The Impact of Pan-Tumor Biomarker Testing

Precision Medicine

INCREASED SCIENTIFIC UNDERSTANDING OF CANCER LED TO INCREASED TREATMENT OPTIONS

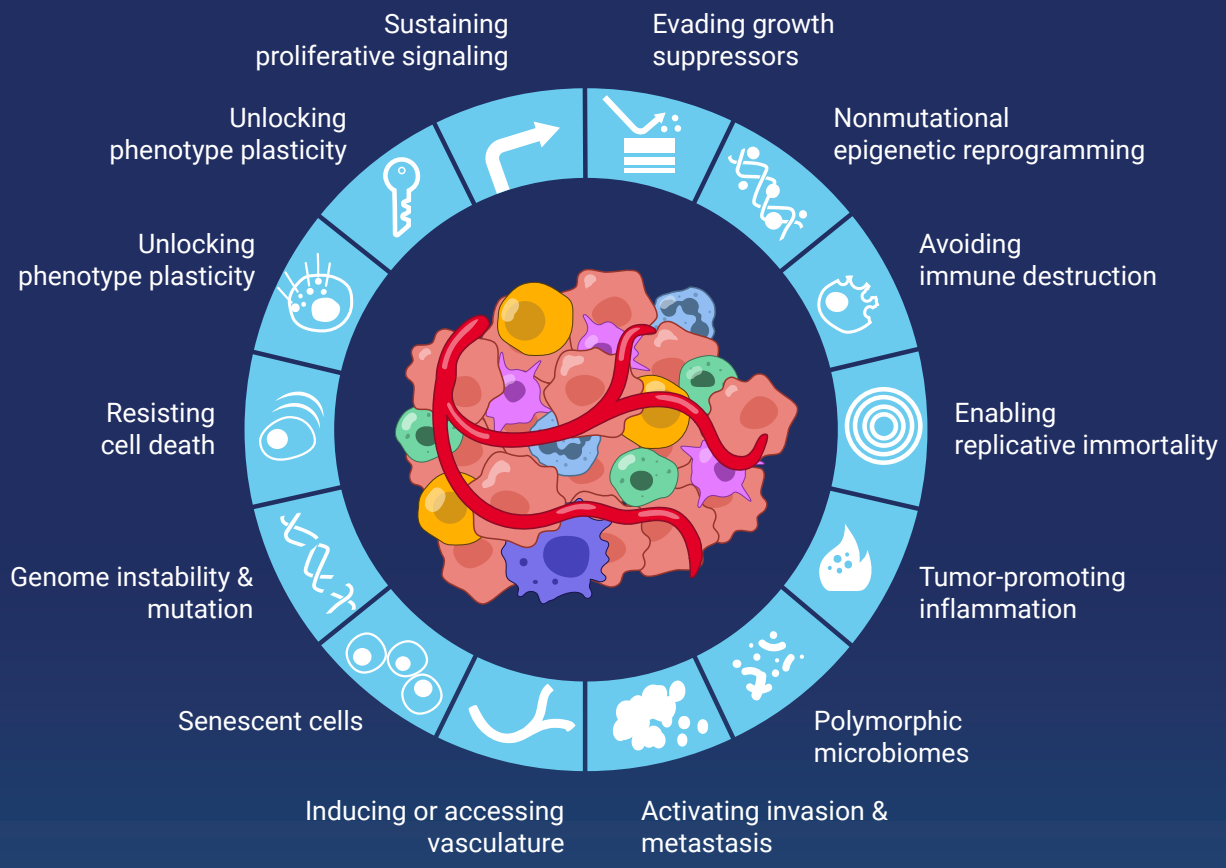


Image adapted from Hanahan D. *Cancer Discov*. 2021;12:31-46.[1]

- Cancers can use multiple pathways to enhance their survival[1]
  - Some tumors can have similar underlying molecular mechanisms driving their growth despite originating in different tissues and having different histologies
  - Advances in pan-tumor analysis revealed some driver alterations are universal and more prevalent in some cancers, while others are tumor type–specific

A greater understanding of cancer biology caused tumors to be further classified by molecular characteristics[2-5]

+

Technical advances in gene sequencing have made genomic sequencing more feasible for use in the clinic[6-9]

↑ Increased homogeneity and number of tumor subtypes[2,3,10]

↓ Fewer number of patients with a specific tumor subtype[2,4,11]

ADVANCES IN CANCER BIOLOGY AND GENOMICS REQUIRED TRIAL DESIGN INNOVATIONS

Trials aiming to test 1 intervention designed for 1 molecularly defined tumor type with a traditional trial design became less realistic from an enrollment perspective[11-14]

↓

New trial designs called master protocols, made possible by statistical advances, were developed to allow for the study of multiple hypotheses in different subpopulations simultaneously[12,13]

↓

Master protocols have improved drug development efficiency and facilitated the study of molecularly defined cancers[9,13,15]

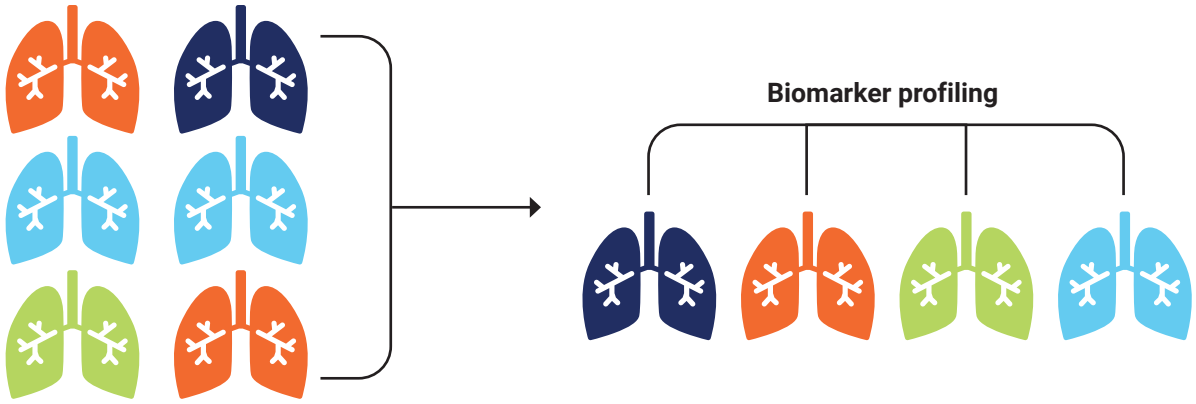
NOVEL TRIAL DESIGNS

Umbrella trials and basket trials are 2 types of master protocols that use biomarkers to determine experimental intervention

Umbrella Trials[13,15,16]

Assess different interventions in participants who have the same tumor type but different predictive biomarkers

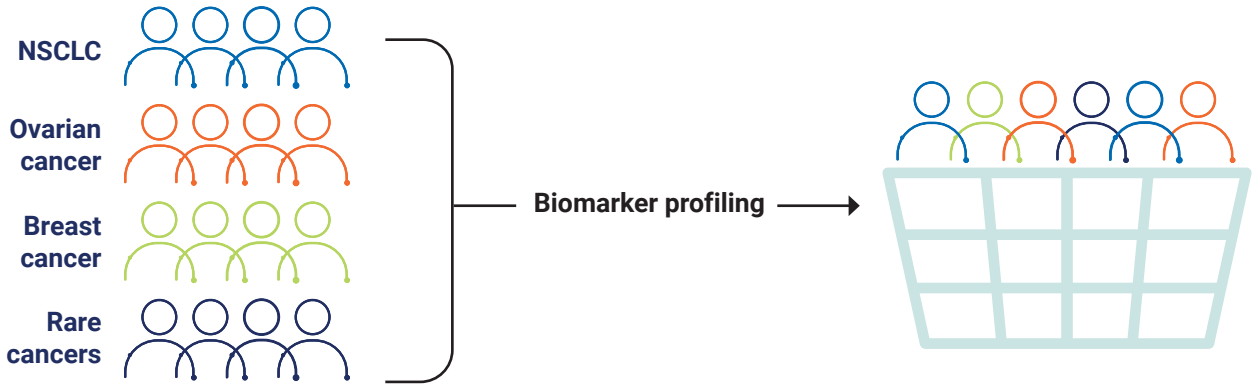
- Predictive biomarkers are used to split patients into subgroups
- More feasible to assign a control group using the current standard of care because only 1 tumor type is being studied



Basket Trials[13,15,16]

Assess 1 intervention in participants with different tumor types but the same predictive biomarker

- Patients split into subgroups based on their tumor type
- May not be possible to assign a control group if standard of care differs among tumor types in trial

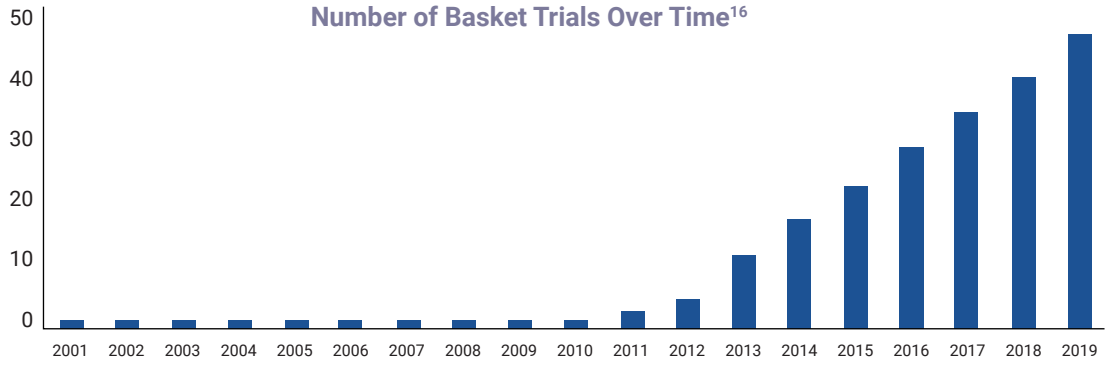


While umbrella and basket trials have key differences, each matches an intervention with a predictive biomarker and enables more efficient and accelerated clinical development[12,14,15,17]

NSCLC, non–small cell lung cancer.

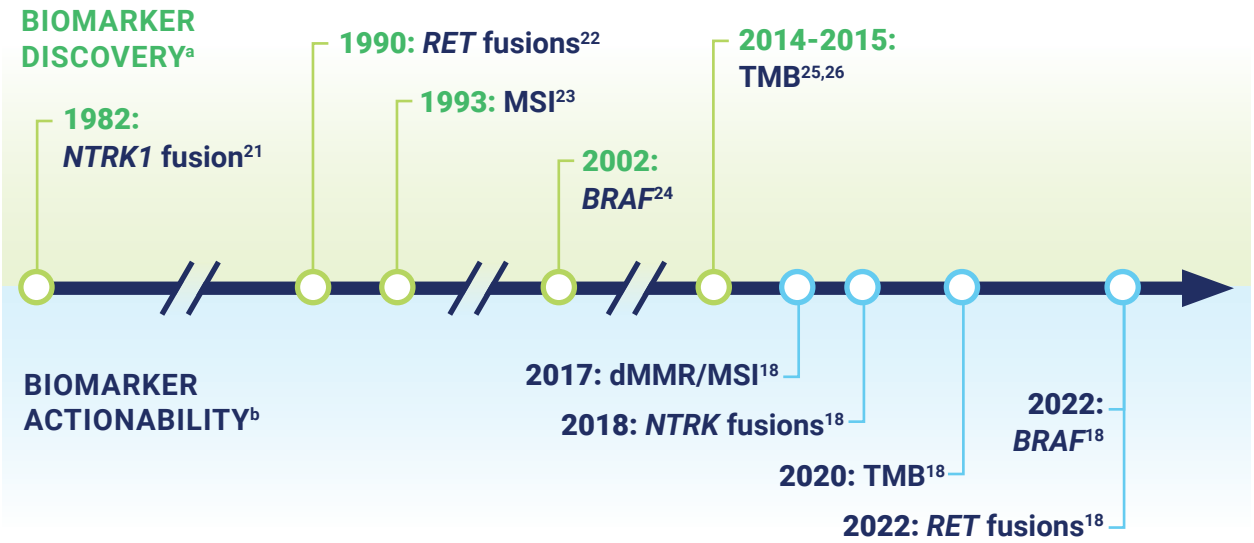
BASKET TRIALS AND THE RISE OF PAN-TUMOR BIOMARKERS

- Basket trials can provide evidence for the FDA approval of tumor-agnostic or "pan-tumor" biomarkers[13,14,18-20]
- Basket trials leading to an FDA approval of a tumor-agnostic therapy have included tumor types like NSCLC, CCA, CRC, and ovarian cancers[18-20]
- As the number of basket trials has risen, so has the number of FDA-approved tumor-agnostic targeted therapies[16,18]



Between 2017 and 2023, ≥5 pan-tumor biomarkers have become actionable[18]

EVOLUTION OF PAN-TUMOR BIOMARKERS: DISCOVERY & ACTIONABILITY



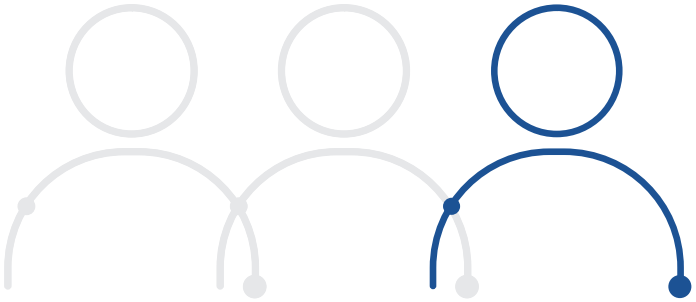
Pan-tumor biomarkers exist for both targeted therapies and immunotherapies[17]

[a]Discovery refers to the first identification in any tumor type.
[b]Actionability is based on the first tumor-agnostic approval of a therapy defined by this biomarker.
BRAF, v-raf murine sarcoma viral oncogene homolog B1; CCA, cholangiocarcinoma; CRC, colorectal cancer; dMMR, deficient mismatch repair; FDA, US Food and Drug Administration; MSI, microsatellite instability; *NTRK*, neurotrophic tyrosine receptor kinase; *RET*, ret proto-oncogene; TMB, tumor mutational burden.

IMPACT OF PAN-TUMOR TESTING ACROSS ONCOLOGY

Nearly 10% of patients with cancer may be positive for a pan-tumor biomarker[18,27-29]

- The prevalence of each pan-tumor biomarker varies across tumor types[18,27-29]
- Patients may be positive for >1 pan-tumor biomarker[5,27,30]
  - TMB and/or MSI-H may occur in patients harboring other driver alterations
- Testing for pan-tumor biomarkers increases the percentage of patients eligible for a biomarker-informed therapy from ≈24% to ≈33%[18,27-29]



1 in 3 patients with cancer may have ≥1 actionable biomarker when including pan-tumor biomarkers[18,27-29,31]

Testing all patients for pan-tumor biomarkers may bring precision oncology to more patients

MSI-H, microsatellite instability–high.

>50% of actionable predictive biomarkers are approved for common cancer types[3,32,33]

As of March 2023, there are **>70 FDA-approved therapies** with ≥1 biomarker-linked indication covering >30 cancer types[34]

Of those, **>50% impact 1 of the top 5** most common solid tumors[3,32,33]

Fewer actionable biomarkers are approved for patients with less common cancers[3,32]

EXAMPLES OF LESS COMMON CANCERS

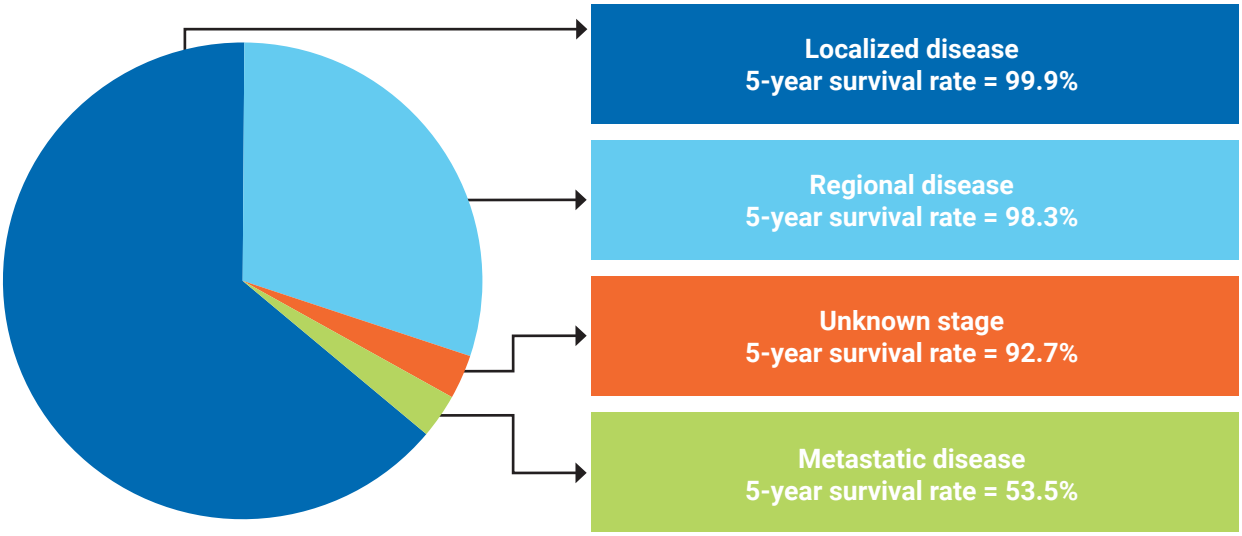
Metastatic thyroid cancer		Impacts 3% of TCs ≈1,310 new diagnoses annually in the United States[35]
Ovarian cancer		≈19,710 new diagnoses annually in the United States[36]
CCA		≈8,000 new diagnoses annually in the United States[37]

TC, thyroid cancer.

METASTATIC THYROID CANCER

TCs are typically diagnosed in early-stage disease[35,38]

- TC impacts ≈44,000 patients in the United States annually
  - The rate of new TC cases increased between 2000 and 2010 before becoming more stable
- ≈97% of patients have a 5-year survival rate of >93%
- The 3% of patients who are diagnosed with **metastatic disease** have a **significantly shorter 5-year survival rate** of 53.5%



In metastatic thyroid cancer, prognosis varies significantly by subtype

The WHO groups TC histologic subtypes into 8 larger categories based on cell of origin, pathologic or molecular features, and biologic behavior[39]

Malignant follicular cell–derived neoplasms consist of the most prevalent subtypes at diagnosis[38,40,41]

Malignant follicular cell–derived neoplasms are further divided into DTCs and ATCs, the latter having the worse prognosis[39,40]

ATC, anaplastic thyroid cancer; DTC, differentiated thyroid cancer; WHO, World Health Organization.

Prevalence and 5-Year Survival Rate of Metastatic TC Histologic Subtypes

		Subtype	Incidence[41]	5-year Survival Rate[40]
Follicular cell–derived neoplasms	DTC	PTC	84%	74%
		FTC	11%	67%
	Undifferentiated	ATC	1%	4%
Thyroid C-cell–derived carcinomas		MTC	2%	43%
		Other[a]	2%	

There are 3 actionable biomarkers in thyroid cancer[3]

RET fusions	**Actionable** for all **TCs and solid tumors**[3,18]	Occur in **≈5% of patients with TC**[3]	Testing methods include FISH, RT-PCR, and NGS **Guideline recommends NGS**[42]
RET mutations	**Actionable in MTC**[3] (=2% of all TCs)[40]	Occur in **56% of patients with MTC**[43]	Testing methods include gPCR and NGS **Guideline recommends gPCR or NGS**[42]
BRAF V600	**Actionable in ATC**[3] (=1% of all TCs)[41] **Actionable in all solid tumors**[18]	Occurs in **32% of patients with ATC**[44] Occurs in **33% of patients with TC**[45]	Testing methods include **IHC, RT-PCR, and NGS**[42,46]

[a]Includes unspecified, poorly specified (eg, insular), and others.
FISH, fluorescence in situ hybridization; FTC, follicular thyroid cancer; IHC, immunohistochemistry; MTC, medullary thyroid cancer; NGS, next-generation sequencing; PTC, papillary thyroid cancer; qPCR, quantitative polymerase chain reaction; RT-PCR, real-time polymerase chain reaction.

METASTATIC THYROID CANCER (CONTINUED)

4 of the 5 pan-tumor biomarkers have been detected in TCs[3,18,28,45,47,48]

- Of those detected, the prevalence varies by subtype[3,28,43,44]
  - *RET* fusions are not observed in MTC but occur in other TC subtypes
  - *BRAF* V600 mutations occur in PTC and ATC but not in MTC[43,44]

Prevalence of Actionable Predictive Biomarkers in TC[3,18,28,43-45,47,49]

Biomarker	TC[3]	Pan-Tumor[18]	Prevalence	
		MTC (2% of TCs)[41]	**ATC** (1% of TCs)[41]	**TC (all)**
RET mutations (MTC only)	–	56%[43]	–	–
BRAF V600E (ATC only)	–	–	32%[44]	–
BRAF V600	x	–	–	33%[45]
RET fusions (all TCs)	x	–	–	5.1%[3]
TMB-H	x	–	–	2.7%[48]
NTRK fusions	x	–	–	2.3%[28]
MSI-H	x	–	–	0%[47]

Testing for *BRAF* V600 in thyroid cancer can identify 33% of patients who may be eligible for a biomarker-informed therapy[18,45]

≈43% of patients with TC an actionable biomarker[3,18,28,45,47,48]

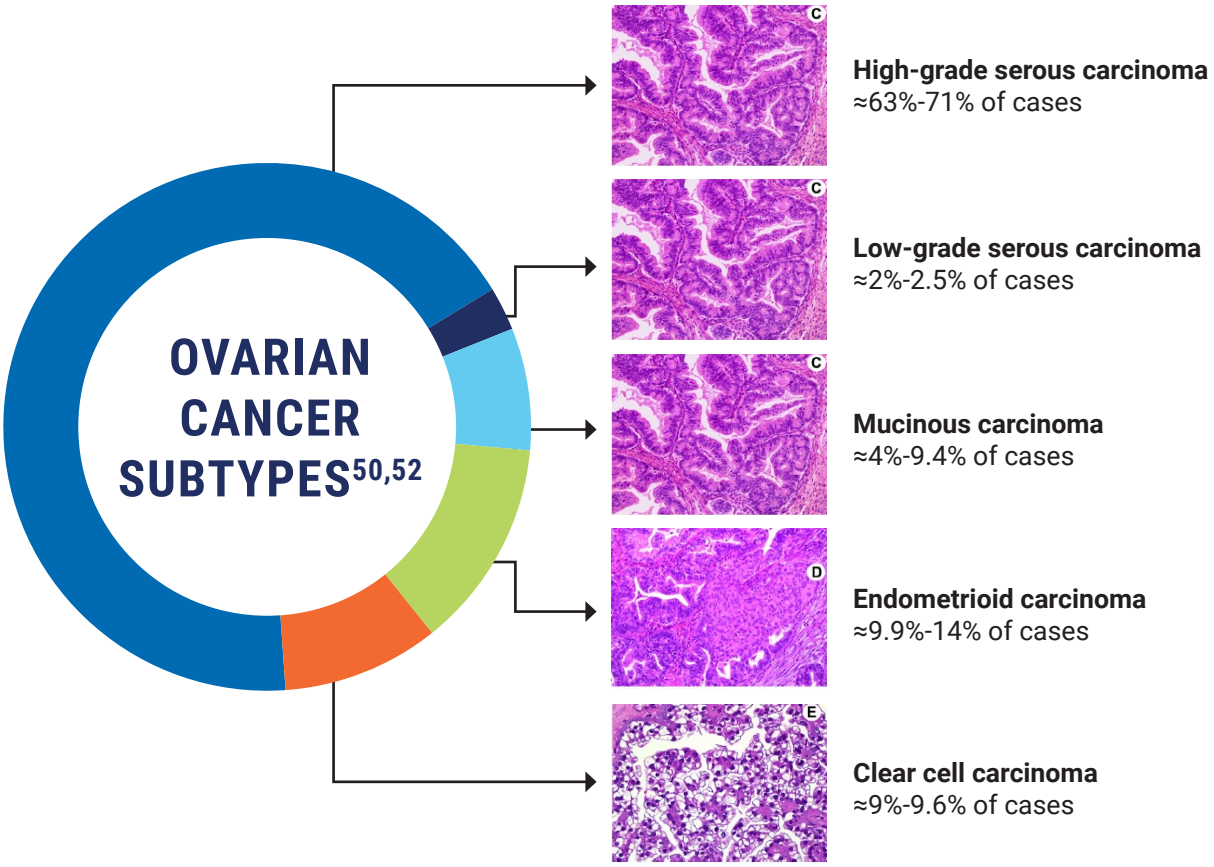
≈38% of patients with TC are positive for a pan-tumor biomarker[3,18,45,47,48]

TMB-H, tumor mutational burden–high.

OVARIAN CANCER

Ovarian cancer is a less common cancer with a poor prognosis that is classified into 5 histologic subtypes

- In 2022, ovarian cancer accounted for 1% of new cancer cases but 2.2% of all cancer-related deaths[36]
- Ovarian cancer subtypes each have a distinct pathogenesis and prognosis[49-51]



All patients with ovarian cancer eventually become resistant to current therapies[49]

The prevalence and actionability of predictive biomarkers in ovarian cancer varies by histologic subtype

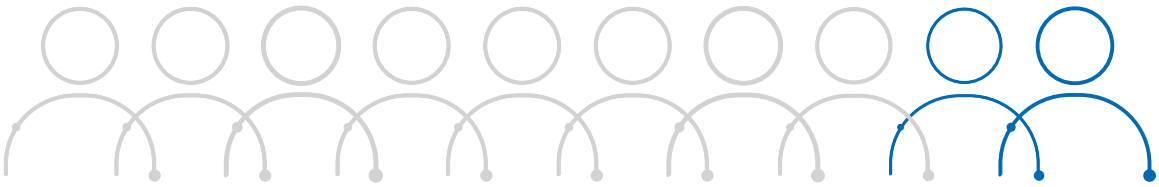
There are 2 actionable biomarkers in ovarian cancer[3,53,54]

BRCA1/2 Mutations[3]

- *BRCA1* and *BRCA2* play key roles in homologous recombination; pathogenic variants contribute to tumorigenesis[55,56]
- ***BRCA1/2* mutations occur in 14%** of women with ovarian cancer[57]
  - ASCO recommends BRCA testing for all patients with ovarian cancer[58,59]
- *BRCA* pathogenic variants can be detected with **RT-PCR or NGS**[60,61]

FRα1-H[53,54]

- FRα1, a GPI-anchored protein encoded by *FOLR*, participates in cell division and proliferation[62]
- ≈55% of patients with ovarian cancer are positive for any FRα1 expression. In patients with high-grade serous ovarian cancer, **36% are FRα1-H–positive**[53,63,a]
- **IHC**, the only assay that can assess FRα1 expression, is only semiquantitative and prone to interobserver variability[53]



20% of patients may be positive for both FRα1-H and *BRCA1/2* mutations[53]

[a]FRα1-H is 75% of tumor cells having high membranous staining (IHC 2+).[53]
ASCO, American Society of Clinical Oncology; *FOLR*, folate receptor; FRα1, folate receptor alpha 1; FRα1-H, folate receptor alpha 1–high; GPI, glycosylphosphatidylinositol.

Every pan-tumor biomarker has been detected in ovarian cancer

- Although the precise prevalence varies considerably among subtypes[18,27,64]
  - For example, *BRAF* V600E can be found in 5% to 20% of low-grade serous subtypes but only 1.7% of all ovarian cancers[65-70]

Actionable Predictive Biomarkers in Ovarian Cancer

Biomarker	Ovarian[3,54]	Pan-Tumor[18]	Prevalence
BRCA1/2	x	—	14%[57]
FRα1-H	x	—	36%[53]
BRAF V600	—	x	1.7%[70]
TMB-H	—	x	1.6%[27]
MSI-H	—	x	≈2%[27]
NTRK fusions	—	x	≈3%[71]
RET fusions	—	x	0.5%[27]

In addition to *BRCA1/2* and FRα1-H, pan-tumor biomarker testing can offer therapeutic options to an additional ≈6% of patients with ovarian cancer[18,27,45,64,71]

≈50%[a] of patients with ovarian cancer have an actionable biomarker[3,18,27,45,53,54,57,64,71]

≈6% of patients with ovarian cancer are positive for a pan-tumor biomarker[18,27,45,64,71]

[a]Calculation did not include the ≈20% of patients with FRα1-H who are also positive for *BRCA1/2*.

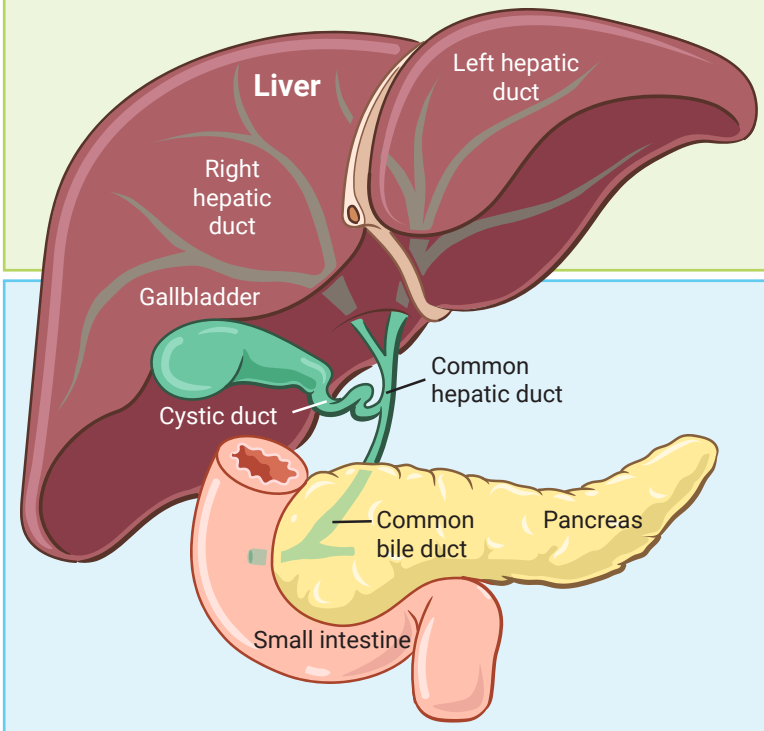
CHOLANGIOCARCINOMA

CCA is a diverse group of aggressive malignancies associated with a poor prognosis[72]

CCA accounts for **3%** of all GI tumors[72]

- CCA is classified by **anatomic site: intrahepatic vs extrahepatic**[72]
- The **prevalence and prognosis vary** by anatomic site[73-75]

CCA Classification, Prevalence, and Prognosis in the United States



Intrahepatic CCA:
- Accounts for 53%-55% of CCAs[73,74]
- 5-year survival: 9%[75]

Extrahepatic CCA (includes perihilar and distal):
- Accounts for 45%-47% of CCAs[73,74]
- 5-year survival: 11%[75]

GI, gastrointestinal.

There are 2 actionable biomarkers in CCA[76]

20% of patients with CCA have an ***IDH1* mutation**[76]	Mutually exclusive with *NRAS/KRAS* mutations[77] Can be detected with NGS, PR, and other sequencing technologies[76]
15% of patients with CCA have an ***FGFR2* fusion**[76]	*FGFR2* fusions account for 12% of all iCCA cases[78] Mutually exclusive with *IDH1*, *KRAS*, and *BRAF* mutations[77,79] Can be detected with FISH and NGS[80]

- ASCO recommends the use of NGS for tissue preservation when there is >1 biomarker-informed therapy for a disease[3]
- Although NGS is recommended, obtaining sufficient tissue for biomarker testing in CCA may be challenging[81]
  - In one study, 27% of patients with CCA did not have sufficient tissue for NGS testing[81,a]
  - When liquid biopsy was used as an alternative, 85% of patients who were tested were positive for an actionable biomarker[81]

[a]Based on a retrospective analysis of 149 tumor samples from 104 patients with advanced CCA.
FGFR2, fibroblast growth factor receptor 2; iCCA, intrahepatic cholangiocarcinoma; *IDH1*, isocitrate dehydrogenase 1; *KRAS*, Kirsten rat sarcoma viral gene homolog; *NRAS*, neuroblastoma ras viral oncogene homolog.

All pan-tumor biomarkers can be detected in CCA

- While all pan-tumor biomarkers can be detected in CCA, they are less common, with each occurring in <5% of patients[3,18,45,48,64,82]
  - *RET* fusions and *NTRK* fusions are particularly rare[3,82]
- However, up to 10% of patients with CCA may be positive for 1 of the 5 markers

Actionable Predictive Biomarkers in Ovarian Cancer

Biomarker	CCA[76]	Pan-Tumor[18]	Prevalence
IDH1 mutations	x	–	20%[76]
FGFR2 fusions	x	–	15%[76]
BRAF V600	–	x	2%[45]
TMB-H	–	x	4%[48]
MSI-H	–	x	1.6-3.8%[64]
NTRK fusions	–	x	0.25%[82]
RET fusions	–	x	0.1%[3]

Testing for pan-tumor biomarkers in CCA identifies an extra ≈10% of patients who may be eligible for biomarker-informed therapy[3,18,45,48,64,82]

≈45% of patients with CCA have an actionable biomarker[3,18,45,48,64,76,82]

≈10% of patients with CCA are positive for a pan-tumor biomarker[3,18,45,48,64,82]

IMPACT OF PAN-TUMOR BIOMARKER TESTING IN SELECT EXAMPLES OF LESS COMMON CANCERS

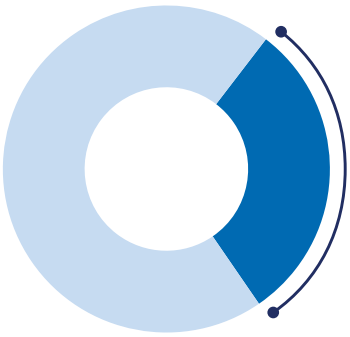
Testing for actionable pan-tumor biomarkers identifies more patients eligible for a biomarker-informed therapy[18]

Metastatic thyroid cancer	 **≈38%** more patients identified[3,28,45,47,48]
Ovarian cancer	 **≈6%** more patients identified[27,45,64,71]
CCA	 **≈10%** more patients identified[3,27,45,48,64]

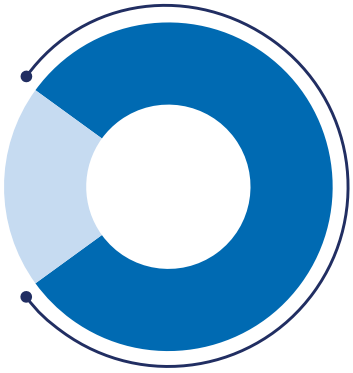
EVOLUTION OF BIOMARKER TESTING IN NEURO-ONCOLOGY

There are ≈7,515 new glioma diagnoses annually in the United States[83,84]

Gliomas account for[83,85]:



30%
of all CNS tumors



80%
of all malignant CNS tumors

- Glioma is an umbrella term covering >40 distinct subtypes, each with a unique pathogenesis and prognosis[83,86]
  - The 5-year survival rate for glioblastoma is 6.4%[87]
  - For other gliomas, the 5-year survival rate is 77.4%[88]

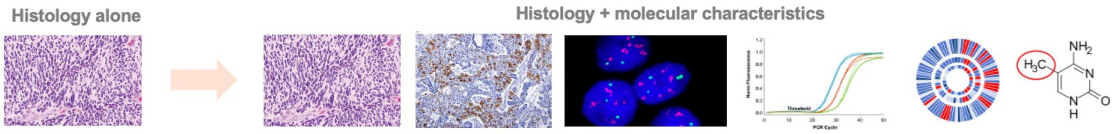
Scientific developments have led to a better understanding of glioma pathogenesis and more precise prognoses[86,89]

CNS, central nervous system.

GLIOMA CLASSIFICATION AND DIAGNOSTICS

In 2016, the WHO created a classification system to identify more homogenous subpopulations of gliomas by integrating molecular characteristics with histology[83,86,90]

Shift in Glioma Categorization[89-91]



- Molecular characteristics included the presence or absence of IDH mutation and 1p/19q codeletion

Glioma diagnostics require biomarker testing[89,90]

In 2021, the WHO updated the glioma classification system to further incorporate the role of molecular characteristics, expanding the number of subtypes[90]

- Key changes included[90]:
  - Using a within-tumor grading system for most tumors
  - Molecular markers determining grade in some instances
  - Additional subtypes
  - Utilization of a layered report structure

Layered Report Structure

- Integrated diagnosis (combined tissue-based histologic and molecular diagnosis)
- Histologic diagnosis
- CNS WHO grade
- Molecular information (listed)

Example Change in Diagnosis[89,90]

2016	Glioblastoma, IDH mutant	**2021**	Astrocytoma, IDH mutant, *ATRX* loss, *TP53* mutated, *CDKN2A/B* deleted, WHO grade 4[a]

[a]WHO grade 4 is based on *CDKN2A/B* status. Histopathologic grading can be WHO grade 2 or 3.
ATRX, alpha-thalassemia mental retardation X-linked protein; *CDKN2A/B*, cyclin-dependent kinase inhibitor 2A/B; *TP53*, tumor protein p53.

Most gliomas are diffuse gliomas[83,86,90]

- Diffuse gliomas are further grouped into 3 different classes, each consisting of multiple distinct subtypes[83,89,90]
- The prevalence and prognosis vary widely among subtypes
  - For example, the 5-year survival for glioblastoma is ≈6%, while the 5-year survival for pediatric low-grade gliomas is 90%[92,93]

Adult-Type Diffuse Gliomas[90]	• Astrocytoma, IDH mutant • Oligodendroglioma, IDH mutant, and 1p/g19 codeleted • Glioblastoma, IDH WT	
Pediatric-Type Diffuse Low-Grade Glioma[90]	• Diffuse astrocytoma, *MYB* or *MYBL1* altered • Angiocentric glioma	• Polymorphous low-grade neuroepithelial tumor of the young • Diffuse low-grade glioma, *MAPK* pathway altered
Pediatric-Type Diffuse High-Grade Gliomas[90]	• Diffuse midline glioma, H3 K27 altered • Diffuse hemispheric glioma, H3 G34 mutant	• Diffuse pediatric-type high-grade glioma, H3 WT and IDH WT • Infant-type hemispheric glioma

Molecular testing is required to distinguish among subtypes within each grouping[89,90]

Multiple distinct molecular alterations define diffuse glioma subtypes by the WHO classification[89,90]

Diagnostic Genomic Alterations

Adult-Type Diffuse Gliomas[89,90]

- Mutations in *IDH1, IDH2, ATRX, TP53, CIC, FUBP1, NOTCH1*, and the *TERT* promoter
- Gene deletion of *CDKN2A/B*
- Gene amplifications in *EGFR*
- Chromosome copy number changes: gain of 7 and loss of 10

Pediatric-Type Diffuse Low-Grade Glioma[90]

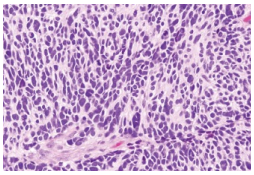
- Mutations in *MYB, MYBL1, BRAF, FGFR* family, and *FGFR1*
- Gene fusions/rearrangements in *BRAF* and *FGFR1*

Pediatric-Type Diffuse High-Grade Gliomas[90]

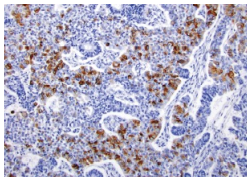
- Mutations in *H3F3A, TP53, ACVR1, PDGFRA, EGFR, ATRX*, and *MYCN*
- Protein overexpression of *EZHIP*
- Gene fusions/rearrangements in *NTRK, ALK, ROS*, and *MET*
- Methylation changes in *EGFR*

ACVR1, activin A receptor type 1; *ALK*, anaplastic lymphoma kinase; *CIC*, capicua; *EGFR*, epidermal growth factor receptor; *EZHIP*, enhancer of zeste homologs inhibitory protein; *FUBP1*, far upstream element-binding protein 1; *MAPK*, mitogen-activated protein kinase; *MET*, mesenchymal epithelial transition; *MYB*, myeloblastosis proto-oncogene; *MYBL1*, myeloblastosis proto-oncogene like 1; *MYCN*, myelocytomatosis viral oncogene neuroblastoma-derived homolog; *NOTCH1*, neurogenic locus notch homolog protein 1; *PDGFRA*, platelet-derived growth factor receptor alpha; *ROS*, ROS proto-oncogene; *TERT*, telomerase reverse transcriptase; WT, wild type.

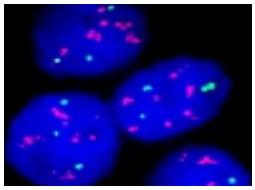
Relevant biomarker testing technologies include[90,94]:



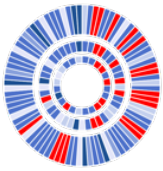
Histologic stains



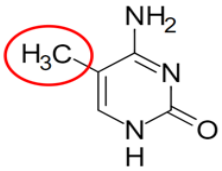
IHC



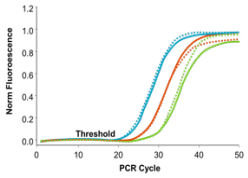
FISH



NGS



Methylomics



RT-PCR

NGS can detect most glioma biomarkers simultaneously[95]

- NGS has similar specificity and sensitivity as IHC, FISH, and RT-PCR but may not be able to determine methylation status[95-97]
- NGS cannot replace histologic analysis[98]

NGS positively impacts patient care

- NGS results have changed the diagnosis and treatment decisions for some patients with glioma in multiple studies[95,99,100]
- NGS is more cost-effective than single-gene testing in glioma[95,96]

Testing for pan-tumor biomarkers in patients with gliomas may identify patients eligible for a biomarker-informed therapy

- Pan-tumor biomarkers have been detected in gliomas, but the prevalence varies by subtype[18,101]
  - For example, *BRAF* V600E occurs in 0% of patients with astrocytoma but 69% of patients with eGB[102]
- Some pan-tumor biomarkers are enriched in specific glioma classes[18,71,102]
  - Both *BRAF* alterations and *NTRK* fusions occur more frequently in pediatric low-grade gliomas
- As of April 2023, the only biomarker-informed therapy approved for any type of glioma is specific to pediatric low-grade gliomas[34,103]

Actionable Predictive Biomarkers in Diffuse Gliomas

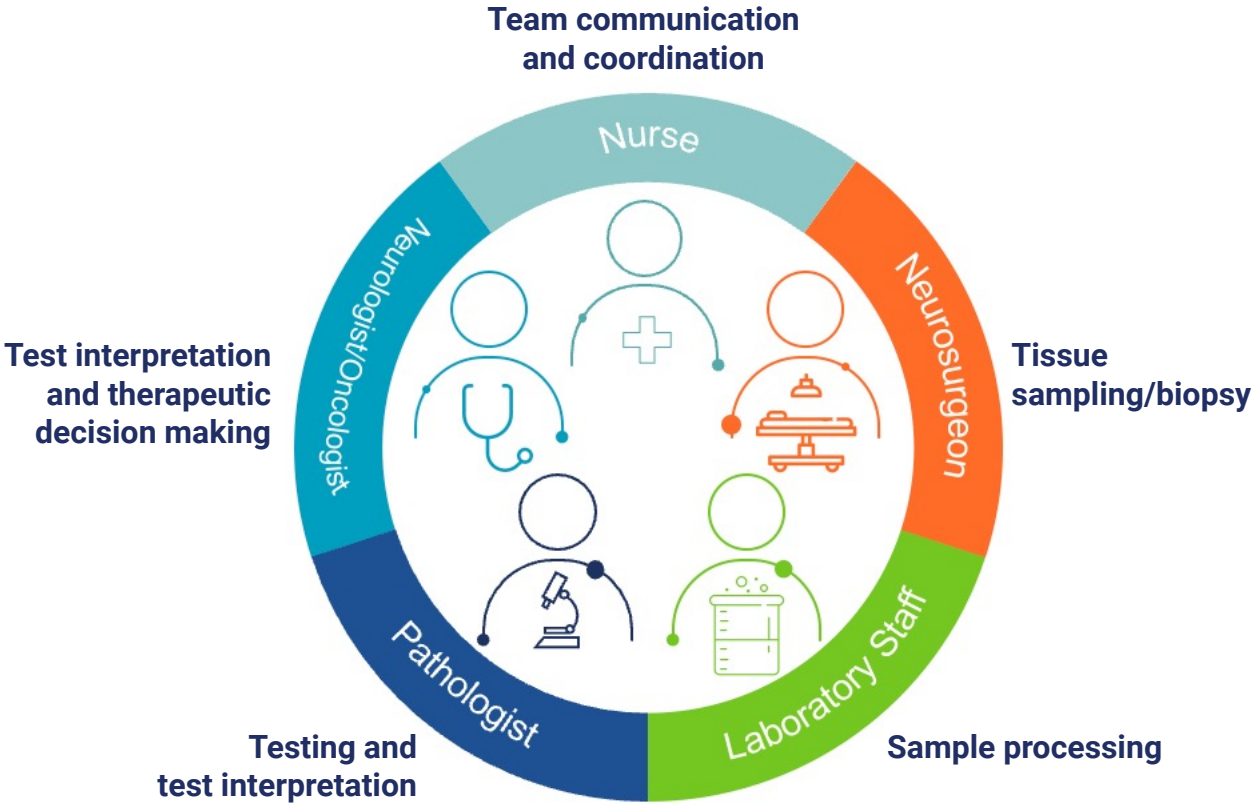
Biomarker	Glioma[103]	Pan-Tumor[18]	Prevalence	
			Adult	Pediatric
BRAF V600[102]	X[b]	X	4%	7%
TMB-H[104]	—	X	3%[a]	—
MSI-H[47]	—	X	0.3%	—
NTRK fusions[71,101]	—	X	0.3%-0.8%	1.2%-3.9%
RET fusions[105,106]	—	X	0%	0%

[a]Only includes glioblastoma.
[b]Only approved for pediatric low-grade gliomas.
eGB, epithelioid glioblastoma.

In gliomas, all actionable biomarkers are pan-tumor biomarkers[18,103]

≈10% of patients with any glioma have an actionable biomarker[18,47,71,101,102,104-106]

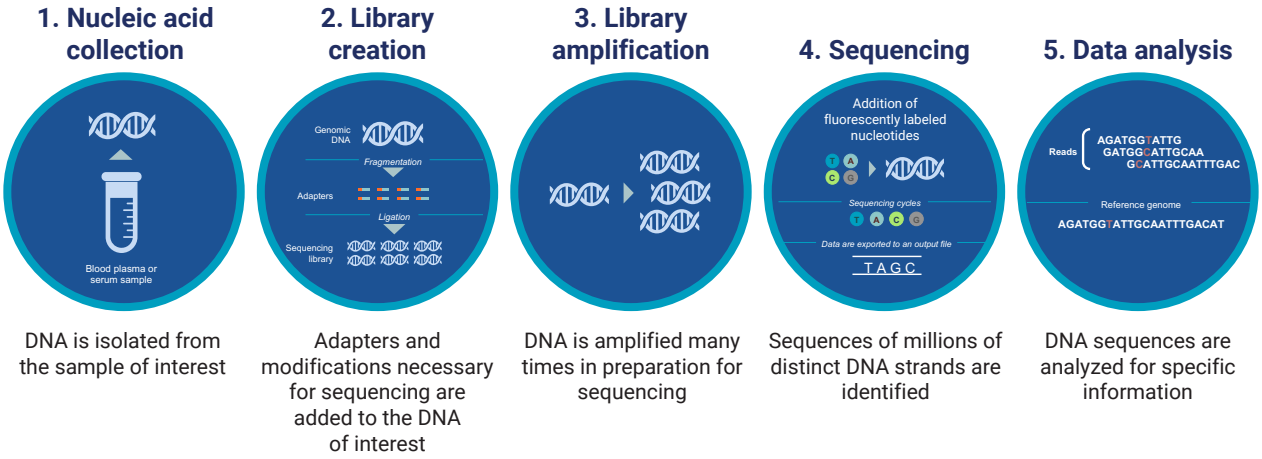
BIOMARKER TESTING REQUIRES MULTIDISCIPLINARY COLLABORATION[107]



PANEL TESTING WITH PAN-TUMOR BIOMARKERS

NGS can simultaneously detect multiple oncogenic drivers

- NGS is a guideline-recommended, high-throughput sequencing method that can simultaneously screen for multiple mutations and genomic alterations with a minimal amount of tissue from eligible patients with metastatic cancer[3,108,109]
- While it can be used to sequence the whole genome, exome, or transcriptome, **targeted NGS** can detect clinically relevant biomarkers in an adequate timeframe to aid therapeutic decisions[110]



DNA is isolated from the sample of interest

Adapters and modifications necessary for sequencing are added to the DNA of interest

DNA is amplified many times in preparation for sequencing

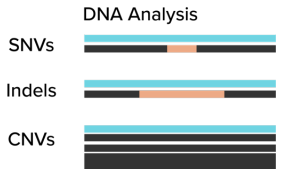
Sequences of millions of distinct DNA strands are identified

DNA sequences are analyzed for specific information

- Sequencing results may be influenced by nucleic acid selection
  - RNA-based NGS can identify patients with actionable biomarkers missed by DNA-based NGS[111]

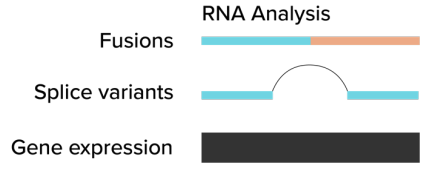
DNA-based NGS[112-114]

- Structural variant/CNV detection may be limited to hybrid-capture assays
- Less sensitive in the detection of fusions and splice variants



RNA-based NGS[112,113,115]

- Directly assays fusions and splice variants
- Technically challenging
- Requires robust bioinformatic analysis



ASCO recommends RNA-based fusion testing for patients with no other oncogenic driver detected by DNA multigene panel–based genomic sequencing[3]

CNV, copy number variation; DNA, deoxyribonucleic acid; RNA, ribonucleic acid; SNV, single nucleotide variant.

Both tissue and liquid biopsies can be used for NGS

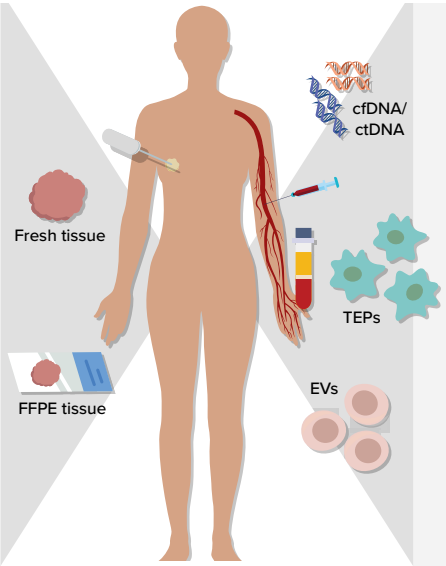
Tissue Biopsy Testing

May provide a snapshot of the cellular and molecular characteristics of 1 part of a single tumor[116]

Does not provide information from all cancer cells

May miss an alteration if it is not present in the tested sample[117]

Processing of biopsies of bone metastases may lead to DNA degradation[117]



Liquid Biopsy Testing

May reflect overall genomic landscape of the tumor and all metastatic sites (bone or other tissues)[118,119]

- Does not provide information on TME[120]

May miss an alteration if ctDNA concentration is below the LOD, leading to a false negative

- ctDNA levels may vary significantly[119,121,122]

CTCs and ctDNA levels may be impacted by the number and sites of metastases, including bone[119,121-123]

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

- CSF has emerging use in NGS sequencing

There are several variables impacting NGS results

		Nucleic Acid Selection[111-115]	
		DNA	**RNA**
Variant detection	SNVs, small indels	✓	✓
	Fusions/rearrangements	Enrichment strategy dependent	✓
	Exon skipping	Enrichment strategy dependent	✓
	CNV	✓	
	TMB	Enrichment strategy dependent[a]	
Bioinformatic analysis complexity		Less	More
Ease of use		More	Less
Biopsy type		Tissue and liquid	Tissue only

- DNA-based NGS assays can be run sequentially with RNA-based NGS assays[3,112]
- Some NGS assays are hybrid assays that use both DNA and RNA inputs simultaneously[113,124]

[a]TMB estimations from panel NGS assays may vary significantly based on assay coverage. Amplicon assays do not cover enough of the genome to estimate TMB.[125]

CTC, circulating tumor cell; ctDNA, circulating tumor deoxyribonucleic acid; EV, extracellular vesicle; FFPE, formalin-fixed paraffin-embedded; LOD, limit of detection; TEP, tumor-educated blood platelet; TME, tumor microenvironment.

TESTING FOR PAN-TUMOR BIOMARKERS

The 5 pan-tumor biomarkers include different types of genomic alterations

BRAF V600E

Point mutations leading to a missense mutation[45]

NTRK fusions

Gene fusions involving *NTRK1*, *NTRK2*, or *NTRK3*[3]

RET fusions

Gene fusions involving *RET*[18]

MSI/dMMR

Increased mutations at microsatellite loci OR MMR protein expression loss[3]

TMB

Quantification of mutations throughout the genome[3]

The testing technology capable of detecting pan-tumor biomarkers differs by biomarker[126-132]

BRAF

BRAF is one of the most common driver oncogenes, with *BRAF* V600E being the predominant *BRAF* mutation[5,45]

Mutations in the *BRAF* gene cause activation of the MAP kinase pathway, leading to uncontrolled tumor growth and proliferation[24]

≈4%-8% of all cancers have a ***BRAF* mutation**[29,45]

Before *BRAF* targeted therapies, *BRAF* mutations were associated with a poor prognosis[133-136]

55%-65% of all *BRAF* mutations are ***BRAF* V600E**, an **actionable pan-tumor biomarker**[29,45,133]

Prevalence of *BRAF* V600 mutation in select solid tumors[45,134]

Cutaneous melanoma[45]	≈40%
Thyroid carcinoma[45]	≈32%
Low-grade serous ovarian carcinoma[70,134-137]	≈5%-20%
Colorectal adenocarcinoma[45]	≈7%
Cholangiocarcinoma[45]	≈2%
Glioma[45]	≈2%

TESTING OPTIONS FOR *BRAF* V600 MUTATIONS[126-128,138,139]

NGS	RT-PCR	IHC
Advantages: Maximum specificity (100%) and high sensitivity (98%) Can detect all *BRAF* mutation classes and other actionable biomarkers simultaneously	**Advantages:** High sensitivity (98%) Fast turnaround	**Advantages:** VE1 clone antibody has high sensitivity (98%) and specificity (99%) Cost-effective first-line screening method
Considerations: Long turnaround time and high cost (depending on assay)	**Considerations:** Only identifies limited number of *BRAF* V600 mutations	**Considerations:** Limited to *BRAF* V600E mutation Risk of false negatives

A liquid biopsy can be used with NGS when a tissue biopsy is not available[140,141]

NTRK

The *NTRK* gene family contains 3 members (*NTRK1, NTRK2, NTRK3*)[129]

Pathogenic gene fusions result in the production of altered TRK proteins and uncontrolled cell growth

Common pathogenic fusion partners include *ETV6, TPM3*, and *LMNA*[71]

NTRK gene fusions occur in only 0.3% of solid tumors but are highly prevalent in rare cancers[101]

While *NTRK* fusions may co-occur with MSI-H (17.6%) and high TMB (20%), it is unclear how these patients respond to immunotherapies[142,143]

***NTRK* gene fusions are actionable tumor-agnostic biomarkers[3]**

ETV6, E26 transformation-specific variant transcription factor 6; *LMNA*, lamin A/C.

NTRK PREVALENCE

Solid tumors[101]

≈0.3% in head and neck neoplasms, pulmonary cancer, CRC, sarcoma, and cutaneous melanoma

Extremely rare cancers[a] impacting <0.02% of patients with cancer[101,144]

>80% in mammary analogue secretory carcinoma and secretory breast carcinoma

Guideline Recommendations for *NTRK* Testing[3]

Use NGS (preferably RNA-based NGS)

↓

IHC can be used to screen when NGS is not feasible

Considerations for Assay Choice

- Some NGS assays can detect both novel and known *NTRK* fusions and other actionable biomarkers[3,129]
- DNA-based NGS may have a higher risk of false negatives than RNA-based NGS[129]
- Screening with IHC should be confirmed with NGS testing[129]
- FISH and RT-PCR cannot detect novel fusion partners[3,129]

While *NTRK* fusions can be detected with NGS, IHC, FISH, and RT-PCR, only NGS assays can assess *NTRK* fusions and other actionable biomarkers simultaneously[3,129]

[a]The NCI defines a rare cancer as a cancer that occurs in fewer than 15 out of 100,000 people each year.

RET

RET is a proto-oncogene that codes for an RTK and plays a role in a variety of cancers[27]

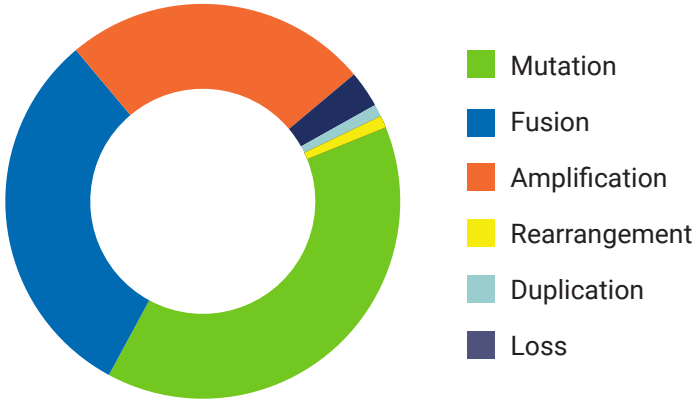
Oncogenic activation of *RET* occurs via 3 main mechanisms, but ***RET* fusions** are the only **actionable pan-tumor biomarkers**[3,27,137,145]

0.5% of all cancers harbor *RET* fusions[27]

In some cancers, *RET* fusions were associated with poor prognosis[146]

RET aberrations may co-occur with other genomic alterations[27,145]

Pathogenic *RET* Aberrations[27]



Cancers With the Highest Prevalence of *RET* fusions[27]

Lung carcinosarcoma	17%
PTC	9%
Lung adenocarcinoma	4%
Salivary gland adenocarcinoma	3%

TESTING OPTIONS FOR *RET* FUSIONS[43,130]

NGS	RT-PCR and FISH	IHC
Use NGS[a] in NSCLC, non-MTC, or other solid tumors **Considerations:** RNA-based NGS is the recommended method for detecting fusions	**Use RT-PCR or FISH when NGS is not available** **Considerations:** RT-PCR and FISH analyses are limited to known fusion partners FISH is susceptible to high false positive/negative rates	**Not recommended** **Considerations:** IHC currently has limited use and value in detecting *RET* fusions due to low sensitivity, low specificity, and the inability to detect a fusion partner

Most preferred — **Guideline testing preference** — Least preferred

MSI/dMMR

MMRD is caused by the dysfunction of MMR proteins (MLH1, MSH2, MSH6, PMS2) and results in increased mutations at microsatellite loci[131]

MSI is the hallmark of constitutional MMRD, and its prevalence varies depending on the tumor type[131]

MSI-H is a hypermutable genomic signature where there is a high level of mutations present at the sequenced microsatellite loci

dMMR is identified by the absence of MMR proteins

MSI-H and dMMR are 2 ways to assess MMRD status in patients

MSI-H is often associated with TMB-H[47,147]

MSI-H and TMB-H generally co-occur in stomach, duodenum, and small intestine adenocarcinomas

dMMR, mismatch repair deficient; MLH1, MutL homolog 1; MMRD, mismatch repair deficiency; MSH, MutS homolog; PMS2, postmeiotic segregation increased 2.

≈3% of all tumor types are marked by MSI-H/dMMR

Most Common MSI-H/dMMR Cancers[47]

Uterine corpus endometrial carcinoma	31.4%
Colon adenocarcinoma	19.7%
Gastric adenocarcinoma	19.1%
Rectal adenocarcinoma	5.7%

Cancers with the highest prevalence of MSI-H are also associated with Lynch syndrome

TESTING OPTIONS TO DETERMINE dMMR/MSI STATUS[131,148,149]

IHC	NGS	PCR
Guideline recommendation[b]: Preferred method for patients with CRC, upper GI[a], and endometrial cancers	**Guideline perspective[b]:** Similar performance to IHC and PCR but requires more resources; not preferred for upper GI[a], and endometrial cancer screening	**Guideline recommendation[b]:** Useful to screen patients with CRC, upper GI[a], and endometrial cancers
Considerations: Need to assess expression of all 4 MMR proteins – PMS2, MLH1, MSH2, and MSH6	**Considerations:** Can detect germline mutations / other genomic alterations simultaneously May mis-categorize MSI-L as MSI-S	**Considerations:** Specific microsatellite loci may differ between tissue types, so may need to tailor assay to tumor type

[a]Does not include ESCC.
[b]AMP/CAP recommendations are endorsed by ASCO.[146]
AMP, Association for Molecular Pathology; CAP, College of American Pathologists; MSI-L, microsatellite instability–low; MSI-H/MSI-S, microsatellite instability–high.

TMB

High TMB is a predictive biomarker, but prevalence varies by tumor type

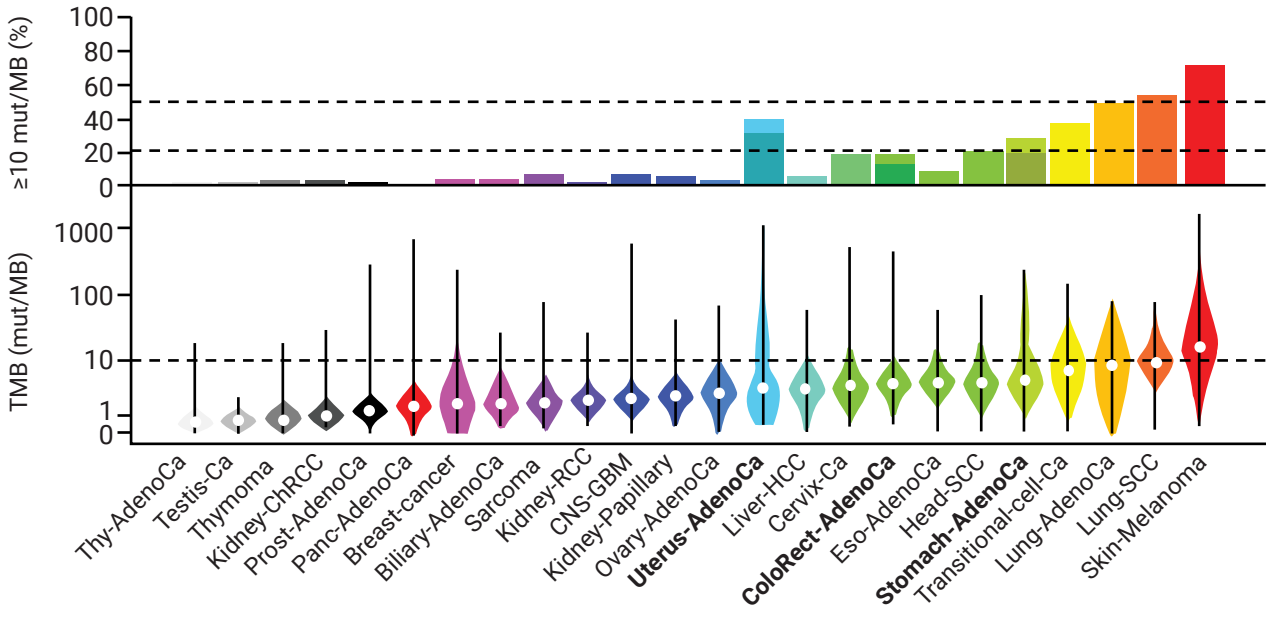
TMB is the **total number of somatic mutations per megabase of DNA sequenced**[3,48,147]

High TMB (≥10 mutations/megabase) is an actionable pan-tumor predictive biomarker for immunotherapy[3,48,104,147]

Some tumor types may have high TMB but low response rates to immunotherapies

High TMB may co-occur with other predictive biomarkers / actionable genomic alterations[104,147]

TMB Exhibits High Variability Among Tumor Types[48]

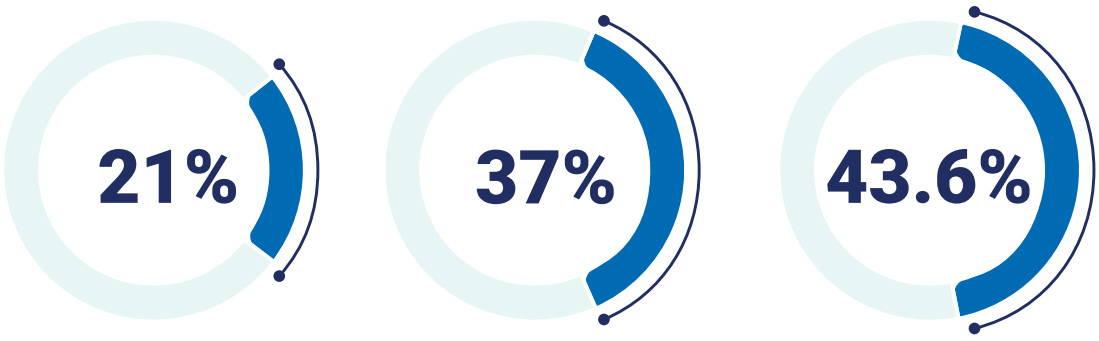


Ca, cancer; ChRCC, chromophobe renal cell carcinoma; GBM, glioblastoma multiforme; HCC, hepatocellular carcinoma; SCC, squamous cell carcinoma.

POTENTIAL BIASES IN TMB ESTIMATIONS

- Estimating the number of somatic mutations requires filtering germline mutations, which involves comparing tumor DNA to a reference genome or DNA from matched normal tissue[150-152]
- **Comparing tumor DNA to a reference genome overestimates TMB**, with higher overestimation in patients of non-European ancestry[152-154]

In one study, using a reference genome to estimate TMB misclassified[154]:



of patients of European ancestry as TMB-H

of patients of Asian ancestry as TMB-H

of patients of African ancestry as TMB-H

When treated with ICIs, misclassified patients with TMB-H had similar outcomes to patients with TMB-L[154]

Self-identified ethnicity may not correlate with genetic ancestry, so comparing tumor DNA with matched normal DNA is the most accurate way to estimate TMB[152-154]

CONSIDERATIONS FOR MEASURING TMB WITH NGS[132,150,155,156]

Sample	Assay Type	Report
Most NGS assays are performed on FFPE tissue Consider fixing for 24 hours in neutral buffered formalin for surgical specimens or 12 hours for biopsies for optimal results Liquid biopsies are challenging because of low levels of ctDNA[152]	**WES is the gold standard but may be impractical for use in the clinic** Consider using larger targeted panels (hybrid capture) with genome coverage of >0.8 Mb to accurately estimate TMB Panels designed to detect "hotspot mutations" could lead to an overestimation of TMB	**Inclusion of TMB definition and calculation in report** Consider including key bioinformatic information like inclusion/exclusion of synonymous mutations There is a need for direct comparisons between panels to establish concordance data

ICI, immune checkpoint inhibitor; TMB-L tumor mutational burden–low.

THE ONLY TESTING TECHNOLOGY THAT MAY BE ABLE TO DETECT ALL PAN-TUMOR BIOMARKERS ARE NGS ASSAYS COVERING A SIGNIFICANT PART OF THE GENOME[42,126,130-132,155,157,158]

***BRAF* V600E**	Can be detected with **NGS**, IHC, or PCR[126-128,138,139]
***NTRK* Fusions**	Can be detected with select **NGS** assays,[a] IHC, or FISH[129,158]
***RET* Fusions**	Can be detected with select **NGS** assays[a] or FISH[42,130]
MSI/dMMR	Can be detected with **NGS**, IHC, or PCR[139,141]
TMB	Can be detected with large **NGS** assays[b] or whole-exome sequencing[132,155-157]

ASCO prefers multigene genomic sequencing whenever patients with cancer are eligible for an approved genomic biomarker informed therapy[3]

The choice between multigene panel–based sequencing vs limited testing should be individualized, considering the relative costs and availability of tissue[3]

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

[a]NGS assays that can detect fusions include RNA-based NGS assays and DNA-based hybrid capture NGS assays.[15]

[b]Large NGS assays cover ≥0.8Mb of the genome.

NOTES

NOTES

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SUMMARY

When incorporating pan-tumor biomarkers, **one out of every three patients** may have an **actionable predictive biomarker**[27-29]

NGS assays have the potential to **detect all pan-tumor biomarkers**[3,111,114,125]

Consider testing all your eligible patients for pan-tumor biomarkers with NGS

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