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Agnostic Biomarkers in Molecular Pathology

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ABSTRACT

Advances in molecular techniques have revealed that different molecular mechanisms are responsible for the behavior of cancer cells. Molecular alterations play a critical role in both the differential diagnosis of cancer and in the development of targeted therapies. Studies have identified the same potentially targetable mutations across various tumor types, supporting the emergence of tumor-agnostic therapies. To date, five biomarkers have been approved for tumor-agnostic therapy: microsatellite instability (MSI), neurotrophic tyrosine receptor kinase (*NTRK*) fusion, tumor mutation burden (TMB), *BRAF* V600E mutation, and rearranged during transfection (*RET*) fusions. The United States Food and Drug Administration (FDA) has approved pembrolizumab for MSI-high tumors or tumors with a high TMB. Larotrectinib and entrectinib have been approved for the treatment of *NTRK* gene fusion-positive tumors. Additionally, the combination of dabrafenib and trametinib has been approved for *BRAF* V600E mutations, and selpercatinib has been approved for *RET* fusion-positive cancers as of 2022. Positive responses to agnostic therapy, a significant milestone in cancer treatment, depend on the identification of new agnostic biomarkers. Ongoing research is focused on defining additional molecular changes, such as programmed death-ligand 1 (PD-L1), Kirsten rat sarcoma virus (*KRAS*), neuregulin 1 (*NRG1*), fibroblast growth factor receptor (*FGFR*), anaplastic lymphoma kinase (*ALK*), AKT serine/threonine kinase (*AKT*), human epidermal growth factor receptor 2 (*HER2*), phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA*), and breast cancer gene (*BRCA*), as potential agnostic biomarkers in various cancer types.

Keywords: Agnostic, biomarker, microsatellite instability, neurotrophic tyrosine receptor kinase, tumor mutation burden.

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INTRODUCTION

Agnostic biomarkers are specific molecular changes that can be targeted by therapeutic interventions, irrespective of the tumor's location or histological classification. Synonyms such as "tissue-agnostic," "histology-agnostic," "site-agnostic," "histology-independent," and "tumor-agnostic" are frequently used to describe this concept. In agnostic therapy, the same drug is used for all types of cancer with similar genetic mutations. Agnostic biomarkers and treatment methods are making significant advancements in the field of cancer treatment.[1–3]

Studies have demonstrated a high response rate to programmed cell death protein 1 (PD-1) blockade in cases of melanoma, renal cell cancer, and lung tumors. However, only one out of 33 patients with colorectal cancer responded to this treatment. Le et al.[4] hypothesized that the patient who benefited from PD-1 blockade might have microsatellite instability (MSI), as the immune system is activated in tumors with somatic mutations, and MSI tumors exhibit 10 to 100 times more somatic mutations than microsatellite stable (MSS) tumors. Confirming their hypothesis, the detection of MSI in this patient suggests that a drug targeting this deficiency could potentially benefit many individuals, as MSI can occur in tumors across various organs. Le et al.[4] demonstrated the predictive value of mismatch repair status for the clinical benefit of immune checkpoint blockade with pembrolizumab. This study was pivotal in the United States Food and Drug Administration (FDA) approval process.

Tumors in different anatomical regions behave differently in terms of response to treatment and prognosis. As a result of advancements in molecular studies, mutations in tumors have been identified, leading to the development of targeted therapies. This approach was first introduced in the 1990s with the development of imatinib for patients with breakpoint cluster region/abelson murine leukemia viral oncogene homolog 1 (BCR/ABL-1) fusion chronic myeloid leukemia and trastuzumab for human epidermal growth factor receptor 2 (*HER-2*)-positive breast cancer. These molecular alterations have also been identified in other histological types of tumors.[5] Since there was no effective response to treatment in other tumor types with *HER-2* mutations, it became evident that not every mutation has the same effect in every tumor type.[6] For this reason, discovering an agnostic marker that elicits a similar response across different tumor types is very difficult.

As with the five approved agnostic treatment agents, basket trials, which investigate the impact of a single drug on a specific mutation across different cancer types, are a widely adopted study design for exploring new treatment options. The National Cancer Institute Molecular Analysis for Therapy Choice (NCI-MATCH) trial is a basket trial investigating the efficacy of cancer treatment based on specific genetic alterations present in patients' tumors, regardless of the underlying cancer type. Through these studies, it has become clear that some biomarkers exhibit agnostic properties.[7]

In this review, five biomarkers—microsatellite instability, neurotrophic tyrosine receptor kinase (*NTRK*) fusion, tumor mutation burden, *BRAF* V600E mutation, and rearranged during transfection (*RET*) fusion—that are targeted by approved agnostic treatment approaches are described in detail (Table 1). Additionally, agnostic treatment candidates currently under clinical investigation and showing promise are discussed.

Study data were retrieved from various online databases, including Google, PubMed, Web of Science, and the bioinformatics database cBioPortal (The *cBioPortal* for Cancer Genomics). The aim is to describe the agnostic and candidate agnostic biomarkers, discuss the methods by which their mutations are detected, and summarize the agnostic treatments approved to date.

MICROSATELLITE INSTABILITY

Microsatellite instability is defined as the inability of mismatch repair (MMR) proteins to correct DNA replication errors. Research on this topic began with bacterial studies in the 1970s and 1980s, but MSI was characterized in the 1990s. MSI can occur sporadically or be associated with a germline syndrome. The syndrome linked to germline MMR gene abnormalities was first documented in the United States in 1913 and was associated with an inherited predisposition to colon, stomach, and endometrial malignancies. Similar features were observed and reported in two families by Henry T. Lynch in 1966, leading to its designation as Lynch syndrome.[8]

MSI or mismatch repair deficiency (dMMR) is the first reported agnostic biomarker.[2] Microsatellites are repetitive DNA sequences of 1–6 to 10 base pairs (short tandem repeats) located in both intronic and exonic regions of the genome, which can typically be repeated 5–50 times. These regions exhibit a higher mutation rate compared to other DNA regions, which is one of the mechanisms contributing to genetic diversity. The mismatch repair system is responsible for detecting and correcting mismatch errors during DNA replication, particularly in microsatellites. At least seven types of DNA MMR-associated proteins exist, including human mutL homolog 1 (h-MLH1), h-MLH3, human mutS homolog 2 (h-MSH2), h-MSH3, h-MSH6, human postmeiotic segregation increased 1 (h-PMS1) and h-PMS2. When mutations or epigenetic changes occur in genes associated with DNA MMR, these genes fail to synthesize MMR proteins, resulting in MSI.[9]

Table 1. FDA approved agnostic biomarkers and agnostic therapy

Agnostic biomarkers	Molecular methods	FDA approved agnostic therapy and general indication	Mechanism
MSI/dMMR	IHC, PCR, NGS	Pembrolizumab (MSI/dMMR solid tumors	PD-1 inhibition
		Dostarlimab (MSI/dMMR solid tumors)	PD-1 inhibition
NTRK fusion	FISH, PCR, NGS	Larotrectinib (*NTRK* fusion-positive solid tumors)	Pan-TRK inhibition
		Entrectinib (*NTRK* fusion-positive solid tumors and NSCLC with *ROS1*-alterations)	Pan-TRK, *ROS1*, *ALK* inhibition
Tumor mutation burden	FoundationOne CDx, NGS	Pembrolizumab (Unresectable or metastatic TMB-H (≥10 mut/Mb) solid tumors)	PD-1 inhibition
BRAF V600E mutation	IHC, PCR, NGS	Dabrafenib + Trametinib (Patients with *BRAF V600E* mutated tumors)	*BRAF* + MEK inhibition
RET fusion	FISH, PCR, NGS	Selpercatinib (Patients with *RET* fusion-positive tumors)	*RET* inhibition

FDA: The United States Food and Drug Administration; dMMR: Mismatch repair defect; FISH: Fluorescent in-situ hybridization; IHC: Immunohistochemistry; NSCLC: Non-small cell lung carcinoma; MSI: Microsatellite instability; NGS: Next generation sequencing; PCR: Polymerase chain reaction; PD-1: Programmed cell death protein.

Although immunohistochemistry (IHC) can be used to detect MSI, cases showing loss of protein expression should be confirmed by polymerase chain reaction (PCR). Intact nuclear expression of MMR proteins, as identified by IHC, indicates a low probability of MSI-high (MSI-H) status; however, it does not entirely exclude the possibility of Lynch syndrome. In colorectal carcinomas, the loss of nuclear expression of MLH1 and PMS2 as detected by IHC should prompt testing for methylation of the MLH1 promoter and/or mutation of *BRAF*. The presence of a *BRAF* V600E mutation and/or MLH1 methylation suggests that the tumor is sporadic. In contrast, the absence of both MLH1 methylation and *BRAF* V600E mutation indicates the potential for Lynch syndrome. It is also important to assess the immunohistochemical nuclear expression of MMR proteins in the non-neoplastic tissues adjacent to the tumor. This is necessary because it is inappropriate to report a tumor as having a loss of nuclear expression of MMR proteins if there is no nuclear expression in the adjacent normal tissues.[10]

The most commonly used method for the detection of MSI in routine practice is PCR. MSI status can be analyzed using Bethesda microsatellite markers, which include BAT26, D17S250, D2S123, BAT25, and D5S346. MSI-H is defined as the presence of mutations in two or more microsatellite markers; MSI-low (MSI-L) is described as a mutation in only one of the respective markers, and tumors are considered microsatellite stable if no mutations are detected. MSI assessment by PCR involves comparing normal and neoplastic tissues at the same microsatellite regions to determine whether the tumor is microsatellite stable or unstable.[11,12] Next-generation sequencing (NGS) technologies are also advancing MSI detection. Companies are now producing large parallel sequencing panels and kits specifically designed for MMR genes. Based on the number of loci evaluated through NGS, tumors are classified as MSI-L if 1–29% of the markers exhibit instability and as MSI-H if greater than or equal to 30% of the markers show instability.[13] MSI is a key predictive marker for immunotherapy and serves as a prognostic marker for various solid tumors.[8]

Pembrolizumab, a programmed cell death protein 1 inhibitor, became the first tumor-agnostic therapy approved by the US Food and Drug Administration in 2017. Dostarlimab also received FDA approval in 2021 as an agnostic therapy for the treatment of MSI-H tumors.[14] If a patient with MSI has disease progression and no other treatment options, immunotherapy can be used as a first-line treatment, regardless of the tumor's organ. The application of immunotherapy has led to investigations of MSI in various organs, establishing MSI as a common therapeutic target. Pembrolizumab is used to treat children and adults with unresectable or metastatic solid tumors and has shown effectiveness in treating 15 different types of cancer.[2] The PD-1 receptor is a T-cell surface molecule. It binds to programmed death ligand 1 (PD-L1), which is released by tumor cells and tumor-infiltrating lymphocytes, suppressing the activation of T lymphocytes and thereby preventing an immune response against cancer. Blocking the PD-1/PD-L1 pathway leads to the activation of anti-tumor immunity. There is an established association between MSI and PD-L1 expression. MSI-positive cancers are known to contain a high number of mutation-related neoantigens that are recognized by the immune system.[15] MSI and dMMR are important agnostic biomarkers for immune checkpoint inhibitor therapy.[9]

Neurotrophic Tyrosine Receptor Kinase (*NTRK*)

The *NTRK* genes include *NTRK*1, *NTRK*2, and *NTRK*3. The proteins encoded by these genes are tropomyosin receptor kinases (TRKA, TRKB, and TRKC, respectively), members of the cell surface receptor tyrosine kinase family. Neurotrophin nerve growth factor binds to the TRKA receptor, brain-derived neurotrophic factor or neurotrophin-4 binds to the TRKB receptor, and neurotrophin-3 binds to the TRKC receptor. Activation of these receptors leads to intracellular dimerization and autophosphorylation of tyrosine kinase receptors, initiating downstream signal transduction that promotes cell proliferation, differentiation, and survival. *NTRK* gene fusions result in the transcription of chimeric tropomyosin receptor kinase (TRK) proteins.[16]

NTRK fusions have been reported in 17 tumor types.[17] Rare cancers such as infantile fibrosarcoma, congenital mesoblastic nephroma, secretory breast carcinoma, and breast analogue secretory carcinoma frequently exhibit *NTRK* fusions, with an incidence of 90%. However, *NTRK* gene fusions are rare in common solid tumors such as lung, breast, and colorectal cancers.[2,16] Fluorescence in situ hybridization (FISH), IHC, reverse transcriptase- polymerase chain reaction (RT-PCR), and DNA or RNA next-generation sequencing (NGS) methods can be used to detect *NTRK* gene fusions.[17] DNA NGS using targeted sequencing is not ideal because it cannot identify unique, unknown fusions and is limited to detecting previously known common breakpoints. Whole genome DNA sequencing can identify these fusions but has limitations regarding sensitivity and depth of coverage. The combination of RNA sequencing and NGS for detecting *NTRK* fusions increases sensitivity to 93% and specificity to 100%.[18] FISH and RT-PCR are also effective, particularly for cancer subtypes where *NTRK* fusions are common. However, the primary disadvantage of these methods is that they can detect only one driver mutation at a time.[18,19] Pan-TRK immunohistochemical staining is recommended by some authors as a screening tool due to its rapid turnaround time and relatively low cost, which are advantageous in laboratory settings. However, false-positive results caused by wild-type *NTRK* expression in certain tissues and false-negative results, especially in tumors with *NTRK*3 fusions, limit the utility of immunohistochemistry as a diagnostic tool. For this reason, although IHC may be used as an initial step for certain tumor types where the likelihood of *NTRK* fusions is high, a confirmatory molecular test is necessary.[20]

Following the approval of pembrolizumab, the detection of *NTRK* fusions and the development of agnostic treatment trials for these fusions have gained importance. In 2018, FDA approved larotrectinib, a pan-TRK inhibitor, for adult and pediatric patients with tumors harboring *NTRK* fusions. This marked the second approval for a tissue-agnostic agent, following pembrolizumab. Larotrectinib is indicated for use in pediatric and adult patients with unresectable or metastatic solid tumors with *NTRK* gene fusions, particularly when the disease has progressed despite prior therapy or when no alternative treatments are available. Although *NTRK* fusions are found in less than 1% of common cancers, such as lung cancer, tumors with *NTRK* fusions demonstrate significant benefit from agnostic treatment.[19] Studies have shown that the most effective methods for detecting *NTRK* fusions are NGS and FISH.[18,20]

Entrectinib became the third agnostic treatment to receive FDA approval in 2019. It is a multi-target pan-TRK inhibitor that is effective against anaplastic lymphoma kinase (*ALK*) and c-ros oncogene 1 (*ROS1*) fusions in addition to *NTRK* fusions. Entrectinib is also used to treat *ROS-1* rearranged metastatic non-small cell lung cancer (NSCLC). It may be a viable option for tissue-agnostic treatment across 10 different tumor types.[2,3,17]

TUMOR MUTATION BURDEN

Tumor mutation burden (TMB), defined as the number of mutations per million bases of the genomic sequence analyzed, is a biomarker for predicting the efficacy of immune checkpoint blockade. The evaluation of this biomarker is based on the assumption that elevated mutation rates in somatic exonic regions result in increased neoantigen formation, which is recognized by $CD8^+$ T cells, thereby initiating an immune response. The PD-1 inhibitor pembrolizumab received accelerated FDA approval in June 2020 for the treatment of unresectable or metastatic solid tumors exhibiting 10 or more mutations per megabase of the genome, as determined by the FoundationOne CDx assay. This is the second instance in which the FDA has approved a tissue-agnostic cancer immunotherapy, following the approval of pembrolizumab for MSI-H or dMMR solid tumors in 2017. Studies indicate that TMB is a predictive biomarker for responses to immunotherapy. TMB is independent of MSI/dMMR status and PD-L1 expression. Whole-exome sequencing with NGS is considered the gold standard for assessing TMB. However, targeted gene panels, such as the cancer gene mapping test FoundationOne CDx, are more commonly used.[21]

Tumor mutation burden is currently assessed via NGS using one of the following approaches: whole-genome sequencing (WGS), whole-exome sequencing (WES), or various targeted gene panels (e.g., FoundationOne CDx, MSK-IMPACT, etc.) performed on formalin-fixed, paraffin-embedded (FFPE) samples. Ongoing studies are investigating circulating tumor DNA for TMB using liquid biopsies.[22] Tumor mutation burden can include non-synonymous mutations, such as missense, nonsense, frameshift, and splice-site mutations. Splice-site and minor insertion or deletion (indel) mutations may also

be variably introduced. Studies are underway to evaluate whether including synonymous variants would improve predictive performance. Currently, major structural variants, amplifications, and genomic copy number gains and losses are not included.[23]

Initial measurements of tumor mutation burden were conducted using WES methods. For this calculation, comparing tumor tissue to matched non-tumor normal tissue is recommended. WES methods analyze coding sequences spanning 30–50 megabases. While WES is less expensive than WGS, it is more costly than targeted gene panel approaches. WES is the definitive standard for TMB prediction, as it is based on sequencing matched FFPE tumor and normal samples and calculating nonsynonymous mutations while excluding germline mutations.[23]

Targeted gene panels are often preferred due to their lower cost, shorter turnaround times, and reduced DNA input requirements. Additionally, gene panels target genes at a higher sequencing depth than WES. FoundationOne CDx and MSK-IMPACT (Memorial Sloan Kettering Cancer Center-Integrated Mutation Profiling of Actionable Cancer Targets) are examples of targeted gene panels. FoundationOne CDx is FDA-approved, while MSK-IMPACT is FDA-authorized.[22] Several commercial companies recommend TMB testing for targeted therapies, using panels that analyze 315 to 600 genes and costing between 4,800 and 6,500 USD.[24] The Friends of Cancer Research TMB Harmonization Project, initiated in 2017, involves collaboration among the FDA, pharmaceutical companies, and academic researchers. This project aims to standardize and harmonize the clinical application and assessment of TMB by comparing different platforms and panels. The goal is to improve the consistency and reliability of TMD estimation across panels and to facilitate the integration of this complex biomarker into clinical decision-making processes.[25]

v-Raf Murine Sarcoma Viral Oncogene Homolog B (*BRAF*)

BRAF is a prognostic and predictive biomarker and a member of the mitogen-activated protein kinase (MAPK) signaling pathway. Its proteins are serine/threonine kinases. The most common *BRAF* mutation is V600E. Melanoma has a higher incidence rate (40–70%) compared to other cancer types. *BRAF* mutations have been detected in 7–15% of all tumor types. Hematological malignancies such as hairy cell leukemia and multiple myeloma, along with papillary thyroid cancer, colorectal cancer (CRC), ovarian serous carcinoma, and non-small cell lung cancer, are among the most common cancers exhibiting *BRAF* mutations, following melanoma. It is well-established that oncogenic *BRAF* mutations result in an aggressive phenotype and reduced disease-free survival. Immunohistochemical studies can employ a specific monoclonal antibody to detect the *BRAF* V600E mutation. Molecular diagnostic techniques include PCR and NGS. Numerous studies have demonstrated a high level of concordance between IHC analysis of *BRAF* and the genotypic analysis of *BRAF* mutations.[17,26]

Different pathways are activated when the *BRAF* mutation is inhibited, with feedback systems that vary according to tumor type. In targeted therapy, different drug combinations are used in addition to *BRAF* inhibition. For example, a combination of *BRAF* and mitogen-activated protein kinase kinase inhibitor (MEK) inhibitors is used to treat melanoma, whereas a combination of *BRAF*, *MEK*, and epidermal growth factor receptor (*EGFR*) inhibitors is employed for CRC cases.[27] It is not appropriate to use a single-agent *BRAF* inhibitor as an agnostic treatment approach. Extensive studies have been conducted on combination therapies for *BRAF*-mutant cancers. Over 20 different tumor types demonstrated antitumor activity with the combination of dabrafenib and trametinib in the ROAR trial (Rare Oncology Agnostic Research), NCI-MATCH trial (National Cancer Institute Molecular Analysis for Therapy Choice), and Study X2101, which considered the totality of the evidence.[28] In 2022, the combination of dabrafenib and trametinib (*BRAF* + *MEK* combination) received approval for agnostic use in tumors harboring the *BRAF* V600E mutation.[29]

Rearranged During Transfection (*RET*)

The *RET* gene is a proto-oncogene located on 10q11 that encodes a receptor kinase with extracellular, transmembrane, and intracellular domains. These domains activate several pathways, including the MAPK, phosphoinositide 3-kinase (PI3K), Janus kinase/signal transducer and activator of transcription (JAK-STAT), and protein kinase A (PKA) pathways.[30] The most common alteration in *RET* is fusion, with over 35 different *RET* fusions identified to date. *RET* mutations have been detected in 1–2% of NSCLC cases, 10–20% of papillary thyroid carcinoma (PTC) cases, and 60% of sporadic medullary thyroid carcinoma cases. Recent studies have also identified *RET* mutations in Spitz tumor, spitzoid melanoma, chronic myelomonocytic leukemia, CRC, and breast cancer. The incidence of this mutation is low in common cancer types such as lung cancer.[31] Mutations in the *RET* gene can be detected using FISH, NGS, or PCR methods. In clinical studies, RT-PCR and FISH techniques were initially used for *RET* fusion detection. However, the use of IHC for *RET* fusion detection is not recommended, as studies have shown that IHC expression may increase even in the absence of *RET* fusion.[32] The RNA-NGS technique is now predominantly used due to its ability to identify fusion transcripts, making it the gold standard method for detecting fusion molecular alterations. In 2022, the FDA approved selpercatinib, a *RET* inhibitor, for the agnostic treatment of tumors with *RET* fusions.[30]

IN-SILICO EVALUATION

The in-silico evaluation of the frequency of tumor-agnostic biomarkers in metastatic patients was conducted using the MSK MetTropism dataset,[33] a pancancer study available in the public database cBioPortal.[34,35] Next-generation sequencing was employed to evaluate approximately 25,755 patients across 50 tumor types from the MSK MetTropism dataset. The MSK-IMPACT panel was used to profile tumor samples and detect somatic alterations on a hybridization capture-based NGS platform. The dataset includes both primary and metastatic tumor samples sequenced using three generations of the MSK-IMPACT panel, covering 341, 410, and 468 genes.[36] Figures 1 illustrate the frequency of agnostic biomarkers across cancer types in 21,546 metastatic (M1) patients.

The *BRAF* V600E mutation is most commonly observed in thyroid cancers (51.04%), followed by melanoma (19.09%), colorectal cancer (7.14%), gastrointestinal neuroendocrine tumors (5.34%), small intestine cancers (2.33%), non-small cell lung cancer (1.34%), appendiceal cancer (1.06%), and hepatobiliary cancer (0.91%) (Fig. 1a). RET fusions are most frequently detected in thyroid cancers, with a rate of 7.55%, while the rates are 2.06% in NSCLC, 0.4% in esophagogastric cancer, 0.37% in small cell lung cancer, 0.26% in hepatobiliary cancer, 0.19% in CRC, 0.17% in prostate cancer, and 0.15% in breast cancer (Fig. 1b). Neurotrophic tyrosine receptor kinase (*NTRK1*, *NTRK2*, *NTRK3*) fusions are most commonly observed in thyroid cancers (2.86%), melanoma (0.76%), uterine sarcomas (0.68%), and esophagogastric cancer (0%). The dataset includes tumor samples from various cancer types, with the highest percentage being soft tissue sarcomas (67%), followed by appendiceal cancer (0.53%), gastrointestinal stromal tumors (0.44%), hepatobiliary cancer (0.39%), pancreatic cancer (0.38%), and prostate cancer (0.34%) (Fig. 1c).[34,35]

POTENTIAL AGNOSTIC BIOMARKERS

Programmed Death-Ligand 1 (PD-L1)

Further biomarker discoveries are necessary to identify patients who do not exhibit MSI but may still benefit from anti-PD-1/PD-L1 therapies, as well as MSI patients who might not respond to such treatments. Studies have identified predictive biomarkers such as PD-L1 expression, TMB, lymphocyte infiltration, RNA expression signatures, and mutation-associated neoantigens (MANA). It is estimated that dMMR cancers contain a significant number of MANA, which can be recognized by the immune system.[17] In 2020, the FDA approved the use of anti-PD-1 therapy for cancers based on TMB evaluation. However, studies to develop easy and reliable tests are ongoing.[19] The lack of standardized cut-off value for assessing PD-L1 expression through IHC poses a significant challenge in this process. PD-L1 clones and cut-off values vary across different histologic tumor types. For the treatment of non-small cell lung carcinoma, the tumor proportion score (TPS) should be ≥50% when using the PD-L1 SP142 clone. In gastric adenocarcinomas and head and neck squamous cell carcinomas (SCC), the combined positive score (CPS) should be ≥1% when using the SP22C3 clone. The FDA approval of different cut-off values and clones for different tumor types adds complexity to the process.[37] Moreover, IHC detection of PD-L1 expression alone may not be sufficient to differentiate responders from non-responders to therapies.

Kirsten Rat Sarcoma Virus (*KRAS*)

KRAS is a membrane-bound protein with guanosine triphosphatase (GTPase) activity. It belongs to the Ras protein family, which includes three closely related isoforms: *HRAS*, *KRAS*, and *NRAS*. Among these, *KRAS* is the most frequently mutated. *KRAS* mutations are found in 86–96% of pancreatic carcinoma cases, as well as in colorectal cancer, non-small cell lung cancer, and lung squamous cell carcinoma. Unfortunately, *KRAS* mutant tumors are typically associated with poor prognoses.[38] Tests for *KRAS* mutations most commonly use PCR. The high prevalence of *KRAS* mutations has driven its evaluation as a potential drug target across multiple cancer types. However, despite decades of research, no therapeutic agent directly targeting *KRAS* has demonstrated positive results. This is due to the absence of protein pockets on the *KRAS* protein surface, rendering it 'undruggable'.[39] Ongoing drug trials are being conducted to target the molecular configuration of *KRAS*. In 2019, data was presented on AMG 510, a novel small-molecule drug that specifically targets the *KRAS* G12C mutation. This mutation is responsible for approximately 10–15% of lung adenocarcinomas and 1–3% of other solid tumors.[3] Sotorasib, an irreversible inhibitor of *KRAS* G12C, is also used as a *KRAS* G12C inhibitor. It holds potential as a future agnostic therapy.[40]

Neuroregulin 1 (*NRG1*)

NRG1 is a growth factor that binds to *HER3*, inducing the heterodimerization of *HER2* and *HER3* and activating the PI3K-AKT and MAPK pathways. *NRG1* fusion is rare, occurring in only 0.2% of tumor samples analyzed, with higher prevalence in gallbladder cancer, pancreatic cancer, NSCLC, breast cancer, and CRC. *NRG1* fusion can be detected using PCR, NGS of DNA or RNA, and FISH methods. Studies have demonstrated that targeted therapy for *HER3* in *NRG1* fusion-positive lung adenocarcinoma cases yields positive results. Additionally, combined therapy with *EGFR* inhibitors has proven effective in treating patients. This rare mutation holds significant potential for use in agnostic therapy.[41,42]

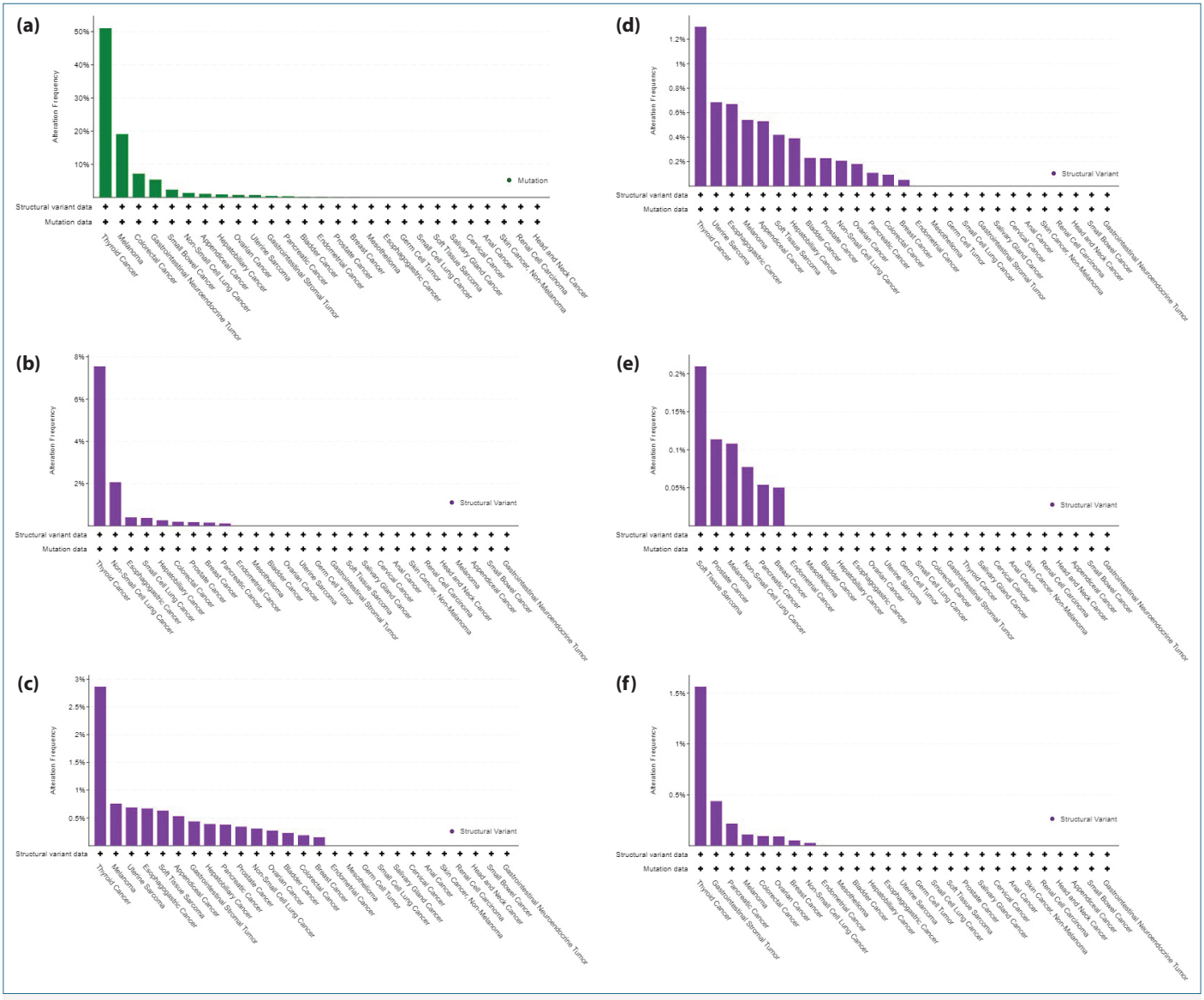

Figure 1. A comparison of *BRAF* V600E mutations and rearranged during transfection (*RET*), as well as neurotrophic tyrosine receptor kinase (*NTRK*) 1, 2, and 3 fusions, was performed using the OncoPrints tool to visualize mutual exclusion and co-occurrence trends between gene pairs in the cBioPortal database.[34,35] In the figure, genes are displayed in rows, while data points are represented in columns. Genomic changes in the sequences are identified as follows: missense mutations are marked in green, and fusions are highlighted in purple. **(a)** The distribution of *BRAF* V600E mutations across various cancer types. **(b)** The distribution of *RET* fusions among cancer types, represented with a bar graph. **(c)** The total distribution of *NTRK* (*NTRK1*, *NTRK2*, and *NTRK3*) fusions among different cancer types, represented with a bar graph. **(d)** The distribution of *NTRK1* fusions among cancer types. **(e)** The distribution of *NTRK2* fusions among cancer types. **(f)** The distribution of *NTRK3* fusions among cancer types.

Anaplastic Lymphoma Kinase (*ALK*)

The *ALK* gene is located at 2p23 and encodes a transmembrane tyrosine kinase receptor composed of 1620 amino acids. Upon binding the appropriate receptor, it activates numerous intracellular signaling pathways, stimulating cell proliferation and differentiation. However, mutations, amplifications, and fusions, which are the most common alterations of *ALK*, lead to ligand-independent activation of signaling pathways, resulting in uncontrolled cell proliferation.[43] *ALK* alterations are most frequently observed in NSCLC, anaplastic large cell lymphoma (ALCL), and inflammatory myofibroblastic tumor (IMT). The most common fusion gene involving *ALK* arises

from the fusion of the 3' end of the *ALK* gene, which encodes the kinase catalytic domain, with the 5' end of the fusion partner gene. In 95% of cases, the fusion partner for NSCLC is the echinoderm microtubule-associated protein-like 4 (EML4) gene. The most commonly used molecular methods to detect fusions are IHC, FISH, RT-PCR, and NGS.[44] Although each method has its advantages and disadvantages, IHC is noted for being fast and easily accessible, while RT-PCR and NGS offer higher diagnostic accuracy. Ongoing clinical studies are investigating the potential of *ALK* as an agnostic marker.[45]

The NCI-MATCH study, involving 6,000 patients with largely treatment-resistant malignant solid tumors, was completed in 2023. It remains the largest tumor-agnostic study ever undertaken. The study tested treatment protocols in patients with mutations of agnostic and candidate agnostic genes. Significant survival rates were observed in patients with *BRAF* V600E mutations, fibroblast growth factor receptor (*FGFR*) mutations/fusions, serine/threonine kinase (*AKT*) mutations, *ALK* fusions, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA*) mutations, and microsatellite instability, all of whom received therapies for these genes.[28] Anti-cancer drugs developed to target molecular changes associated with different *NTRK* gene fusions, *ALK*, *ROS1*, *MET*, and v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog (*KIT*) mutations have demonstrated efficacy across multiple tumor types. Ongoing clinical trials are exploring whether these biomarkers can serve as agnostic biomarkers.[3]

CONCLUSION

Site-agnostic therapy has emerged as a breakthrough in cancer treatment, driven by agnostic biomarkers that target specific mutations rather than the histological type or location of the tumor. This treatment option is particularly valuable for rare tumor types that currently lack histology-specific therapies. Studies on biomarkers and targeted therapy have demonstrated that identical oncogenic mutations can occur across various tumor types. However, the effectiveness of targeted therapy varies depending on the organ and tumor type. Consequently, the number of tumor-agnostic biomarkers and drugs available today is limited. This limitation may be attributed to the complex relationship between specific molecular abnormalities and cancer morphology. Another perspective involves the nature of molecular features. Most molecular changes that have received FDA approval or show potential as agnostic markers are related to immune signaling pathways and kinase fusions. A smaller subset of drugs targeting single nucleotide variants and amplifications shows promise for tumor-agnostic applications. Ongoing clinical basket studies are investigating agnostic biomarkers, and it is anticipated that agnostic treatment approaches will play an increasingly pivotal role in cancer therapy.

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