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Tumor-Type Agnostic, Targeted Therapies: BRAF Inhibitors Join the Group

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Abstract
In the present review, we briefly discuss the breakthrough advances in precision medicine using a tumor-agnostic approach and focus on BRAF treatment modalities, the mechanisms of resistance and the diagnostic approach in cancers with *BRAF* mutations. Tumor-type agnostic drug therapies work across cancer types and present a significant novel shift in precision cancer medicine. They are the consequence of carefully designed clinical trials that showed the value of tumor biomarkers, not just in diagnosis but in therapy guidance. Six tumor-agnostic drugs (with seven indications) have been approved through October 2022 by FDA. The first tumor-agnostic treatment modality was pembrolizumab for MSI-H/dMMR solid tumors, approved in 2017. This was followed by approvals of larotrectinib and entrectinib for cancers with *NTRK* fusions without a known acquired resistance mutation. In 2020, pembrolizumab was approved for all TMB-high solid cancers, while a PD-L1 inhibitor dostarlimab-gxly was approved for dMMR solid cancers in 2021. A combination of BRAF/MEK inhibitors (dabrafenib/trametinib) was approved as a tumor-agnostic therapy in June 2022 for all histologic types of solid metastatic cancers harboring $BRAF^{V600E}$ mutations. In September 2022, RET inhibitor selpercatinib was approved for solid cancers with *RET* gene fusions.
Conclusion. Precision cancer medicine has substantially improved cancer diagnostics and treatment. Tissue type-agnostic drug therapies present a novel shift in precision cancer medicine. This approach rapidly expands to provide treatments for patients with different cancers harboring the same molecular alteration.

Key Words: Precision Medicine ▪ Targeted Therapy ▪ BRAF ▪ BRAF Inhibitors ▪ Molecular Diagnostics.

Introduction

Precision Medicine and Tumor-Agnostic Approach

Precision (or personalized) medicine in oncology represents a novel approach to cancer treatment. It implies using the right anticancer drug for the right patient at the right time. In contrast to the traditional oncologic treatment, this innovative approach considers individual differences in patients' genes, environment and lifestyle. Precision medicine was coined in 2011 by the USA's National Research Council's (NRC) report "Towards Precision Medicine" (1). In 2015-2020, 290 different precision and matched clinical trials were conducted (2), resulting in the approval of numerous targeted treatment modalities for various solid and hematologic malignancies; the list is provided here (3).

Much of the progress in precision medicine is due to rapid advances in high-throughput genomic sequencing technologies (e.g., next-generation sequencing/NGS) that enabled clinical implementation of assays. These assays can rapidly interrogate cancers for various molecular genomic alterations and targetable biomarkers and allow for more appropriate clinical decision-making and patient outcomes (4).

The precision medicine approach has led to a substantially higher proportion of responding cancer patients, with markedly improved clinical outcomes compared with traditional clinical trials involving unselected patients (5). In particular, clinical trials based on comprehensive molecular profiling may provide "customized multidrug regimens" with a substantial positive impact on the outcome of hard-to-treat and refractory cancers (6). Tissue/tumor type-agnostic drug therapies present a significant, albeit gradual, shift in precision cancer medicine. It is a consequence of carefully designed clinical trials showing the value of tumor biomarkers, not just in diagnosis but also in therapy guidance. Advances in molecular-genetic testing capabilities coupled with understanding complex molecular pathways interactions have led to the stratification of histologically diverse malignancies into biomarker/pathway-similar tumors. Three essential criteria should be fulfilled for tumor agnostic treatment: (1): Cancers must be enriched for at least one genomic alteration; (2) Such an alteration should be predictive of response to a matched therapy, (3) and the genomic alterations should be found across the cancers (7). Tissue type-agnostic drugs are usually assessed in "basket trials" in which small patient cohorts with diverse cancers are treated with the same targeted therapy (8).

Consequently, most basket trials are prospective phase II clinical trials designed to assess durable and objective therapeutic responses to a targeted treatment across different histologic cancer subtypes (9). Up to 2019, 49 basket trials were completed and their results were published (10). Our literature search revealed 76 different basket trials registered in the database ClinicalTrials.gov, most of which are related to cancer treatment (11).

The Food and Drug Administration (FDA) approved six different agnostic-based drugs (seven indications) in oncology from the period 2017 – October 2022 (12) (summarized in Table 1). The first drug approved in 2017 in a tissue-agnostic manner was pembrolizumab for the treatment of unresectable or metastatic solid tumors that have been identified as a microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) (13, 14). Three years later, FDA approved pembrolizumab for adult and pediatric patients with advanced and/or metastatic solid tumors exhibiting a high tumor mutational burden (TMB) (defined as ≥10 mutations/Mb) (15, 16). In 2018, larotrectinib was approved for pediatric and adult tumors harboring neurotrophic tyrosine receptor kinase (*NTRK*) gene fusions without a known acquired resistance mutation (17, 18), while another NTRK inhibitor, entrectinib, was approved in August 2019 for a similar indication (Table 1) (19, 20). In 2021, FDA granted accelerated approval for the PD-L1 inhibitor dostarlimab-gxly for adult patients having dMMR advanced or recurrent solid cancers (21, 22). FDA also approved the VENTANA MMR RxDx assay as a companion diagnostic (CDx) test to select patients with dMMR solid cancers for treatment with dostarlimab-gxly. In June 2022, FDA granted accelerated approval to dabrafenib in combination with trametinib for the treatment of adult and pediatric patients ≥6 years of age with unresectable or metastatic solid tumors with *BRAF* ^{V600E} mutations who have progressed following prior treatment and have no satisfactory alternative treatment options (Table 1) (23). This approval was based on marked therapeutic responses to targeted BRAF/MEK inhibition of various solid malignancies with *BRAF* ^{V600E} mutations, including low-grade gliomas, biliary, gynecological and gastrointestinal cancers (24-26).

Highly potent RET inhibitors were developed, targeting the *RET* oncogene that encodes a receptor-type tyrosine kinase. *RET* (rearranged during transfection) acts as an essential oncogene in several cancers, including medullary thyroid, non-small cell lung (NSCLC), pancreatic, breast, and ovarian carcinomas (27). *RET* is usually rearranged via mutations or gene fusions (28). FDA has already approved the RET-inhibitor selpercatanib for RET-positive (fused or mutated) NSCLC, medullary thyroid and differentiated thyroid carcinomas (29), while another RET inhibitor pralsetinib was approved in 2020 for metastatic RET-fused NSCLC (30). In September 2022, FDA granted accelerated approval for selpercatanib for treating

Table 1. Overview of the Agnostic-Based Approved Targeted Treatments in Oncology

Name of the drug(s)	Year of approval	Mechanism of action	Indications
Pembrolizumab	2017	PD-1 inhibition	Adult and pediatric patients With solid cancers harboring MSI-H/dMMR status
Larotrectinib	2018	pan-TRK (NTRK1-3) inhibition	Adult and pediatric patients with NTRK1-3-fused solid cancers
Entrectinib	2019	NTRK1-3, ALK, and ROS1 inhibition	Adult and pediatric patients with NTRK1-3-fused solid cancers
Pembrolizumab	2020	PD-1 inhibition	Adult and pediatric patients with TMB-H solid cancers*
Dostarlimab-gxly	2021	PD-1-PD-L1/PD-L2 inhibition	Adult patients with dMMR recurrent or advanced solid cancers
Dabrafenib and trametinib	2022	BRAF and MEK (MAP2K1) inhibition	Metastatic solid cancers with *BRAF*^{V600E} mutations
Selpercatinib	2022	RET kinase inhibition	Adult patients with locally advanced or metastatic solid cancers with *RET* gene fusions

*TMB-H defined as ≥10 mutations/Mb; PD-1=Programmed cell death protein 1; NTRK1-3=Neurotrophic Tyrosine Receptor Kinase 1-3; MSI-H=Microsatellite instability-high; dMMR=Deficient mismatch repair; TMB-H=Tumor mutational burden high.

locally advanced or metastatic solid cancers harboring *RET* gene fusions. The tissue type-agnostic approval was based on the LIBRETTO-001 basket trial enrolling 45 patients with colorectal, breast, pancreatic, salivary gland, ovarian, small intestine, and cholangiocarcinomas, cancer of unknown primary, soft tissue sarcoma, and bronchial carcinoid (31). The basket trial revealed that selpercatinib exhibited clinically impactful activity in the *RET* fusion-positive tumor-agnostic patients, with a safety profile similar to the one previously reported for selpercatinib (31).

Herein, we review the distribution of *BRAF* mutations and other genomic alterations across tumor types, methods of detection and potential pitfalls and caveats associated with biomarkers testing.

BRAF and Precision Medicine

BRAF Gene

The *BRAF* gene (B-Raf proto-oncogene, serine/threonine kinase), located on chromosome 7q34, is a constitutive part of the mitogen-activated protein kinase (MAPK/ERK) signaling pathway involved in cancer initiation and progression via cell survival and proliferation (Figure 1) (32). The *BRAF* gene encodes a cytoplasmic protein with serine-threonine kinase activity. BRAF is usually activated via surface ligand binding to receptors with tyrosine kinase activity, such as Epidermal Growth Factor Receptor 1 (EGFR/HER1) or Human Epidermal Growth Factor Receptor 2 (HER2/ERBB2), followed by the activation of RAS-family GTPases. This chain of reactions leads to the dimerization of BRAF with BRAF or CRAF and activation of downstream components of the MAPK/ERK pathway MEK1/2 and ERK1/2 (Figure 1). The activation of the MAPK pathway upregulates various transcription factors involved in cellular survival, proliferation, and growth (32).

BRAF Mutations and Other Genomic Alterations

BRAF is frequently mutated in human cancer, with an estimated frequency of ~3-7% (33-37). Since 2002 when Davies et al. described *BRAF* mutations in a subset of human neoplasms (33), numerous studies explored *BRAF* status in various solid tumors (melanoma, carcinomas, brain tumors) and hematological malignancies (e.g., hairy cell leukemia, multiple myeloma, systemic histiocytoses) (35, 38-41). *BRAF* mutations have also been described in various soft tissue tumors, including malignant peripheral nerve sheath tumors (~10%), Ewing sarcomas (3%), and gastrointestinal stromal

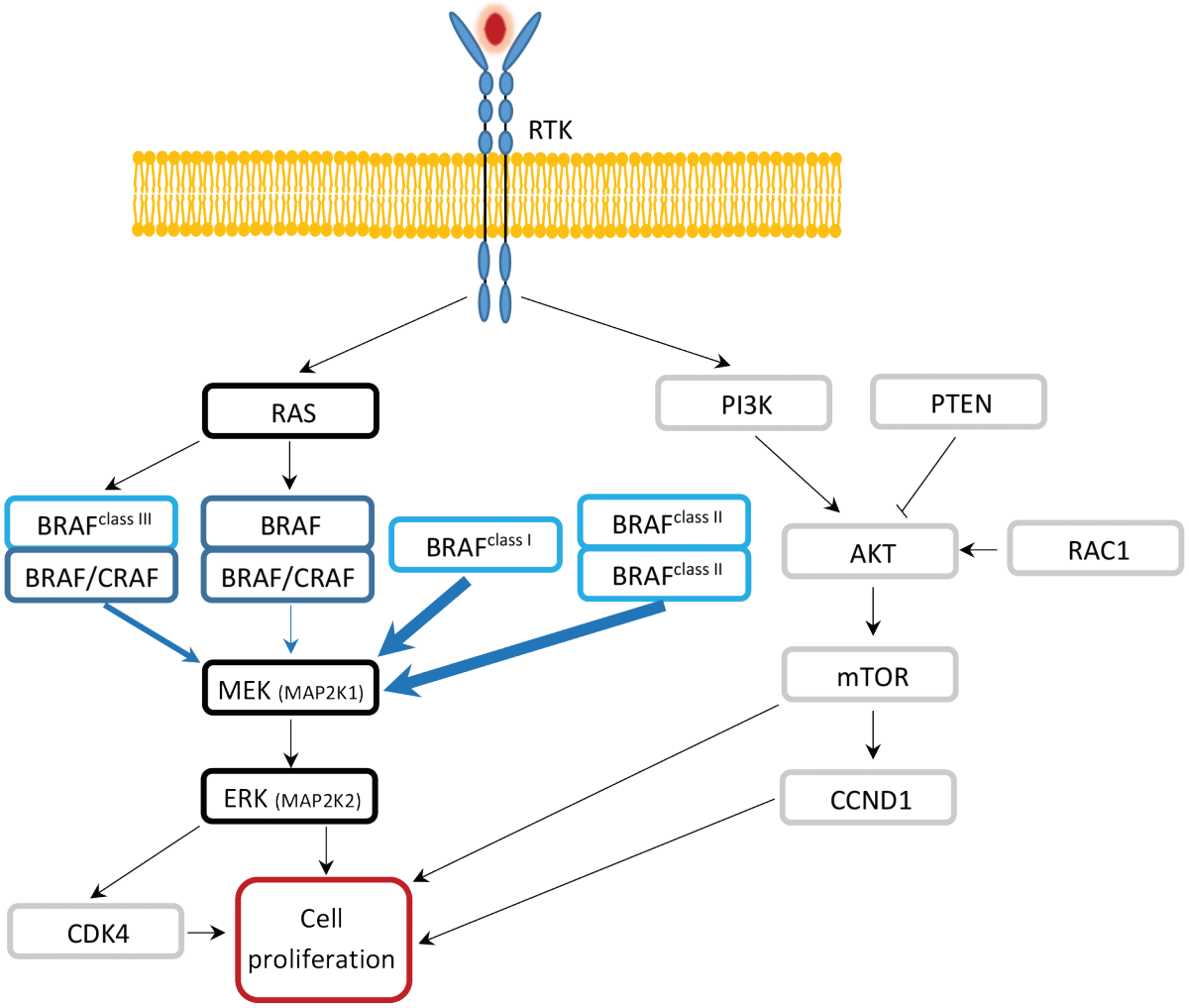

Figure 1. Schematic of MAPK signal (black/dark blue) and related (gray) pathways. Wild type *BRAF* (in dark blue) acts as a dimer (with BRAF or CRAF) to activate MEK in response to activation of RAS, eventually leading to cell proliferation. *BRAF* mutations (light blue) may act in a RAS-independent manner as monomers (Class I) or dimers (Class II), or in a RAS-dependent manner as a dimer (with wild type *BRAF/CRAF*). Mutant *BRAF* (in light blue) appears to be a stronger activator of MEK than wild type, with Class III less strong than classes I and II. BRAF inhibitor resistance may involve mutations at several of the genes encoding the proteins shown (see text).

tumors (7%) [reviewed in (42)]. *BRAFV600E* mutations were also detected in rare, poorly differentiated sarcomas with spindle cell morphology (43).

Tumors with the highest *BRAF* mutation rate (~50-80%) include malignant melanoma, papillary thyroid carcinoma, pilocytic astrocytoma and low-grade serous carcinoma of the ovary (35). However, in other tumors, the frequency of *BRAF* gene mutations is usually seen in the minority of cases (<5%) (44-47). *BRAF* mutations have also been described in several benign tumors, such as melanocytic nevi, metanephric adenomas, and pituitary adenomas, as well as in low-grade neoplasms, such as Erdheim-Chester disease and Langerhans cell histiocytosis (48-51) or locally aggressive neoplasms such as ameloblastomas and craniopharyngiomas (52-55). The data from molecular studies indicate that *BRAF* mutations alone cannot initiate malignant transformation and are usually preceded by the inactivation of

tumor suppressor genes (e.g., *CDKN2A, PTEN* and *BAP1*), *TERT* promoter mutations or inactivation of genes involved in DNA repair (48, 56-58).

Based on their effects on the MAPK pathway, three classes of *BRAF* gene mutations have been described: Class 1, associated with kinase activity (e.g., BRAF V600E, V600K/D/R/M mutations); Class 2 (e.g., K601E, K601N, K601T, L597Q, L597V, L485F, G469A, G469V, G469R, G464V, G464E, and fusions), with constitutively active dimers (codons 601, 597, 469, and 464) (Figure 1); These mutations are resistant to vemurafenib but may be sensitive to MEK inhibitors. Class 3 (D287H, V459L, G466V, G466E, G466A, S467L, G469E, N581S, N581I, D594N, D594G, D594A, D594H, F595L, G596D, and G596R), with low-to nil kinase activity/RAS-dependent mutations, frequently affect exons 11 and 15 and these mutations are commonly observed in non-small cell lung and colorectal carcinomas (48, 59); these mutations are sensitive to MEK inhibitors. Class 1 mutations are usually mutually exclusive with other driver mutations (e.g., *EGFR, KRAS, ALK*). The majority (~80-90%) of *BRAF* mutations are class 1 missense V600E mutations (35, 60). V600E mutation is caused by the transversion of T to A nucleotide 1799 (T1799A), resulting in a substitution of valine (V) for glutamic acid (E) at position 600. The remaining (15-20%) of *BRAF* mutations include V600K, V600R, V600M, V600D and non-V600 mutations (e.g., K601, D594N, G469). Some of these mutations may also be amenable to treatment with BRAF and/or MEK inhibitors (e.g., V600K). However, the efficacy appears to be lower compared to the sensitivity of V600E mutations (61). In contrast, some other mutations (e.g., G469 mutations) are predictors of resistance to anti-BRAF therapies but sensitivity to EGFR inhibitors (57, 62, 63).

In addition, rare *BRAF* gene fusions have been described in various cancer subtypes (frequency 0.3%), particularly in Spitzoid melanomas, pilocytic astrocytomas, papillary thyroid carcinomas, acinar pancreatic carcinomas, gastric carcinomas, serous ovarian carcinomas (both low- and high-grade), salivary gland carcinomas, and histiocytic neoplasms (pediatric and adult xanthogranulomas) (64-73). *BRAF* gene fusions and point mutations have recently been found in a subset of adult and pediatric soft tissue tumors with spindle cell morphology and infantile fibrosarcoma-like growth pattern (74-76). Antonescu also described a poorly differentiated sarcoma with a *BRAF* gene rearrangement; the neoplasm exhibited a whorling growth pattern with the spindle cells within a fibrotic stroma (77). Various *BRAF* gene fusions have also been described in other sarcoma morphologies (78-80). *BRAF*-fused cancers confer resistance to BRAF and EGFR inhibitors but may be sensitive to MEK or pan-RAF inhibitors (65, 81-88).

Not all cancers with *BRAF* mutations are responsive to BRAF inhibitors. Thus, in colorectal carcinoma, there is a strong interplay between *BRAF* and *EGFR,* and BRAF inhibitors alone are ineffective due to the activation of the EGFR pathway. However, a combined treatment with BRAF, MEK and EGFR inhibitors may overcome the potential resistance and induce a much better therapeutic response (89). In contrast, BRAF inhibitors effectively inhibit melanoma cells due to the low expression of EGFR receptor in these cells (89).

BRAF mutations have been associated with a more aggressive clinical course and poor outcomes in cancer patients (90-93). *BRAF* mutations are also strong predictors of response to anti-BRAF treatment modalities, such as BRAF (vemurafenib, dabrafenib and encorafenib) and MEK inhibitors (trametinib, cobimetinib, binimetinib) alone or in combination (45, 94). BRAF inhibitors and five combinations of a BRAF inhibitor plus an additional agent(s) to manage cancers such as melanoma, non-small cell lung cancer, anaplastic thyroid cancer, colorectal cancer, and Erdheim-Chester disease have been approved (Table 2). To date, each regimen is effective only in patients with tumors harboring *BRAF* V600 mutations, and the benefit duration is often short-lived. Further limitations preventing optimal management of *BRAF* mutant cancers are that treatments of non-V600 BRAF mutations have been less profound. Combined therapy is likely

Table 2. Overview of the Cancers with Approved Anti-BRAF Treatment Modalities

Tumor type (indication)	Drug(s)/Combinations	Predictive testing
Malignant melanoma	BRAF/MEK inhibitors /vemurafenib, dabrafenib, encorafenib/trametinib, cobimetinib, binimetinib/	*BRAF* mutational status
Colorectal carcinoma	BRAF/MEK/EGFR inhibitors (encorafenib/binimetinib/ cetuximab)	*KRAS, NRAS* and *BRAF* mutational status
Non-small cell lung carcinoma	BRAF/MEK inhibitors (dabrafenib/trametinib)	*BRAF* mutational status
Anaplastic thyroid carcinoma	BRAF/MEK inhibitors (dabrafenib/trametinib)	*BRAF* mutational status
Erdheim-Chester Disease	BRAF inhibitors (vemurafenib)	*BRAF* mutational status
Solid tumors	BRAF/MEK inhibitors (dabrafenib/trametinib)	$BRAF^{V600E}$ mutations

necessary to overcome resistance mechanisms, but multi-drug treatment options are often too toxic (95). The combination of a BRAF inhibitor and a MEK inhibitor (which acts by inhibiting kinases further downstream of BRAF in the MAPK pathway) substantially inhibits MAPK signaling with a more potent and durable inhibition of MAPK/ERK signaling and delayed acquired resistance (96-98). Dual MAPK pathway inhibition is a standard treatment option for *BRAF*-mutated melanoma (94, 99). Multiple studies also revealed the therapeutic benefit of vemurafenib in patients with several non-melanoma, *BRAF*-mutated cancer types such as NSCLC, Erdheim-Chester disease, Langerhans' cell histiocytosis, and hairy cell leukemia (39, 44, 100).

Resistance to BRAF/MEK Inhibitors

The resistance to BRAF/MEK inhibitors is an emerging problem associated with various genetic and/or epigenetic alterations within the two major signaling pathways, RAF/MEK/ERK and PIK3CA/PTEN/AKT (Figure 1) (35, 101-104). While the intrinsic resistance to BRAF/MEK inhibitors is relatively rare, the acquired resistance (following the treatment) is widespread and nearly inevitable. In particular, mutations of *KRAS, NRAS, MAP2K1,* and *MAP2K2,* along with *BRAF* amplifications (MAPK reactivation or "addiction"), contribute to the resistance to BRAF inhibitors [(reviewed in (57, 104)]. Another potential resistance mechanism is a *BRAFV600E* splice variant that promotes RAF dimerization (105). Mutations within the PIK3CA/PTEN signaling pathway involving *PIK3CA, PTEN, AKT1, PIK3R1, PIK3R2,* and *AKT3* genes are also involved in the resistance to BRAF inhibitors. Genetic alterations of *RAC1, CDK4, CCND1*, and *c-MET* genes also contribute to the resistance to anti-BRAF treatment modalities. Recently, androgen receptor (AR) expression has been described as a potential resistance mechanism in preclinical (animal) models with significantly reduced anticancer activity of BRAF/MEK inhibitors in male mice compared with female mice (106). The study also revealed significantly higher AR expression in melanomas affecting male mice than female mice. The preclinical observations were further translated and confirmed in a clinical cohort of melanoma patients treated with BRAF/MEK inhibitors (106). Further studies should confirm whether androgen suppression could be combined with BRAF/MEK inhibitors in melanoma patients. In NSCLC, the most common causes of resistance to BRAF/MEK inhibitors are mutations of *MEK1, PTEN, NRAS*, and *KRAS* genes (107).

Epigenetic or transcriptome-based changes were speculated to be the likely drivers of the resistance to BRAF inhibitors among ~40% of melanomas that progressed on the treatment and lacked any identifiable genetic abnormality to explain such resistance (104). Among these resistance mechanisms, DNA methylation, post-translational

histone modifications, and various miRNAs appear to play prominent roles (108).

Diagnostic Approaches for BRAF Mutations

For treatment purposes, a routine determination of *BRAF* status is the standard of care (99, 109-111). *BRAF* analysis is usually performed on formalin-fixed paraffin-embedded tissue (FFPE) samples (either primary or metastatic). If FFPE of the primary or metastatic cancer is unavailable, a blood sample or liquid biopsy using circulating tumor DNA (ctDNA) may be an alternative (Guardant 360, Table 3). Although ctDNA presents an essential innovation in cancer diagnostics and management (e.g., diagnosis and molecular profiling of advanced non-small cell lung cancer or the monitoring of BRAF status in melanoma patients during the targeted treatment with BRAF/MEK inhibitors) (34703985), it has certain limitations, including lower sensitivity (47-84%) compared with the PCR-based assays performed on FFPE (112-116).

BRAF analysis is usually performed using various DNA-based molecular assays. The FDA has also approved several diagnostic assays for detecting *BRAF* mutations as CDx tests or authorized assays (summarized in Table 3). Various laboratory-developed assays have also been developed and routinely utilized for *BRAF* gene testing in patients with melanoma and other cancers with approved anti-BRAF treatment modalities (Table 2) (57).

Among the DNA/RNA-based assays, Sanger sequencing, pyrosequencing, mutation-specific Polymerase Chain Reaction (PCR) and mutation-specific real-time PCR, digital PCR (dPCR), High-Resolution Melting curve analysis (HRM), Matrix Assisted Laser Desorption Ionization-Time Of Flight Mass Spectrometry (MALDI-TOF MS; Sequenom), and many Next-Generation Sequencing (NGS) based assays are available (117). Each of these assays has its characteristics and performances but shares very high sensitivity and specificity (~85-100%) in detecting genomic alterations, including *BRAF* gene mutations (117). Some of these assays were also approved by FDA as CDx tests (summarized in Table 3).

The Cobas 4800 BRAF V600 Mutation Test (Roche Molecular Systems, Inc.) was the first FDA-approved CDx for BRAF assessment. This test was used in the clinical trial that led to the approval of vemurafenib by FDA and later by the European Medicines Agency (EMA) (118). The Cobas 4800 BRAF V600 Mutation Test was approved for the vemurafenib/cobimetinib combination, while another RT-PCR-based assay approved for dabrafenib/trametinib combination is the THxID-BRAF kit (bioMerieux Inc.) (Table 3). The therascreen BRAF V600E RGQ PCR Kit (QIAGEN GmbH) is the third RT-PCR-based assay approved by FDA as a CDx. It assesses *BRAFV600E* mutations in patients with colorectal cancer for the potential treatment with encorafenib in combination with cetuximab (a monoclonal antibody against EGFR).

NGS refers to large-scale (high-throughput) DNA and RNA sequencing technology that allows for querying the whole genome, the exons within all known genes (whole exome), or only exons of selected genes (target panel). The use of NGS revolutionized cancer genomic profiling and has become a cornerstone diagnostic tool in precision medicine management (119, 120). It is a highly efficient and precise assay (sensitivity of 98% and specificity of 100%) that enables comprehensive cancer genomic profiling. It is, therefore, a reliable and affordable tool for detecting various genomic alterations, including those affecting the *BRAF* gene (117). Several NGS-based assays achieved either CDx status or were authorized by FDA. These include CDx assays FoundationOne CDx (by Foundation Medicine, Inc.), and Oncomine Dx Target Test (by Life Technologies Corporation), and FDA-authorized assays MSK-IMPACT (by Memorial Sloan Kettering Center), and Guardant360 CDx (by Guardant Health, Inc.) (Table 3). These assays include gene panels of various sizes (from 55 to 505 genes) and also provide additional valuable information about other predictive biomarkers (e.g., tumor mutational burden or microsatellite instability status) (See Table 3

Table 3. The List of FDA-Approved Companion Diagnostic and Authorized Tests/Assays for *BRAF* Testing [Adopted and Modified From (4)].

Test (Manufacturer)	Indication(s)	Diagnostic method
CDx tests/assays		
Cobas 4800 BRAF V600 Mutation Test (Roche Molecular Systems, Inc.)	Malignant melanoma (covering V600E and V600K mutations, respectively)	PCR-based assay
FoundationOne CDx (Foundation Medicine, Inc.)	NSCLC and melanoma (covering V600E and V600 mutations, respectively)	NGS based assay
Oncomine Dx Target Test (Life Technologies Corporation)	NSCLC (covering V600E mutations)	NGS based assay
The therascreen BRAF V600E RGQ PCR Kit (QIAGEN GmbH)	Colorectal cancer (covering V600E mutations)	Real-time PCR
The THxID-BRAF kit (bioMerieux Inc.)	Malignant melanoma (covering V600E and V600K mutations)	Real-time PCR
FDA-authorized tests/assays		
MSK-IMPACT (Memorial Sloan Kettering/ MSK/)	Melanoma and other cancers with *BRAF* and other mutations (the panel of 505 genes)	NGS based assay
Guardant360 CDx (Guardant Health, Inc.)	NSCLC, CRC (*BRAF* and 54 additional targetable genes)	NGS assay based on liquid biopsy

PCR=Polymerase chain reaction; CDx=Companion diagnostics; NGS=Next-generation sequencing; NSCLC=Non-small cell lung carcinoma; CRC=Colorectal carcinoma.

with the list of FDA-approved CDx assays based on NGS technology).

The VE1 antibody is the only immunohistochemical assay currently available for BRAF protein testing and detection (57) but has not received regulatory approval as a CDx despite its widespread availability. BRAF V600E-specific antibody VE1 has a good concordance with detecting the *BRAFV600E* mutation by some genetic tests (34). A meta-analysis based on 21 studies covering 1687 melanoma cases confirmed an excellent diagnostic utility of the VE1 antibody for detecting *BRAFV600E* mutation, with a sensitivity of 0.96 and specificity of 1.00 (121). Similar performance of the VE1 antibody was reported in colorectal (122-124), thyroid carcinomas (125-128), hairy cell leukemias (129, 130) (Figure 2A-B), and low-grade serous ovarian neoplasms (131).

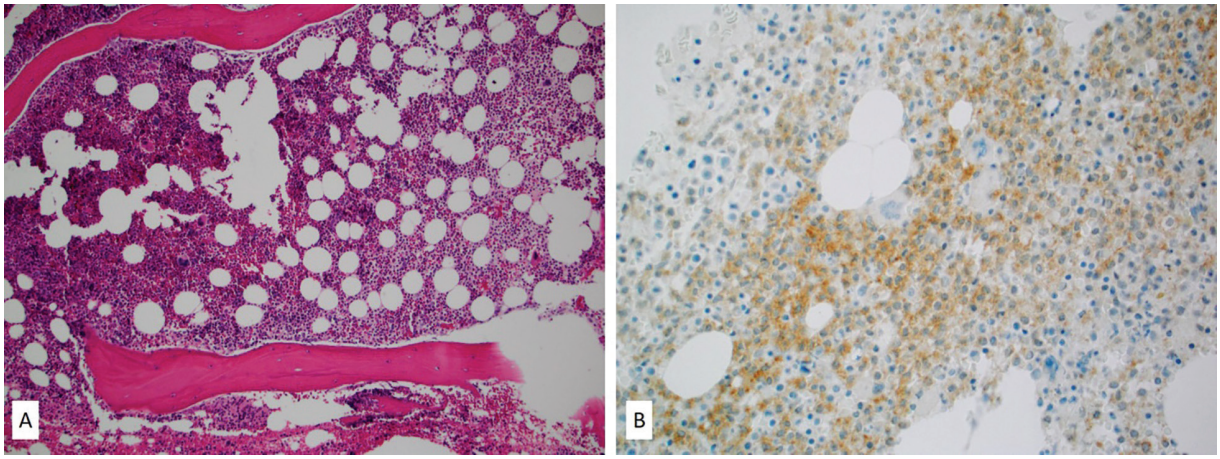

Figure 2A-B. (A): Hematoxylin and Eosin (H&E) slide of a case of hairy cell leukemia with a diffuse bone marrow infiltration (10x magnification); neoplastic cells harbored *BRAFV600E* mutation, which was confirmed immunohistochemically using VE1 antibody (40x magnification).

Our previous study, based on a cohort of diverse cancers, confirmed that the VE1 antibody is 100% sensitive and 91% specific for *BRAFV600E* protein and may serve as a good screening tool, especially in tumor types with a high proportion of *BRAFV600E* mutation (e.g., thyroid carcinoma, colorectal carcinoma, melanoma) (132). However, VE1 IHC screening in tumor types with a higher proportion of non-*BRAFV600* mutation may not be feasible with a high proportion of false-negative results (133-135). For instance, lung adenocarcinomas may have a higher proportion of false negative results due to the finding of the D594V mutation. Rare actionable mutations (e.g., V600K) may also be missed using VE1 IHC alone (132, 136). The discrepancies between VE1 IHC and PCR assays have also been described in the kidney's *BRAFV600E* -mutated metanephric adenomas, pituitary adenomas, and Langerhans cell histiocytosis (51, 137, 138).

Although Martins-de-Barros et al. in the systematic review with a meta-analysis, reported an excellent diagnostic utility of VE1 IHC in ameloblastomas (139), several studies reported its low diagnostic value in maxillary ameloblastomas that are predominantly affected by non-*BRAFV600E*-mutations (52, 140).

Taken together, VE1 IHC appears to be an excellent screening assay, particularly for the detection of *BRAFV600E* mutations, but further confirmation with molecular (PCR)-based methods is still required for the targeted treatment with BRAF and/or MEK inhibitors.

Conclusions

Precision cancer medicine has substantially improved cancer diagnostics and treatment. Tissue type-agnostic drug therapies present a novel shift in precision cancer medicine. It is a consequence of carefully designed clinical trials showing the value of tumor biomarkers, not just in diagnosis but in therapy guidance. Six different tumor-agnostic treatment modalities have been approved for cancer treatment since 2017 when pembrolizumab was approved for MSI-H/dMMR solid tumors regardless of their histotype. In June 2022, a combination of BRAF/MEK inhibitors (dabrafenib/trametinib) was approved in a tumor-agnostic fashion for all solid metastatic cancers harboring $BRAF^{V600E}$ mutations. *BRAF* mutations affect ~3-7% of all cancers, with the highest prevalence in melanoma, papillary thyroid carcinoma, pilocytic astrocytoma, and low-grade serous ovarian carcinoma. However, a low prevalence (≤5%) of *BRAF* mutations has been described in ~50 cancer subtypes. BRAF inhibitors alone or combined with MEK inhibitors have been approved and substantially improved the treatment of several cancers, including malignant melanoma, non-small cell lung cancer, anaplastic thyroid cancer, colorectal cancer, and Erdheim-Chester disease. The diagnosis of *BRAF* mutations remains a cornerstone of anti-BRAF treatment(s), and several highly sensitive and specific diagnostic assays were approved as CDx tests. Resistance to treatment represents an emerging issue among BRAF cancers, mainly when BRAF inhibitors are administered alone. Apart from mutations within MAPK/MEK and PIK3CA signaling pathways, novel and potentially targetable resistance causes have been recently described (androgen receptor overexpression). Further efforts are needed to translate these findings into clinical practice and improve the outcome of patients with *BRAF*-mutated cancers.

Conflict of Interest: Gargi D. Basu and David W. Hall are full-time employees and stockholders of Exact Sciences. Zoran Gatalica is a part-time employee of Exact Sciences. Semir Vranic declares no conflict of interest.

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