Pan-Cancer Biomarkers

Changing the Landscape of Molecular Testing

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• ***Context.***—**The increasing use of large panel next-generation sequencing technologies in clinical settings has facilitated the identification of pan-cancer biomarkers, which can be diagnostic, prognostic, predictive, or most importantly, actionable.**

Objective.—**To discuss recently approved and emerging pan-cancer and multihistology biomarkers as well as testing methodologies.**

Data Sources.—**The US Food and Drug Administration approval documents, National Comprehensive Cancer Network guidelines, literature, and authors' own publications.**

Conclusions.—**Since 2017, the US Food and Drug Administration has approved genotype-directed therapies for pan-cancer biomarkers, including microsatellite instability, neurotrophic receptor kinases fusions, and high-tumor mutation burden. Both the importance and rarity of these biomarkers have increased the prevalence of genomic profiling across solid malignancies. As an integral part of the management team of patients with advanced cancer, pathologists need to be aware of these emerging biomarkers, the therapies for which they determine eligibility, and the strengths and pitfalls of the available clinical assays.**

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Massively parallel or next-generation sequencing (NGS) of large panels of genes in all tumor types has amplified our knowledge about the prevalence of certain oncogenic alterations and played a large part in their detection for eligibility for specific genotype-directed therapies. Our previous report of mutational landscape of distinct histologically defined tumor types from 10 000 patients with metastatic solid cancers revealed recurrent targetable alterations across tumor types, including somatic mutations, copy number changes, and structural variants. At the time of the study, approximately 30% of these alterations were targetable, and 11% of these patients were enrolled in clinical trials based on their genomic profiling data.[1] One such example is the fusions involving neurotrophic receptor kinases (*NTRK*) family genes 1-3, which have been identified in a diverse spectrum of adult and pediatric cancers. A clinical trial of the Trk inhibitor larotrectinib demonstrated marked and durable antitumor activity in these patients, regardless of tumor type.[2] This led to the accelerated approval of larotrectinib as a tissue-agnostic treatment of cancers harboring *NTRK* fusions. In addition, biomarkers associated with immuno-oncology (IO) agents can be assessed through large-panel NGS testing, such as mismatch repair (MMR)/microsatellite instability (MSI) signature and tumor mutation burden (TMB).[1] These 2 biomarkers were approved as pan-cancer biomarkers of pembrolizumab in 2017 and 2020, respectively.[3] Patients without targetable oncogene alterations may benefit from IO therapy if their tumors are MSI-high (MSI-H) or TMB-H.

DNA-based massive parallel sequencing (DNAseq) with large panels can detect these pan-cancer biomarkers. There are 2 different chemistries employed in DNA-based NGS. Hybrid capture, which has the advantage of evaluation copy number alteration, structural variant but limitations of higher input DNA requirement and longer turnaround time, while amplicon capture has the advantage of low DNA input requirement and high sensitivity but limitation of more difficult copy number and structural variant assessment. DNAseq can be matched tumor-normal sequencing or tumor only. Matched tumor-normal sequencing has higher accuracy in evaluating TMB because it can unequivocally identify the somatic mutations in tumor cells, whereas tumor-only sequencing cannot distinguish rare germline variants and somatic mutations coming from white blood cells versus somatic mutations coming from tumor cells.[4]

Besides DNAseq, other methods are also available to detect these biomarkers, including immunohistochemistry (IHC), fluorescence in situ hybridization (FISH), reverse-transcription polymerase chain reaction (RT-PCR), multiplex PCR, and RNA-based NGS, such as anchored multiplex PCR, which is especially useful for the detection of gene fusions arising from genomic breakpoints within very large

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Table 1. Features and Characteristics of Different Microsatellite Instability (MSI)/Mismatch Repair (MMR) Detection Methods

	Targets	Scoring and Results Interpretation	Features
MMR by IHC	MLH1, PMS2 MSH2, MSH6	Loss of both MLH1 and PMS2 (abnormal MLH1) Loss of both MSH2 and MSH6 (abnormal MSH2) Isolated losses of PMS2 or MSH6	Sensitivity 94%; works for low tumor purity samples; fast TAT; cheap
MSI by PCR	5 poly-A mononucleotide repeats (BAT-25, BAT-26, NR-21, NR-24, MONO-27)	MSS: 0/5 exhibit instability MSI-L: 1/5 exhibit instability MSI-H: $\geq$2 exhibit instability	Sensitivity 93% provided tumor purity is adequate; simple workflow and fast TAT
MSI by MANTIS, mSINGS, MSIsensor using NGS data	Hundreds to thousands of MSI loci	MANTIS (aggregate instability): Tumor vs. Normal Cutoff 0.4 mSINGS (per locus) Tumor vs. Baseline Cutoff 0.2 MSIsensor (per locus) Tumor vs. Normal Cutoff 3.5%	MANTIS: Sensitivity: 97.18% Specificity: 99.68% mSINGS Sensitivity: 76.06% Specificity: 99.68% MSIsensor Sensitivity: 96.48% Specificity: 98.73% Provides information on other targetable molecular alterations

Abbreviations: IHC, immunohistochemistry; MANTIS, Microsatellite Analysis for Normal Tumor InStability; MSI-H, MSI-high; MSI-L, MSI-low; mSINGS, MSI phenotype using NGS; MSS, microsatellite stable; NGS, next-generation sequencing; PCR, polymerase chain reaction; TAT, turnaround time.

introns that are difficult to cover by DNA NGS, such as fusions involving *NTRK3*.

Herein, we summarized the emerging pan-cancer biomarkers, multihistology biomarkers, and current testing technologies as an up-to-date review.

FDA-APPROVED PAN-CANCER BIOMARKERS

Microsatellite Instability

MSI is the first tissue/site-agnostic (pan-cancer) biomarker approved by the US Food and Drug Administration (FDA) and it is one of the most important biomarkers to predict response to immune checkpoint inhibitor therapies.[1,3] The indications to test include adult and pediatric patients with unresectable or metastatic MSI-H or dMMR solid tumors that have progressed after prior treatment and who have no satisfactory alternative treatment options. Patients with solid malignancies should receive MSI status testing as part of a workup for either Lynch syndrome or pembrolizumab eligibility (see National Comprehensive Cancer Network tumor-specific guidelines).

Microsatellites are 1- to 6-base pair long short-tandem repeats scattered throughout the human genome. They are prone to replication errors induced by DNA polymerase slippage, which are corrected by the MMR proteins MLH1, PMS2, MSH2, and MSH6. MSI-H tumors result from deficient MMR (dMMR) and are usually hypermutated with a particularly elevated number of frame-shift indels. Previously, MSI was studied mainly in colorectal cancer (CRC) and uterine endometrioid cancer (UEC) due to the high prevalence of MSI-H status in these tumor types. MSI testing was performed mainly by multiplex PCR and evaluation of MMR protein expression was performed by IHC. Testing by MMR IHC and MSI PCR was recommended based on patient's age and family history according to the Bethesda guidelines.[5] Patients with MSI-H/dMMR tumors have an increased chance of harboring Lynch Syndrome, which accounts for 3% to 5% of CRC, while somatic dMMR is responsible for approximately 15% of CRC.[6] These patients have a lower response rate to fluorouracil and are eligible for pembrolizumab if their cancer is unresectable or metastatic.[7–9] With the recognition of predictive value of MSI for IO therapy responses and given the high prevalence of dMMR in CRC, the updated 2020 National Comprehensive Cancer Network guidelines for CRC recommend universal MMR or MSI testing in all newly diagnosed patients with CRC. MMR or MSI testing is also included in 2020 National Comprehensive Cancer Network guidelines for locally advanced, recurrent, or metastatic gastroesophageal cancer, recurrent endometrial cancer, and many other cancer types, such as pancreatic cancer and cancer of unknown primary, which were discovered to have low frequencies of MSI-H through prospective testing by large-panel NGS.[10]

Features and characteristics of different MSI/MMR detection methods are summarized in Table 1, including IHC for MMR proteins[11]; multiplex PCR for microsatellite markers[12]; and software programs that use NGS data to determine MSI status, such as MANTIS (Microsatellite Analysis for Normal Tumor InStability, Ohio State University, https://github.com/OSU-SRLab/MANTIS), mSINGS (MSI phenotype using NGS, Ohio State University), and MSIsensor (Washington University in St. Louis, https://github.com/ding-lab/msisensor).[13–15] Accepted testing material is formalin-fixed, paraffin-embedded (FFPE) tissue. Matched normal is often required in the form of DNA from blood or nontumor FFPE tissue. MMR IHC works better with low-tumor purity samples than PCR or NGS because the result is visualized rather than scraped off slides and diluted by normal DNA. MMR IHC has a fast turnaround time of approximately 1 to 2 days and uses only 4 unstained slides, whereas PCR or NGS requires more tissue material. MMR IHC has a sensitivity of approximately 94%.[11,16] It has been documented that tumors with apparently normal MMR protein expression by IHC occasionally are MSI-H, and this is usually associated with pathogenic missense mutations in *MMR* genes (as opposed to truncating mutations resulting in loss of protein expression). MMR IHC is specific provided that normal internal controls demonstrate staining. One fairly common situation

Table 2. Features and Characteristics of Different Neurotrophic Receptor Kinases (*NTRK*) Fusion Detection Platforms

	Material Required	Tumor Content Requirement	TAT	Sensitivity (Sen)	Specificity (Spe)	Additional Notes
IHC	1 USS	NA	1 d	75%–96% for NTRK1/2; 50%–70% for NTRK3	92%–100%	Interpretation should take histologic tumor type into consideration. False positive seen in tumors with neural or smooth muscle differentiation
FISH	3 USS (for 3 NTRK genes)	NA	1–3 d	High if canonical breakpoints	High	Useful when high suspicion of *ETV6-NTRK3* fusions and supporting histology
RT-PCR	10–20 USS or 1 μg RNA	NA	1 wk	Variable	Variable	Requires knowledge of both involved genes and breakpoints. Low throughput Can be quantitative
DNAseq	10–20 USS (50–250 ng of DNA)	20%	2 wk	Variable	Variable	Sen/Spe depends on depth of introns covered by the panel and whether a transcribed fusion is generated
RNAseq	10–20 USS (200 ng of RNA)	>10%	1–2 wk	Very high	Very high	Comprehensive assessment of other possible gene fusions covered by the panel
DNA/RNA hybrid seq	10–20 USS (10–40 ng RNA)	20%	1–2 wk	98%–100%	96%–100%	Provide comprehensive assessment of genomic alterations

Abbreviations: FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; NA, not applicable; RT-PCR, reverse transcription polymerase chain reaction; TAT, turn-around time; USS, unstained slides.

leading to false loss of MSH6 expression is the use of neoadjuvant chemotherapy.[17] NGS-based MSI assessment programs, such as mSINGS, MANTIS, and MSIsensor, each requires validation when performed on custom NGS panels in a specific molecular lab.[1,10,13,15,18] NGS requires at least 1 to 2 weeks in terms of turnaround time but provides other important data, such as MAPK pathway alterations and TMB.

Neurotrophic Receptor Kinases Fusions

NTRK fusion (neurotrophic receptor kinases) is the second tissue/site-agnostic (pan-cancer) biomarker approved by the FDA and it can predict response to Trk inhibitors. The indications to test are unresectable or metastatic solid malignancies (see National Comprehensive Cancer Network guidelines).

The *NTRK* family includes 3 genes, *NTRK1*, *NTRK2*, and *NTRK3*, encoding the receptor tyrosine kinases *NTRK1*, *NTRK2*, and *NTRK3* (also known as, TrkA, TrkB, and TrkC, respectively). Physiologically, they play important roles in cell proliferation and survival and are expressed in neural and smooth muscle tissues. In-frame fusions of the C-terminal of *NTRK* with the N-terminal of partner genes activate the kinase domain and become novel targets of tyrosine kinase inhibitors (TKIs). While *NTRK* fusions are rare (<1%) in all solid tumors, they are very prevalent in certain tumors as follows: secretory carcinomas of the breast and salivary gland as well as infantile fibrosarcomas and congenital mesoblastic nephromas (>90%); intermediate frequency (5%–25%) in papillary thyroid carcinoma and spitzoid tumors; while low frequency (<5%) in appendiceal cancer, glioma/glioblastoma, gastrointestinal stromal tumor, non-small cell lung cancer (NSCLC), and so on.[19,20] Patients with *NTRK* fusions demonstrated a response rate of more than 75% with long duration to larotrectinib regardless of 5′ partner and across many histologies.[2] *NTRK* fusion can be performed on FFPE tissue by IHC, break-apart FISH, RT-PCR, DNA-based NGS for *NTRK* 1/2 fusions and *ETV6-NTRK3* fusions, RNA-based NGS for all *NTRK* fusions, or DNA/RNA hybrid NGS (Table 2).[21–26]

Like MMR IHC, pan-TRK IHC has a quick turnaround time and works well for low-tumor purity cases, yet its sensitivity is lower for *NTRK3* fusions (approximately 77%) and its specificity is lower in tumors with neural and/or smooth muscle differentiations.[22,27,28] DNA-based NGS works well if the regions of interest can be appropriately covered (in this case, both introns and exons of *NTRK1-3* spanning common breakpoints). *NTRK3* has proven difficult to cover sufficiently due to long and repetitive introns and thus, the sensitivity of DNA-based NGS is lower for *NTRK3* fusions. RNA-based NGS has the best sensitivity and specificity assuming satisfactory quality RNA is available. *NTRK* break-apart FISH has shorter turnaround time than NGS and requires only 3 unstained slides (1 per gene). It can only tell whether a *NTRK* gene rearrangement is present but does not identify the precise oncogenic fusion.

Tumor Mutation Burden

Recently, the FDA approved pembrolizumab for adults and children with tumor mutation burden-high (TMB-H) solid tumors (≥10 mutations/megabase), unresectable or metastatic solid tumors, based on the ongoing phase 2 KEYNOTE-158 trial. In this trial, the overall response rate was 28.3% for TMB-H and 6.5% for TMB-low tumors.[29] This approval marked the second tumor-agnostic approval for pembrolizumab and defined TMB as the third pan-cancer biomarker.

TMB is a continuous, quantitative variable that is associated with the likelihood of tumor cell neoantigen generation. It is defined as the total number of non-synonymous mutations per megabase of tumor genome. Even though not all the mutations generate neoantigens, the total number of mutations in a given tumor correlates with the odds of developing neoantigens and responding to immunotherapy. Therefore, TMB had emerged as a prom-

Table 3. Comparison of Selected Tumor Mutation Burden (TMB) Testing Panels

Panel Name	FDA Approval (Yes/No)	Capture	DNA Input, ng	Genes, n	TMB Region Covered, Mb	Sample Type	Matched Normal	TMB Estimation
MSK-IMPACT (Memorial Sloan Kettering)	Yes	Hybrid	50–250	468	1.14	FFPE	Yes	Nonsynonymous
FoundationOne CDx (Foundation Medicine)	Yes	Hybrid	50–1000	324	0.8	FFPE	No	Nonsynonymous, synonymous
Omics Core (NantHealth, Inc.)	Yes	Hybrid	50–300	468	39	FFPE	Yes	Nonsynonymous
PGDx elio (Personal Genome Diagnostics)	Yes	Hybrid	50	505	1.33	FFPE	No	Nonsynonymous, synonymous
TruSight Tumor 500 (Illumina)	No	Hybrid	40	523	1.33	FFPF	No	Nonsynonymous, synonymous
Oncomine Tumor Mutation Load Assay (Thermo Fisher Scientific)	No	Amplicon	20	409	1.20	FFPE	No	Nonsynonymous

Abbreviations: FDA, US Food and Drug Administration; FFPE, formalin-fixed, paraffin-embedded.

MSK-IMPACT (Memorial Sloan Kettering) reports the estimated TMB for the sample tested (mt/Mb). The median TMB assessed by MSK-IMPACT for all patients and for patients with the same tumor type as the tested sample as of the date the report was issued. This gives an idea to oncologists that where this given TMB lines up in patient's specific tumor type and in the entire cohort tested by MSK-IMPACT.

Omics Core received FDA clearance of whole exome sequencing in vitro diagnostic (IVD) test reporting overall TMB by sequencing 19 396 protein-coding genes (whole exome) targeting 39 million base pairs (39 Mb) in 2019.

Except Oncomine Tumor Mutation Load Assay, which assesses TMB and single nuclear variants (SNVs) only, other panels are more comprehensive, designed for all categories of alterations, including SNVs, copy number changes, structural variants, microsatellite instability (MSI)/deficient mismatch repair protein (dMMR) signature, and TMB detection.

ising biomarker of response to IO therapies in several prospective trials, including multiple tumor types.[30,31]

On the other hand, the predictive value of TMB varies among tumors and may not correlate with response to IO agents in certain tumor types. One example is Merkel cell carcinoma. In Merkel cell carcinoma, the response rates to IO agents are not statistically different ($P = .63$) between TMB-H group, which exhibited an ultraviolet light exposure mutational signature (50%) and TMB low group, which showed positive Merkel cell polyomavirus (41%).[32] Other factors may also affect the predictive value of TMB. In NSCLC, mutations of *EGFR* and *STK11* are associated with poor response to immune checkpoint inhibitors.[33] In general, host response or genomic aberrations affecting specific immune-signaling pathways or leading to immune dysregulation, such as loss-of-function mutations in beta-2 microglobulin or loss of human leukocyte antigen genes, *PTEN* loss, or mutations in *JAK* or other IFNγ-related genes have been associated with resistance to immune checkpoint inhibitors.[34–38] All of these should be considered when selecting patients for IO therapy.

While whole-exome sequencing is the optimal method to estimate TMB, targeted large panel NGS testing has been demonstrated to be comparable for the estimation of TMB.[1,39] However, the value of TMB varies among different panels and is impacted by assay design (tumor-normal matched sequencing or tumor only), panel size, genome coverage, and bioinformatics pipelines to calculate TMB (nonsynonymous only or both nonsynonymous and synonymous, and so on). Tumor-normal matched sequencing can eliminate rare germline variants and somatic mutations from white blood cells (clonal hematopoiesis),[4] while the value of TMB, based on tumor only sequencing, may be compounded by these alterations.

TMB predicts response to IO therapy and the indications to test are all advanced solid malignancies as eligibility criterion for pembrolizumab. TMB can be tested by targeted panel or whole-exome sequencing. The FDA cleared Omics Core whole-exome sequencing as an in-vitro diagnostic test, reporting overall TMB and the FDA approved the FoundationOne CDx (Foundation Medicine) assay as a companion diagnostic for pembrolizumab to identify patients with TMB-H malignancies on FFPE tissue with a TMB-H cutoff is 10 or more mutations/megabase. Selected testing methods are summarized in Table 3.

FUTURE CANDIDATES OF PAN-CANCER BIOMARKERS

Programmed Death-Ligand 1 Expression

The interaction between programmed death protein 1 (PD-1) and its ligand (PD-L1) is a well-characterized immune checkpoint. PD-L1 expression on tumors affects neoantigen presentation and the level of PD-L1 expression on tumor cells or immune cells in the tumor microenvironment is associated with clinical responses to IO therapies in many tumor types. The FDA approved 4 PD-L1 IHC monoclonal antibodies to test PD-L1 expression on FFPE tissue samples and several immune checkpoint inhibitors for certain cancer types. The approval list continues to expand to more tumor types in recent years.

PD-L1 IHC reporting systems are based on either PD-L1–stained tumor cells (TC) or PD-L1–stained tumor infiltrating immune cells (IC), depending on the tumor types (Table 4). PD-L1 expression on TC was evaluated as tumor proportion score or percentage of TC, which is the percentage of PD-L1–positive TC showing partial or complete membrane staining in the overall tumor sections; PD-L1 expression on IC was assessed as the proportion of tumor area occupied by PD-L1–positive IC of any intensity,[40] while combined positive score is the number of PD-L1–staining cells (tumor cells, lymphocytes, macrophages) divided by the total number of viable tumor cells, multiplied by 100.[41]

Although PD-L1 expression, MSI, and TMB are all biomarkers that determine eligibility for IO therapies, the correlation between PD-L1 expression and treatment response varies; most studies have found little or no correlation between TMB and PD-L1 expression[29] and the overlap between these markers varies among different

Table 4. FDA Approved PD-L1 Antibodies and Associated Drugs					
Machine	Clone	Drug	Tumor Types	Scoring Assessment	Complementary/ Companion[a]
VENTANA	SP142	Atezolizumab (TECENTRIQ)	Non-small cell lung cancer (NSCLC)	TC ≥50% or IC ≥10%,	Companion
			Urothelial cancer	IC ≥5%	Companion
		Atezolizumab (TECENTRIQ) plus nab-paclitaxel (Abraxane)	Breast cancer (triple negative)	IC ≥1%	Companion
VENTANA	SP263	Durvalumab (IMFINZI)	Urothelial carcinoma	TC ≥25% or IC ≥25%	Complementary
pharmDx	28-8	Nivolumab (OPDIVO)	NSCLC, urothelial, squamous cell cancer of head and neck (SCCHN)	TC ≥1%	Companion
pharmDx	22C3	Pembrolizumab (KEYTRUDA)	NSCLC	TPS ≥1%	Companion
			Gastric or GEJ adenocarcinoma	CPS ≥1	
			Cervical cancer	CPS ≥1	
			Urothelial carcinoma	CPS ≥10	
			SCCHN	CPS ≥1	

Abbreviations: CPS, combined positive score; FDA, Food and Drug Administration; GEJ, gastroesophageal junction; IC, immune cells; NSCLC, non-small cell lung cancer; TC, tumor cells; TPS, tumor proportion score.

[a] A complementary diagnostic is a test that aids in the benefit–risk decision–making about the use of the therapeutic product, where the difference in benefit–risk is clinically meaningful. A companion diagnostic is a medical device, often an in vitro device (IVD), which provides information that is essential for the safe and effective use of a corresponding drug or biological product.

tumor types.[42] Patients with elevated PD-L1 levels may not respond to immunotherapy, while a substantial minority of patients who had low PD-L1 expression may experience clinical benefit.[31] In a study of comparison of MSI status, PD-L1, and TMB in 11 348 patients, only 0.6% of patients were positive for all 3 of them. Therefore, it is important to perform comprehensive assessment of these biomarkers to select the patients most likely to respond to IO therapies.

Rearranged During Transfection Fusions and Mutations

The *RET* gene, a gene name derived from "rearranged during transfection," is mutated in approximately 80% of medullary thyroid cancer, and fusions have been detected in papillary thyroid carcinoma, NSCLC, and a range of other tumor types at lower frequencies.[43–46] The overall detection rate of *RET* fusions in NSCLC is 1% to 2% but fusions are enriched in nonsmokers lacking other known driver mutations.[47,48] *RET* fusions define a new therapeutic target in this subset of lung cancers, especially with the availability of selective *RET* inhibitor selpercatinib, which demonstrated durable response and increased progression-free survival.[49,50] In NSCLC, the overall response rate was 64% in previously treated patients and as high as 84% in never-treated patient group. In medullary thyroid cancer, the overall response rate of selpercatinib for the 55 previously treated patients was 69% and 73% in the patients without prior treatments. In *RET* fusion-positive thyroid cancer, the overall response rate for the 19 previously treated patients was 79% and 100% patients without prior treatments. Most patients showed responses longer than 6 to 12 months.[50,51] Based on these clinical trials, Retevmo (selpercatinib) was recently approved by the FDA for the treatment of *RET* fusion-positive NSCLC, *RET* fusion-positive thyroid cancer, and *RET*-mutant medullary thyroid cancer.[52,53]

Table 5. Current Available Rearranged During Transfection (*RET*) Testing Methods						
Assay	Tissue Requirements	TAT	Sensitivity	Specificity	Cost	Notes
FISH	1 USS	2–3 d	Better for KIF5B, CCDC6 than NCOA4	Pending	$$	Often break-apart, detects rearrangements, works for low tumor purity
IHC	1 USS	1 d	~90% (lung, ptc)	~85% (lung, ptc)	$	Must have cytoplasmic expression, works for low tumor purity
RT-PCR	10 USS	1 wk	Depends on partner, exons covered	>99%	$	Need primer for specific 5′ partner
DNA NGS (hybridization)	10 USS (resection), 20 USS (biopsy)	3 wk	?	~89%	$$$	Detects mutations, MSI, amplifications, and fusions
DNA NGS (amplicon)		1–2 wks	Depends on coverage	?	$$	Faster and cheaper than hybrid-capture. Usually best for detecting hotspot missense mutations
Anchored Multiplex PCR	10 USS (resection), 20 USS (biopsy)	~2 wk	>95%	>99%	$$$	Works agnostic of 5′ partner
DNA/RNA NGS (hybrid capture)	40 ng DNA/ 40 ng RNA	4 days	>95%	>98%	$$$	Detects mutations, MSI, amplifications, and fusions agnostic of 5′ partner

Abbreviations: FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; MSI, microsatellite instability; NGS, next-generation sequencing; PTC, papillary thyroid carcinoma; RT-PCR, reverse-transcription polymerase chain reaction; TAT, turn-around time; USS, unstained slides.

Testing of *RET* mutations and fusions is indicated in NCSLC and thyroid cancers and the available testing methods on FFPE tissue are listed in Table 5.

Fibroblast Growth Factor Receptor

Fibroblast growth factor receptor (*FGFR*) is a family of transmembrane tyrosine kinase receptors. They dimerize when binding with ligands, fibroblast growth factors (FGF), leading to intracellular phosphorylation of receptor kinase domains, triggering a cascade of intracellular signaling, and gene transcription. *FGF/FGFRs* signal through several downstream intracellular pathways, including the Ras/Raf/MEK and the phosphatidylinositol-4,5-bisphosphate 3 kinase (PI3K)–Akt pathway. In solid tumors, *FGFR* abnormalities are mainly copy number amplification, then gain of function mutations, and less common structural rearrangements. Urothelial carcinoma has the highest overall prevalence of *FGFR* alterations, followed by breast carcinoma, endometrial adenocarcinoma, ovarian carcinoma, glioma, squamous cell lung carcinoma, gastric adenocarcinoma, cholangiocarcinoma, and so on.[54] *FGFR3* alteration by RT-PCR has been approved in metastatic urothelial carcinoma. *FGFR2* gene fusions are common in intrahepatic cholangiocarcinoma, ranging from 10% up to 43% of cases. *FGFR2* fusion detection in cholangiocarcinoma was part of companion diagnostics of FoundationOne CDx FDA approval.

Other tumor types with *FGFR2* alterations are breast, gastrointestinal tract, and lung cancers, suggesting that *FGFR* could be another pan-cancer biomarker. The FDA approved the pan-*FGFR* inhibitor Balversa (erdafitinib) for locally advanced or metastatic bladder cancer in 2019 and a more selective FGFR inhibitor, pemigatinib, for cholangiocarcinoma with an *FGFR2* fusion in 2020.

Testing for *FGFR* fusions can be performed with DNAseq, RNAseq, FISH, or RT-PCR, while mutations can be tested by DNAseq and RT-PCR.

SUMMARY

Pan-cancer and multihistology biomarkers are new in the field of molecular testing and treatment. Large-panel comprehensive genomic profiling has made it possible to screen for various rare targetable alterations simultaneously across all histologies. The list of targetable alterations continues to grow along with the list of FDA-approved biomarkers used for various tumor histologies. It is therefore important to not only know which biomarkers are used for guiding management, but also the advantages and pitfalls of the assays used to assess them.

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