	A premature stop codon is introduced

Structural Variants

Copy Number Variation	A deviation from the expected two copies of a gene via an increase (**amplification**) or decrease (**deletion**) in the number of copies
Translocation	A rearrangement in which regions from two nonhomologous chromosomes are joined
Fusion	A novel gene product created from two previously separate and independent genes May arise from chromosomal translocations, interstitial deletions, inversions, or tandem duplications

DETECTING GENOMIC ALTERATIONS

Biomarker Testing in Oncology Is Complex[1,3]

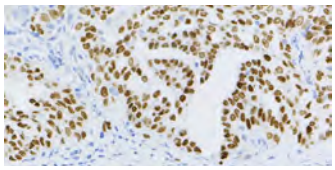
As of June 2022, there are:

≥70
FDA-approved biomarker-linked indications

43
actionable genomic alterations

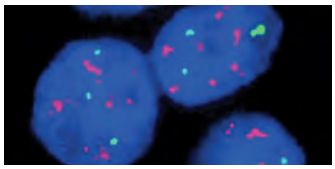
28
cancer types treatable by Precision Oncology

Single-Gene Testing



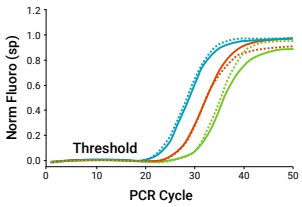
IHC: A test that uses an antibody to detect the expression, or loss of expression, of a specific protein or mutated protein form

Can assess: ***Protein expression***



FISH: An assay using a DNA probe that typically binds to target sequences in chromosome DNA; assessed under a fluorescence microscope

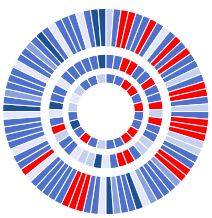
Can assess: ***Rearrangements, CNVs***



RT-PCR: An assay that amplifies and measures DNA from extracted RNA

Can assess: ***SNVs, indels, known rearrangements***

Multigene Testing



NGS[a]: A technology that performs massively parallel DNA sequencing to detect genomic alterations

Can assess: ***SNVs, indels, rearrangements, CNVs***

[a]Genomic alterations and biomarkers tested will vary by assay.

UNDERSTANDING NGS

NGS Assays Are Not Identical[5-9]

Enrichment Strategy[1,7,9,10]

		Amplicon	Hybrid capture
Variant detection	SNVs, small indels	✓	✓
	Fusions/ rearrangements	Nucleic acid dependent	✓
	Exon skipping	Nucleic acid dependent	✓
	CNV		✓
Bioinformatic analysis complexity		Less	More
Nucleic acid requirements		>10 ng	>100-200 ng

Nucleic Acid Selection

RNA-based NGS may be more sensitive than DNA-based NGS in detecting fusions and exon skipping[1,9,11-13]

Understanding assay limitations is critical to identifying patients with actionable biomarkers[1]

Recommendations for NGS[1]

ASCO recommends multigene panel-based genomic testing or NGS for:

Patients eligible for an approved genomic biomarker–linked therapy

- To detect tumor-agnostic actionable biomarkers like dMMR and/or MSI-H, TMB-H, and NTRK fusions, which may not be detected by single-gene tests

Patients eligible for >1 approved genomic biomarker–linked therapy

- To provide the most efficient use of limited tumor biopsy tissue

ASCO recommends using NGS for the most efficient utilization of limited biopsy tissue; it may allow simultaneous testing for multiple approved targeted therapies

UNDERSTANDING TESTING TERMS

Establishing Common Testing Terminology

Specialty-specific definitions for jargon may impact MDT communication and coordination. Establishing a common language, as below, with the MDT may help ensure that patients are not missed because of communication errors.

GENERAL → **SPECIFIC**

Biomarker panel[14,1]

- Tests a defined/ prespecified set of biomarkers, ranging from a few to hundreds
- Technology used may be NGS, microarrays, or a collection of single-gene tests

NGS[14,16]

"Next generation sequencing"

A methodology capable of:

- Whole genome sequencing
- Whole exome sequencing
- Detecting mutations in a small panel of prespecified genes

– Enrichment strategy (amplicon or hybrid capture) and assay design impacts the detection of some genomic alterations

CGP[17]

"Comprehensive genomic profiling"

- A hybrid capture–based NGS assay that typically tests 50+ genes simultaneously
- CGP assays may detect all types of genomic alterations

Additional Terms Explained

Multigene Panel[1]

An NGS test that sequences a defined list of genes with at least 50 genes in total

VS

Hotspot Panel[1]

Sequencing select hotspot codons, which contain recurrently altered amino acids, for all genes on a panel

VS

Genetic Profile[18]

General term referring to the information collected about specific genes, including variations and gene expression

SV[1]

"Structural variant"

Large genomic alterations (> 50 bp) that typically contain CNVs, translocations, inversions, deletions, and/or insertions

VS

SNV[1]

"Single nucleotide variant"

The substitution of one DNA nucleotide for another nucleotide

CDx[19]

"Companion diagnostic"

A test approved by the FDA that provides information that is essential for the safe and effective use of a corresponding therapeutic product

VS

LDT[20]

"Laboratory developed test"

A test that is designed, manufactured, and used within a single laboratory, but does not receive FDA oversight

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TEST REQUISITION FORMS (TRFs)

TRFs serve as a primary mode of communication between clinical and pathology team members during biomarker ordering[21,22]

of HCPs reported communication breakdowns during biomarker test ordering[23,*]

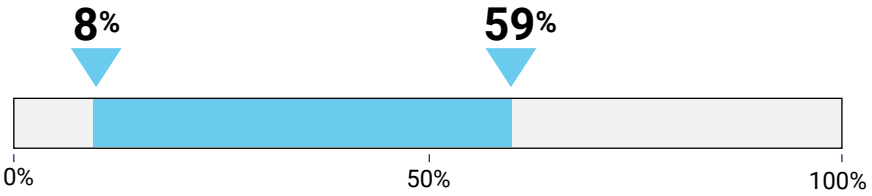
Common TRF Roadblocks

Confusion may result from:

- Too many testing options (eg, multiple testing platforms or vendors, each with unique sample requirements)[24]
- Requisition form variability between different institutions/ reference labs[22]

Incomplete or Incorrect Data Entry

The Rate of Incomplete TRFs Received by Laboratories Across Three Clinical Practices[21,25,26]



- **Data entry errors** are more common with handwritten compared to electronic forms[27]

Under- or Overtesting

Format of TRF may impact test utilization, resulting in potential under- or overtesting[28,29]

- Overtesting or inappropriate testing arises when testing exceeds guideline-recommended testing

*The Association of Community Cancer Centers, the Association of Molecular Pathology, the American Society for Clinical Pathology, and CAP conducted a survey in June 2018 with 659 responses from a multidisciplinary group and different cancer program settings.

THE IMPORTANCE OF TRF LAYOUT

TRF format and layout vary between institutions and can impact ordering, information processing, and results[22,30]

Considerations

Multiple professional societies have developed resources to assist with testing barriers:

Reduce Variability and Complexity

by generating internal standards for testing documentation[31]

One community hospital system saw improvements in biomarker testing after creating a standard ordering process with minimal testing platforms to streamline laboratory processes[32]

Ensure a Common Language

ASCO provided definitions of common terms for clinicians[1,33]

Establishing a common language with the MDT may help ensure that patients are not missed because of communication errors

Keep Forms Up-to-Date

by incorporating multiple guidelines; frequently updated guidelines may be the source for updates to internal SOPs[24]

Consider reviewing and incorporating recommendations from different guidelines at a cadence that keeps pace with updates[24]

Introduction to Hypothetical TRFs

In the next section, 1 hypothetical TRFs is reviewed. This form provides a way to highlight important components of TRFs and details to consider including. In practice, TRFs should be carefully designed to maximize clear communication between members of the MDT.

The form in the following section is purely hypothetical, includes guideline recommendations, and is not intended to be used in practice

HYPOTHETICAL TRF BASIC INFORMATION

Test Requisition Form

Basic Information

Patient Information

Medical Record # _____ Name _____ Sex _____ DOB _____

Address _____ Phone Number _____

Clinical Information

Diagnosis / Diagnosis Code _____ Disease Stage _____

Number of Prior Therapies _____

Original Activating Mutation _____ Reason for Testing _____

Attachments: ☐ Pertinent Laboratory Results ☐ Test results from molecular assays

Physician Information

Requisition Completion Date _____ Completed By _____

Address (results will be sent to this address) _____

Ordering Physician Name _____ NPI # _____ Phone/Fax _____

Treating Physician Name _____ NPI # _____ Phone/Fax _____

Authorizing Physician Signature _____

Referring Pathologist Name _____ NPI # _____ Phone/Fax _____

Specimen Information

Specimen ID _____ Specimen Type _____ Block ID _____

Site of Biopsy _____ Primary or Metastasis _____

Collection Date and Time _____ Retrieved Date_____

Fixation Method _____ Fixation Duration _____

Billing Information

Bill to: ☐ Insurance ☐ Medicare ☐ Medicaid ☐ Patient Self Pay ☐ Direct Bill ☐ Other

Insurance Information _____

Billing contact information _____

Specimen Origin: ☐ Hospital In-Patient ☐ Hospital Out-Patient ☐ Non-Hospital Patient

This form is purely hypothetical and is not intended to be used in practice; content is based on guideline-recommended testing

DOB, date of birth; NPI, national provider identifier.

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HYPOTHETICAL TRF EXPLAINED BASIC INFORMATION

Patient Information lists basic identifying information for the patient

Clinical Information provides details on the disease, stage, and clinical history of the patient

Disease stage and number of prior therapies *are important details for the pathology team, as these may impact mutation status*[34-36]

The ability to include attachments of prior pathology results allows pathologists to see the most relevant and up-to-date information that may impact patient care[37]

Physician Information provides relevant contact information and a mailing address for results

Specimen Information communicates details about the specimen submitted for testing

Fixation method and duration *are important to note, as these factors may impact biomarker testing results*[38,39]

Test Requisition Form

Basic Information

Patient Information
Medical Record # _____ Name _____ Sex _____ DOB _____
Address _____ Phone Number _____

Clinical Information
Diagnosis / Diagnosis Code _____ Disease Stage _____
Number of Prior Therapies _____
Original Activating Mutation _____ Reason for Testing _____

Attachments: ☐ Pertinent Laboratory Results ☐ Test results from molecular assays

Physician Information
Requisition Completion Date _____ Completed By _____
Address (results will be sent to this address) _____

Ordering Physician Name _____ NPI # _____ Phone/Fax _____
Treating Physician Name _____ NPI # _____ Phone/Fax _____
Authorizing Physician Signature _____
Referring Pathologist Name _____ NPI # _____ Phone/Fax _____

Specimen Information
Specimen ID _____ Specimen Type _____ Block ID _____
Site of Biopsy _____ Primary or Metastasis _____
Collection Date and Time _____ Retrieved Date_____
Fixation Method _____ Fixation Duration _____

Billing Information
Bill to: ☐Insurance ☐Medicare ☐Medicaid ☐Patient Self Pay ☐Direct Bill ☐Other

Insurance Information _____
Billing contact information _____

Specimen Origin: ☐Hospital In-Patient ☐Hospital Out-Patient ☐Non-Hospital Patient

Billing Information provides important details for billing of the testing

Specimen origin and date of collection *may affect insurance coverage, particularly with Medicare*[40,41]

HYPOTHETICAL TRF TEST SELECTION

Test Requisition Form

Featured Oncology Testing

Cell-Free DNA (cfDNA)

☐ Single gene test 1

☐ Single gene test 2

☐ Panel test 1 (list number of genes included)

Replicate fields to reflect all cfDNA testing options available at your institution

Pan Tumor Marker 1

☐ Marker by PCR

☐ IHC for protein X

Pan Tumor Marker 2

☐ Marker 2 fusion hotspot panel

☐ Marker 2 pan-protein IHC

Programmed Death-Ligand 1 (PD-L1)

☐ PD-L1 antibody 1 IHC

☐ PD-L1 antibody 2 IHC

☐ Other PD-L1 antibody IHC

Can replicate fields to reflect current number of antibodies available

Solid Tumor NGS Panel*

☐ 359-gene hybrid capture NGS panel

DNA-based assay including all pan tumor markers including TMB as well as actionable markers in multiple disease states. Please see second page for full gene list.

Tumor-Specific Panels and Profiles*,†

†Profile options include all biomarkers linked to FDA-approved or contraindicated therapies, by tumor type (current as of June 2022).

☐ **Melanoma NGS panel**

50 gene hotspot NGS panel; includes all NCCN-recommended biomarkers

☐ **Lung NGS panel**

121 gene hybrid capture DNA NGS panel; includes all NCCN-recommended biomarkers (current as of January 2022)

☐ *Reflex to 15 gene hotspot assay if insufficient DNA for larger panel*

Replicate fields to reflect all reflex testing options at your institution.

☐ **Breast profile**

PCR and IHC; includes all NCCN-recommended biomarkers (current as of January 2022)

☐ *Reflex to FISH if HER equivocal*

Replicate fields to reflect all reflex testing options at your institution.

☐ **Colon profile**

PCR and IHC; includes all NCCN-recommended biomarkers except TMB (current as of January 2022)

☐ *Reflex to FISH if IHC is unclear*

Replicate fields to reflect all reflex testing options at your institution.

Replicate fields to reflect all reflex panels and profile testing options at your institution.

**For full gene list, see appendix.*

Tumor-Specific Panels and Profiles*,†

☐ ATM	☐ BARD1	☐ BRAF	☐ BRCA1	☐ BRCA2
☐ BRIP1	☐ CDK12	☐ CHEK1	☐ CHEK2	☐ EGFR

This form is purely hypothetical and is not intended to be used in practice; content is based on guideline-recommended testing

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HYPOTHETICAL TRF EXPLAINED TEST SELECTION

Featured Oncology Testing provides tests that inform the use of tumor agnostic therapeutics

cfDNA: *cfDNA can be used in single gene testing or in panel testing. All available options should be clearly listed. Reflex testing to tissue testing may be included in the order*[42,43]

PD-L1: *PD-L1 antibodies are associated with specific therapies and are not interchangeable.*[44,45] *Forms should list available options and, potentially, the associated therapy*

Pan-tumor markers: *Pan-tumor markers can be detected with multiple different methods. Each pan-tumor marker and the method(s) may be listed.*[1]

When different isoforms of the same gene function as the same biomarker, consider clarifying if the method can assess 1 isoform or all isoforms.[46,47]

Test Requisition Form

Featured Oncology Testing

Cell-Free DNA (cfDNA)

- ☐ Single gene test 1
- ☐ Single gene test 2
- ☐ Panel test 1 (list number of genes included)

Replicate fields to reflect all cfDNA testing options available at your institution

Pan Tumor Marker 1

- ☐ Marker by PCR
- ☐ IHC for protein X

Pan Tumor Marker 2

- ☐ Marker 2 fusion hotspot panel
- ☐ Marker 2 pan-protein IHC

Programmed Death-Ligand 1 (PD-L1)

- ☐ PD-L1 Antibody 1 IHC
- ☐ PD-L1 Antibody 2 IHC
- ☐ Other PD-L1 antibody IHC

Can replicate fields to reflect current number of antibodies available

Solid Tumor NGS Panel*

- ☐ 359-gene hybrid capture NGS panel

DNA-based assay including all pan tumor markers including TMB as well as actionable markers in multiple disease states. Please see second page for full gene list.

Tumor-Specific Panels and Profiles*†

†Profile options include all biomarkers linked to FDA-approved or contraindicated therapies, by tumor type (current as of June 2022)

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121 gene hybrid capture DNA NGS panel; Includes all NCCN recommended biomarkers (current as of January 2022)

☐ *Reflex to 15 gene hotspot assay if insufficient DNA for larger panel*

Replicate fields to reflect all reflex testing options at your institution.

☐ **Breast profile**

PCR and IHC; Includes all NCCN recommended biomarkers (current as of January 2022).

☐ *Reflex to FISH if HER equivocal*

Replicate fields to reflect all reflex testing options at your institution.

☐ **Colon profile**

PCR and IHC; Includes all NCCN recommended biomarkers except TMB. (current as of January 2022).

☐ *Reflex to FISH if IHC is unclear*

Replicate fields to reflect all reflex testing options at your institution.

Replicate fields to reflect all reflex panels and profile testing options at your institution.

Tumor-Specific Panels and Profiles*†

☐ ATM	☐ BARD1	☐ BRAF	☐ BRCA1	☐ BRCA2
☐ BRIP1	☐ CDK12	☐ CHEK1	☐ CHEK2	☐ EGFR

Solid tumor NGS panel: *Because the ability to detect fusions in an NGS assay is impacted by the genes tested, nucleic acid input, and enrichment strategy, all pieces of information may be listed on the form.*[1,31] *Additionally, if the assay can detect TMB, it may also be explicitly stated. It is important to remember that TMB, which refers to the number of somatic mutations per megabase of DNA sequenced, can be influenced by the size of the panel, or assay coverage. The benchmark method to measure TMB is whole-exome sequencing. However, multigene panel-based sequencing with fewer genes (324-595 genes) can be used. Smaller panels cannot accurately estimate TMB*[1]

Finally, if using an outside vendor, consider including the name of the vendor along with aforementioned information.

HYPOTHETICAL TRF TEST SELECTION

Test Requisition Form

Featured Oncology Testing

Cell-Free DNA (cfDNA)

- ☐ Single gene test 1
- ☐ Single gene test 2
- ☐ Panel test 1 (list number of genes included)

Replicate fields to reflect all cfDNA testing options available at your institution

Pan Tumor Marker 1

- ☐ Marker by PCR
- ☐ IHC for protein X

Pan Tumor Marker 2

- ☐ Marker 2 fusion hotspot panel
- ☐ Marker 2 pan-protein IHC

Programmed Death-Ligand 1 (PD-L1)

- ☐ PD-L1 antibody 1 IHC
- ☐ PD-L1 antibody 2 IHC
- ☐ Other PD-L1 antibody IHC

Can replicate fields to reflect current number of antibodies available

Solid Tumor NGS Panel*

- ☐ 359-gene hybrid capture NGS panel

DNA-based assay including all pan tumor markers including TMB as well as actionable markers in multiple disease states. Please see second page for full gene list.

Tumor-Specific Panels and Profiles*,†

†Profile options include all biomarkers linked to FDA-approved or contraindicated therapies, by tumor type (current as of June 2022).

☐ **Melanoma NGS panel**

50 gene hotspot NGS panel; includes all NCCN recommended biomarkers

☐ **Lung NGS panel**

121 gene hybrid capture DNA NGS panel; includes all NCCN recommended biomarkers (current as of January 2022)

- ☐ *Reflex to 15 gene hotspot assay if insufficient DNA for larger panel*

Replicate fields to reflect all reflex testing options at your institution.

☐ **Breast profile**

PCR and IHC; includes all NCCN recommended biomarkers (current as of January 2022)

- ☐ *Reflex to FISH if HER equivocal*

Replicate fields to reflect all reflex testing options at your institution.

☐ **Colon profile**

PCR and IHC; includes all NCCN recommended biomarkers except TMB. (current as of January 2022)

- ☐ *Reflex to FISH if IHC is unclear*

Replicate fields to reflect all reflex testing options at your institution.

Replicate fields to reflect all reflex panels and profile testing options at your institution.

**For full gene list, see appendix.*

Tumor-Specific Panels and Profiles*,†

☐ ATM	☐ BARD1	☐ BRAF	☐ BRCA1	☐ BRCA2
☐ BRIP1	☐ CDK12	☐ CHEK1	☐ CHEK2	☐ EGFR

This form is purely hypothetical and is not intended to be used in practice; content is based on guideline-recommended testing

Precision Medicine

HYPOTHETICAL TRF EXPLAINED TEST SELECTION

Tumor-Specific Panels and Profiles: *Tumor specific panels and profiles allow physicians to assess a group of select biomarkers and genes that are relevant to different tumor types.*

To minimize confusion among providers, consider implementing ASCO definitions in forms (eg, a panel is an NGS assay of at least 50 genes)[1]

Reflex testing *can improve turnaround time by streamlining the ordering process. Incorporating it on the form provides ordering physicians the ability to select the most appropriate option for their patient.[7,48]*

Description of assays: *Biomarkers are constantly being added across disease states[49]; staying current is a well-established challenge.[24] Guideline recommendations can help practitioners stay current, but it is important to remember that guidelines may not reflect the most recent evidence, as advances may have occurred after a publication or update.[24,50] Therefore, incorporating details on guideline recommendations (and the associated guideline date) provides important context for the assay's clinical relevance. Complete gene lists may accompany TRFs on subsequent pages.[24]*

Test Requisition Form

Featured Oncology Testing

Cell-Free DNA (cfDNA)

- ☐ Single gene test 1
- ☐ Single gene test 2
- ☐ Panel test 1 (list number of genes included)

Replicate fields to reflect all cfDNA testing options available at your institution

Pan Tumor Marker 1

- ☐ Marker by PCR
- ☐ IHC for protein X

Pan Tumor Marker 2

- ☐ Marker 2 fusion hotspot panel
- ☐ Marker 2 pan-protein IHC

Programmed Death-Ligand 1 (PD-L1)

- ☐ PD-L1 Antibody 1 IHC
- ☐ PD-L1 Antibody 2 IHC
- ☐ Other PD-L1 antibody IHC

Can replicate fields to reflect current number of antibodies available

Solid Tumor NGS Panel*

- ☐ 359-gene hybrid capture NGS panel

DNA-based assay including all pan tumor markers including TMB as well as actionable markers in multiple disease states. Please see second page for full gene list.

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**For full gene list, see appendix*

Tumor-Specific Panels and Profiles*†

☐ ATM	☐ BARD1	☐ BRAF	☐ BRCA1	☐ BRCA2
☐ BRIP1	☐ CDK12	☐ CHEK1	☐ CHEK2	☐ EGFR

Single Gene Testing Options *allow for the customized selection of individual genes of interest that may not be included in tumor-specific panels or profiles.*

PATHOLOGY REPORTS

The report is an essential part of the testing process. Incomplete, unclear, or missing reports can lead to incorrect patient management[51]

Electronic Record Compatibility

While pathologists may rely on a **laboratory information system (LIS)**, clinicians routinely use the **electronic health record (EHR)**.

- Interoperability of these systems varies across institutions[52]

Joint consensus from ASCO, CAP, and AMP[51]:
Pathology reports should be in a format that enables integration with the electronic health record

Common Reporting Pitfalls and Solutions

Reports are Lost / Missing in the EHR[24,53,54]:

- Reports may not be fully integrated into EHRs because of a lack of compatibility between LIS and EHR
- Pages may be lost when reports are scanned in the EHR

ACCC-Recommended Solutions[54]:

- Utilizing CAP electronic Cancer Checklists to facilitate structured data capture and reporting
- Exploring ways to improve report readability and searchability across electronic systems
- Minimizing the use of scanned reports
- Considering using pathology LIS modules built by the inpatient EHR vendor

Multiple Reports are Generated at Different Times[52]:

- Confusion may result when several individual reports are created for each specimen or test ordered for a single patient

CAP-Recommended Solutions:

- Provide a single, comprehensive report

Addendum Reports[54]

- Biomarker test reports are often added as an addendum and may be missed if:
- Oncologists are not notified when addendum reports are added to the EHR
- Addendum reports are not linked to pathology reports in the EHR

CAP-Recommended Solutions[52]:

- Link the final pathology report to all subsequent addendum reports in the EHR
- When issuing the final report, pathologists should indicate that an addendum report is pending
- Utilize electronic notifications to alert clinicians that an addendum report has been entered into the chart
- Optimize the use of optical character recognition software to allow clinicians to search scanned reports using keywords

Challenging Reports[53-58]

- Narrative reports may be challenging to interpret quickly
- NGS reports may not state actionable genomic alterations, complicating interpretability
- Not every gene in an NGS report may be actionable
- Not every mutation in a driver gene is actionable

CAP-Recommended Solutions[51,52]:

- Use synoptic style reporting with layout continuity
- Use headlines to emphasize key findings
- Use clear and unambiguous nomenclature
- Provide patient management options, when possible, based on evidence

ASCO-Recommended Solutions:

ASCO recommends using precision oncology knowledge databases to assess a list of genomic alterations considered clinically actionable[1]

Difficult to interpret or "lost" reports may lead to patients not receiving biomarker-informed care[24]

GENETIC REPORTS

Genomic Alterations: Drivers vs. variants of unknown significance (VUS)

Not every mutation in an oncogene or tumor suppressor gene is a driver mutation[1]

For example, the driver gene BRAF (shown below) may contain mutations that are considered driver alterations *(black)* or alterations not associated with oncogenesis *(gray)*[59]

If a patient is positive for an alteration, it is important to determine whether the alteration is clinically meaningful[51]

Categorizing Genomic Alterations[51]**:** A joint consensus from ASCO, CAP, and AMP

Alterations are categorized into four categories based on their clinical impact:

TIER I Variants of Strong Clinical Significance	TIER II Variants of Potential Clinical Significance	TIER III Variants of Unknown Clinical Significance	TIER IV Benign or Likely Benign Variants
Therapeutic, prognostic & diagnostic	*Therapeutic, prognostic & diagnostic*		
Level A Evidence FDA–approved therapy Included in professional guidelines **Level B Evidence** Well-powered studies with consensus from experts in the field	**Level C Evidence** FDA–approved therapies for different tumor types of investigational therapies Multiple small published studies with some consensus **Level D Evidence** Pre-clinical trials or a few case reports without consensus	Not observed at a significant allele frequency in the general or specific subpopulation databases, or pan-cancer or tumor-specific variance databases No convincing published evidence of cancer association	Observed at significant allele frequency in the general or specific subpopulation databases No existing published evidence of cancer association

Joint consensus from ASCO, CAP, and AMP[51]**:**

Only tier I–III alterations should be included within a report; these should be listed in descending order of clinical importance

Databases Help Distinguish Actionable Driver Alterations

Several publicly available knowledge bases maintain up-to-date records that list driver alterations targeted by FDA-approved therapies. These include[1,60]:

The Memorial Sloan Kettering Cancer Center (MSK) OncoKB	MD Anderson Precision Oncology Decision Support (PODS)	The Catalogue of Somatic Mutations in Cancer (COSMIC)

The Impact of Variant Allele Frequency (VAF)

VAF corresponds to the proportion of genetic sequencing reads that harbor a specific allelic variant. VAF may be an indicator of the proportion of tumor cells that carry the variant.[1,61]

Somatic mutations generally have a VAF < 50% due to contaminating normal tissue. A VAF of ~50% or 100% may indicate a potential germline mutation[51]

Consider consulting a molecular tumor board (MTB) when needed.[62]
82 patients with solid tumors tested with NGS in a tertiary care center suggests that MTBs may help in appropriate and actionable clinical decision-making[63]

Joint consensus from ASCO, CAP, and AMP[51]:
VAF should be included in the report when appropriate[51]

Summary

Joint Consensus from ASCO, CAP, and AMP on Reporting Genetic Variants[51]

✓	**Classify alterations into tiers based on clinical impact** • Only include tier I-III alterations in the report
✓	**Provide a list of tested genes, including only those that were capable of being fully analyzed by assay used**
✓	**Prioritize clear communication** • Standard nomenclature should be used, in addition to colloquial nomenclature as needed, to convey meaning with clarity
✓	**Include relevant negative findings** • For tier I variants, pertinent negative results should be reported
✓	**Detail the clinical significance of detected variants** • For tier I and II variants, provide interpretive comments with clinicopathologic context to inform management decisions

GLOSSARY

ACCC, ASSOCIATION OF COMMUNITY CANCER CENTERS

AMP, ASSOCIATION FOR MOLECULAR PATHOLOGY

ASCO, AMERICAN SOCIETY OF CLINICAL ONCOLOGY

BRAF, V-RAF MURINE SARCOMA VIRAL ONCOGENE HOMOLOG B1

CAP, COLLEGE OF AMERICAN PATHOLOGISTS

CNV, COPY NUMBER VARIATION

EHR, ELECTRONIC HEALTH RECORD

FDA, U.S. FOOD AND DRUG ADMINISTRATION

FISH, FLUORESCENCE IN-SITU HYBRIDIZATION

IHC, IMMUNOHISTOCHEMISTRY

LIS, LABORATORY INFORMATION SYSTEM

MDT, MULTIDISCIPLINARY TEAM

NGS, NEXT-GENERATION SEQUENCING

RT-PCR, REVERSE TRANSCRIPTION POLYMERASE CHAIN REACTION

SNV, SINGLE NUCLEOTIDE VARIANT

TRF, TEST REQUISITION FORM

VAF, VARIANT ALLELE FREQUENCY

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SUMMARY

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

TRF format and layout vary and can impact biomarker ordering. Professional societies recommend:

- Reducing form variability and complexity[31,52]
- Ensure use of a common language[33,51]
- Keeping forms up-to-date[24]

Pathology reports are critical to ensure correct patient management. These reports should ideally be integrated into the EHR and should be as consolidated, clear, and synoptic as possible[52]

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