Review Article

A narrative review of BRAF alterations in human tumors: diagnostic and predictive implications

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Abstract: BRAF is a ubiquitous oncogene in human tumors; oncogenic mutations give rise to a broad biologic spectrum of clonal proliferations depending on cellular context and tumor suppressor gene co-mutation. The *BRAF* V600E mutation, a class 1 variant, is the best-understood alteration in the gene, but despite that, extensive pre-clinical and empiric study has been required to optimize combinatorial therapies in order to overcome bypass and downstream signaling induced by BRAF inhibitor monotherapy in tumors with this mutation. Class 2 and 3 BRAF variants predominate in certain tumors such as non-small cell lung carcinoma (NSCLC) and are associated with distinct clinicopathologic features including resistance to RAF inhibitors and co-mutation with other genes in the RTK/RAS/MAPK pathway. Clinical trials of BRAF inhibitors in some of the most common malignancies, including melanoma, colorectal cancers, and lung cancers, have led to a number of FDA-approved combination RAF-MEK or RAF-EGFR inhibitors, with substantial implications for improved survival and quality of life in a large number of cancer patients. Mutational testing for *BRAF* V600E is required for selection of patients with BRAF inhibitor therapies; rapid screening for the mutation may be carried out using mutation-specific immunohistochemistry, whereas next generation sequencing is an optimal testing strategy in order to identify relevant co-mutations and mutational patterns (such as mismatch repair status) likely to influence therapeutic selection and outcomes.

Keywords: BRAF; MEK; melanoma; non-small cell lung carcinoma (NSCLC); colorectal carcinoma

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Introduction

B-Raf proto-oncogene, serine/threonine kinase (*BRAF*) represents one of the most commonly mutated and best characterized oncogenes in human tumorigenesis. The spectrum of tumors harboring *BRAF* mutations spans essentially every organ system and represents both indolent neoplasms and highly aggressive malignancies. Advances in clinical sequencing technologies have enabled routine detection of *BRAF* alterations beyond the V600E hotspot, leading to novel clinicopathologic correlations. Developments in targeted therapeutics against RAF and MEK pathway activation have led to a number of BRAF biomarker-driven clinical trials, beginning with targeted BRAF inhibitor monotherapy in melanoma, followed by exploratory pan-cancer basket trials, and ultimately culminating in specific targeted inhibitor combination therapies now approved for patients with advanced or metastatic *BRAF* V600E-mutated tumors. Understanding the spectrum and targetability of *BRAF* alterations is now fundamental to the practice of diagnostic and therapeutic oncology. This article will review the role of *BRAF* alterations in neoplasia, examine the recently defined classes of BRAF alteration with regard to downstream signaling and targetability, review select examples of BRAF targeting in clinical practice, and discuss molecular diagnostics for detection of *BRAF* mutations. We present the following article in accordance with the Narrative Review reporting checklist (available at http://dx.doi.org/10.21037/pcm-20-39).

Methods

Literature used to inform the text herein was drawn from PubMed.gov from the National Library of Medicine and included full length manuscripts published in the English language between 1997 and 2020. Congress abstracts were identified through targeted searches of their sponsoring organization websites, where necessary. Regulatory documents were identified via Google search.

BRAF structure and function

BRAF belongs to the rapidly accelerated fibrosarcoma (RAF) family of serine/threonine kinases and functions as a Mitogen-activated pathway kinase kinase kinase (MAPKKK) in the MAP kinase/ERK signaling cascade. BRAF is normally triggered following ligand binding to receptor tyrosine kinases (RTKs) such as epidermal growth factor receptor (EGFR) or ERBB2. RTK phosphorylation leads to activation of the RAS-family of GTPases, which trigger dimerization of RAF family members and downstream activation of kinases including MEK1/2 and ERK1/2, leading to direct and indirect transcriptional regulation involved in cell survival and proliferation (1). Three distinct Raf genes have been described: ARAF, BRAF, and CRAF (RAF-1); all have been demonstrated to play essential roles in development and tumorigenesis. However, BRAF is maximally activated by oncogenic Ras signaling, whereas ARAF and CRAF appear to require Raf-dependent tyrosine phosphorylation (2). *BRAF* is located on chromosome 7q34; it is comprised of 18 exons and contains three regions that are conserved across the Raf family members: C1, containing the Raf-like Ras binding domain and an auto-inhibitor of the kinase domain (encoded by amino acids 150–290); C2, containing the serine and threonine-rich hinge region (encoded by amino acids 360–375); and C3, containing the protein tyrosine kinase domain (encoded by amino acids 457–717).

BRAF in disease

Germline *BRAF* mutations falling within the C1 domain and protein tyrosine kinase domain may give rise to Cardio-facio-cutaneous syndrome, which is associated with facial dysmorphism, mental retardation, and cardiac defects. Individuals with this disorder only rarely go on to develop malignancies (3,4). Somatic mutations in *BRAF*, on the other hand, are among the most common oncogenic alterations reported in humans and can be found in adult and pediatric cancer patients, in both solid and liquid tumors, and as apparent drivers of both highly aggressive and indolent neoplasms. Oncogenic mutations in *BRAF* are reported overall in 6% of human malignancies and are located principally within the C3 region (1). Although *BRAF* Val600Glu (V600E) mutations are the most well-recognized both for diagnostic and therapeutic purposes, over 200 oncogenic alterations have been reported in this gene with a range of implications for downstream pathway activation and targetability (5).

Oncogenesis versus senescence

BRAF mutations were first recognized as oncogenic in 2002, when investigators involved in The Cancer Genome Project described V600E mutations (annotated at the time as V599E) in a range of cancer types including in two-thirds of melanomas (6). However, these same mutations were also described in over 80% of benign nevi, suggesting that *BRAF* V600E is not sufficient to drive oncogenesis (7). In benign clonal processes such as nevi, *BRAF* oncogene-driven senescence blocks cellular proliferation via induction of the tumor suppressor $p16^{INK4a}$ and stable cell cycle arrest (7,8). The vast majority of benign nevi fail to progress to malignancy and some may even regress. In melanoma, in contrast, it appears that precursor cells first acquire hits in tumor suppressor genes such as *CDKN2A* and/or *PTEN*; in this context, subsequent mutations in MAPK genes, including *BRAF*, can drive malignant transformation (9). Indeed, it appears that in most tumor contexts with recurrent *BRAF* mutations, inactivation of a variety of tumor suppressor genes are required for transformation. Oncogene-induced senescence is also associated with telomere dysfunction; therefore in a subset of tumors the de-repression of hTERT gene expression, such as through *TERT* promoter mutation, restores telomerase activity and escape from senescence (10).

Besides melanocytic nevi, *BRAF* mutations are associated with a wide range of benign proliferations and neoplasms of low malignant potential impacting most organs of the body. These include endosalpingiosis (11), metanephric adenoma (12) and metanephric stromal tumors (12), papillary craniopharyngiomas (13), ganglioglioma (14), pituitary adenoma (15), bronchial adenomas/ciliated muconodular papillary tumor of the lung (16,17), Erdheim-Chester disease (18), Langerhans cell histiocytosis (19), and sessile serrated adenomas of the colon (20). Selected

examples of indolent or low-grade neoplasms containing *BRAF* V600E mutations are described in more detail below.

Endosalpingiosis

Endosalpingiosis, defined as the presence of morphologically-benign glandular structures comprised of fallopian tube epithelium involving peritoneum or lymph nodes, is considered the precursor of ovarian low-grade serous neoplasms. Accordingly, similar driver oncogenic events are identified in both lesions, and in patients with both endosalpingiosis and low-grade serous neoplasms, a common *KRAS* G12/G13 or *BRAF* V600E mutation can be detected in both populations (11). In cell culture models of fallopian tube or ovarian surface epithelial cells, the presence of mutant *KRAS* or *BRAF* triggers growth arrest (11,21); consistent with this observation, the proliferation rate in endosalpingiosis is low. Additional defects in tumor suppressor pathways are likely necessary to drive the evolution from salpingiosis to clinically detectable serous tumors.

Histiocytoses

Erdheim chester disease (ECD) and Langerhans cell histiocytosis (LCH) are progressive, systemic neoplastic processes affecting both children and adults, currently characterized by the World Health Organization as inflammatory myeloid neoplasms. ECD is comprised of CD68+ histiocytes whereas LCH is comprised of CD1a+ CD207+ histiocytes; both are associated with multi-organ involvement. Fifteen percent of ECD patients also have LCH, a situation classified as "overlap histiocytosis" (22). Pulmonary LCH is uniquely associated with cigarette smoking, and the symptomatic and radiographic sequelae can often be managed through smoking cessation alone. The biologic relatedness of ECD and LCH is supported in part by evidence for similar genomic profiles, including frequent *BRAF* V600E mutations (in ~70% and ~60%, respectively, when using highly sensitive molecular techniques) and mutually exclusive *MAP2K1* hotspot mutations (in 20% and 12%, respectively) (22,23). Other *BRAF* activating events including indels, duplication, or fusion are reported in a minor subset of LCH cases (24). *BRAF* V600E mutations were initially reported as sole alterations in histiocytosis (25); however, later studies have reported *TP53*-comutations (26) or loss of p16(INK4a) in aggressive cases (27), consistent with at least a two-hit model of tumorigenesis and the need for impaired tumor suppressor function to release the *BRAF*-mutated cells from senescence.

Colonic sessile serrated adenomas/polyps

BRAF V600E mutations are reported in approximately 60-80% of serrated neoplasms (28) but are absent in traditional adenomas of the colon. Sessile serrated adenomas/polyps (SSA/P) are thought to represent the benign precursor to CpG Island Promoter Methylation-high /microsatellite instability-high (MSI-H) colon cancers. Accordingly, SSA/P shows frequent *BRAF* V600E mutation, with acquisition of WNT pathway activity and widespread CpG island methylation, including *MLH1* promoter methylation, during progression to cytologic dysplasia (29,30). A minority of *BRAF*-mutated SSP/A may progress down a microsatellite stable pathway via acquisition of tumor suppressor gene mutations including in *TP53* and *PTEN* (31).

Overview of types of oncogenic alterations found in solid malignancies

Activating somatic *BRAF* alterations, including mutations, fusions, and amplifications, are reported in a diverse set of solid tumors. These are common in primary brain tumors, followed by non-follicular thyroid tumors, melanoma, and colorectal carcinoma. There is a long tail of other solid tumors that show mutations and/or fusions in 1–5% of cases (lung adenocarcinomas, acinar cell carcinomas of the pancreas, intrahepatic cholangiocarcinomas) or in fewer than 1% of cases (prostate and bladder carcinomas, high grade serous ovarian carcinoma) (32) (*Figure 1*).

A subset of lower grade central nervous system tumors shows a particularly high frequency of *BRAF* activating alterations. *BRAF* V600E mutation is seen in nearly all papillary craniopharyngiomas. Pilocytic astrocytoma harbors a *BRAF* fusion or mutation in more than 80% of cases, and ganglioglioma and pleomorphic xanthoastrocytoma harbor *BRAF* fusions or mutations in up to 50% and 66% of cases, respectively. In contrast, *BRAF* mutations are rare in glioblastoma multiforme. *BRAF* alterations have variable and conflicting prognostic implications in lower grade primary brain tumors; however, identification of these changes can inform diagnosis and impact selection of BRAF targeted therapies (33). As a result, molecular analysis and/or fluorescence *in situ* hybridization maybe employed routinely in clinical practice to detect these changes and confirm a morphologic diagnosis.

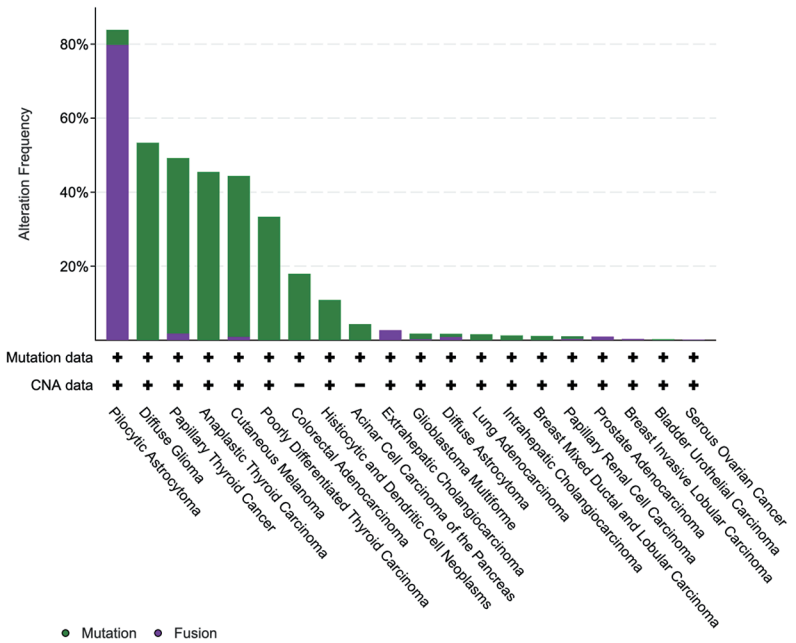

Figure 1 Frequency of *BRAF* fusions and mutations across solid tumors and histiocytic neoplasms based on The Cancer Genome Atlas sequencing studies (http://cbioportal.org). Myeloid and lymphoid neoplasms are excluded here (32).

Oncogenic activity of BRAF alterations

Mutations

Oncogenic *BRAF* mutations are characterized based on whether they demonstrate kinase activity and require upstream RAS activity and BRAF dimerization. Kinase-activating mutations are independent of RAS signaling. Within this class of mutations, a subset signal as monomers (class 1) and others signal as constitutively active dimers (class 2). *BRAF* V600E falls into the class 1 activating group of mutants, along with other less common substitutions at this codon including V600K/D/R/M. Other small insertion deletion mutations involving the V600 codon are rare, but at least isolated examples may stabilize the activated kinase in a similar fashion to other class 1 mutants (33). Class 1 mutations tend to occur in a mutually exclusive fashion with other oncogenic driver alterations (e.g., KRAS, EGFR, ALK fusions, etc.), at least in the pre-treatment setting (34). Class 2 mutants cluster at codons 601, 597, 469 and 464 (5). Finally, a subset of *BRAF* mutations demonstrates low to absent kinase (kinase-dead) activity, and activation of downstream signaling is dependent on RAS activity. These so-called class 3 mutants are scattered throughout hotspots in exons 11 and 15 and represent up to 30% of *BRAF* mutations observed in colorectal carcinoma and up to 30% of *BRAF* mutations observed in non-small cell lung carcinoma (1) (*Figure 2*), occasionally in tandem with oncogenic mutations in RAS family member genes. Mechanistically, these RAS-activated kinase-low/dead mutants appear to heterodimerize with CRAF to trigger ERK signaling and thereby amplify other inputs into the ERK pathway (5). While class 1 (V600) mutants are sensitive to targeted RAF inhibitors, class 2 mutants may require dual inhibition of RAF and downstream MEK signaling (36), and class 3 mutants appear responsive to MEK inhibitors (5).

Fusions and other structural variants

Oncogenic BRAF fusions were first reported in papillary thyroid tumors, enriched in individuals exposed to radiation following the Chernobyl nuclear accident (37). *AKAP9-BRAF* was the first characterized fusion, comprised of the first 8 exons of AKAP9 and the C-terminal portion of BRAF including exons 9–18 and resulting from paracentric chromosomal inversion event on the long arm of chromosome 7. This event led to loss of the CR1 and CR2 regulatory domains within the N-terminal portion

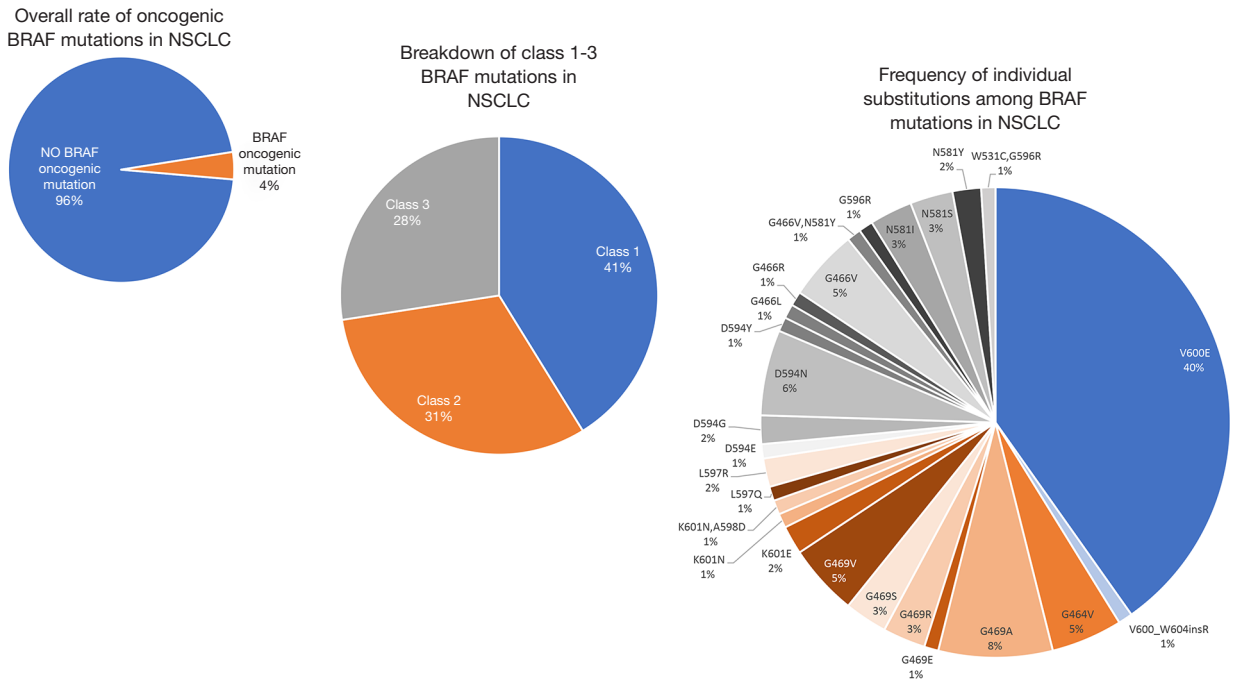

Figure 2 The spectrum of *BRAF* mutations in non-small cell lung carcinoma. About 4% of NSCLC have an oncogenic *BRAF* mutation in exons 11 or 15 of the gene. These can be further subclassified into classes based on their kinase activity and responsiveness to BRAF inhibitor therapy. The most common mutation, the class 1 V600E mutation, represents about 40% of oncogenic *BRAF* mutations. The remaining mutations represent less common hotspot alterations; these may have implications for clinical trial enrollment but currently do not represent criteria for BRAF inhibitor therapy (5,35).

of BRAF, with retention of the kinase domain. Consistent with loss of the regulatory region of the protein, the fusion product demonstrates constitutive RAF kinase activation and transforming ability. Structurally similar fusions were subsequently described in pilocytic astrocytomas, where a duplication event on 7q34 leads to fusion of the N terminus of *KIAA1549* and the C terminus of *BRAF* beginning at either exons 9 or 11 (38). Other less common but recurrent fusions include *FAM131B-BRAF*, resulting from an interstitial deletion on 7q (39) in pilocytic astrocytoma and *SND1-BRAF* mutations in lung adenocarcinoma and pancreatic acinar cell carcinomas (40). Over 50 additional fusion partners have been identified in one to two reports each in a spectrum of tumors including pilocytic astrocytoma, spitzoid melanomas, and other solid tumor types at an exceptionally low frequency (41,42). Internal tandem duplications of the *BRAF* kinase domain and/or intragenic deletion of the N-terminal regulatory domains have been reported rarely in melanoma and infantile fibrosarcoma (43,44).

Fusion events are considered Class 2 alterations; the loss of the N-terminal regulatory domains enables RAS-independent homodimerization, which is required for kinase activation in this class of mutants. As with the point mutations in this class, BRAF fusions drive MAPK pathway signaling but are resistant to first generation BRAF inhibitors such as vemurafenib (45). Next generation RAF inhibitors that inhibit BRAF homo- and heterodimers may have more activity in BRAF-fusion driven tumors (46).

Therapeutic implications of *BRAF* mutations

Melanoma

Oncogenic mutations in *BRAF* are reported in over 40% of cutaneous melanomas, are enriched in tumors arising on skin without chronic sun damage and are clustered in and around codon V600 (47). The most common substitution is V600E followed by V600K; other substitutions including V600R/M/D/G and small indels at this position are uncommon (48). Clinical trials of single agent vemurafenib in patients with metastatic melanoma and *BRAF* V600E/

K mutations showed objective response rates of around 50% (49-51). Overall survival with vemurafenib therapy was improved relative to standard of care at the time of the published monotherapy trials (52) but was limited by development of resistance (53,54). Melanomas exposed to BRAF inhibitors employ heterogeneous mechanisms to reactivate MAPK and alternative pathways (55). Combination RAF and MEK therapy (dabrafenib, trametinib) provides improved overall survival relative to vemurafenib monotherapy and >60% response rate (56), but shows only modest efficacy in patients with BRAF inhibitor-refractory melanoma following monotherapy (57). Current (2020) clinical testing and treatment guidelines recommend *BRAF* mutational testing for any patient with stage III or IV cutaneous melanoma; detection of a *BRAF* V600-activating mutation justifies use of BRAF-MEK therapies including dabrafenib/trametinib, vemurafenib/cobimetinib, or encorafenib/binimetinib. These therapies may be used in the first line or after progression on immunotherapy with PD-1 inhibitors (58). Mutations occurring at codons L597 and K601 may also predict response to MEK or combination MEK/RAF inhibitors, however other exon 11 and 15 mutations do not (36). Post-hoc analyses of clinical trials of the PD-1 inhibitor pembrolizumab demonstrate efficacy of this immunotherapeutic irrespective of *BRAF* V600 mutational status or prior treatment with BRAF/MEK inhibitors (59).

Targeting BRAF beyond melanoma

Following positive trials of BRAF inhibitor therapy in melanomas, basket trials were opened to enroll patients across various *BRAF* V600-mutated nonmelanoma cancers to determine if targeted BRAF inhibitors might be a promising approach across diagnoses. Initial results for vemurafenib monotherapy were disappointing, however, with most activity seen in NSCLC and histiocytosis. Occasional durable responses were seen in other tumor types including cholangiocarcinoma, anaplastic thyroid carcinoma, and ovarian cancer, and no activity was observed for combined vemurafenib and cetuximab in colon cancer (60). Given these at best modest initial results, investigators began instead looking to combinatorial therapy in a variety of clinical contexts.

Colon

BRAF V600E mutations are reported in 10% of colorectal carcinomas, are enriched in right sided tumors and those with sporadic mismatch repair deficiency due to *MLH1* promoter methylation, and serve as exclusionary criteria for selection of patients for treatment with anti-EGFR (such as cetuximab). This is based on the assumption that activation of RAF/RAS kinase members such as KRAS, NRAS and BRAF leads to constitutive MAPK pathway activity independent of RTK signaling, such as through EGFR (1). In retrospective studies of colon cancer patients treated with anti-EGFR monoclonal antibodies, *BRAF* V600 mutations serve as negative predictors of response (61,62). Preclinical studies have demonstrated that inhibition of BRAF V600E in colon cancer leads to feedback activation of EGFR and bypass activation of MAPK pathway via CRAF. Indeed, the combination of anti-BRAF and anti-EGFR agents have a synergistic effect in killing of *BRAF* mutant colon cancer cells (63,64). Phase I/II studies of combined MEK and BRAF inhibitors showed a modest improvement in response over BRAF inhibitors alone in patients with *BRAF* V600E mutant colon cancers (65). Trials of triplet BRAF, EGFR and MEK inhibition in patients with *BRAF* V600E mutant metastatic colorectal carcinoma (BEACON) demonstrated tolerability and improved efficacy over standard of care therapy (66,67). In 2018, the FDA granted breakthrough therapy designation to BRAF, MEK, and EGFR inhibitor combination (encorafenib, binimetinib, and cetuximab, respectively) as second line therapy (68). Patients in the BEACON study receiving doublet encorafenib and cetuximab therapy in the second or third lines also showed significant improvements in response rates and survival relative to standard of care, leading to FDA approval in 2020 (69). Non-V600 mutations are not so clearly associated with lack of response to anti-EGFR therapies (70,71). In theory, a subset of tumors with class 3 mutations lacking co-mutations in other RAS pathway members and retaining dependence on RTK signaling may derive benefit from anti-EGFR therapy (72).

Sixty percent of sporadic *MLH1* promoter methylated mismatch repair-deficient (MMR-D)/MSI-H colorectal carcinomas have a *BRAF* V600E mutation; this contrasts with only about 1% of colon cancers arising in patients with germline MMR mutations (73). Therefore, knowledge of the *BRAF* V600E status can inform the likelihood of Lynch Syndrome-associated versus sporadic colorectal carcinoma. *BRAF* mutation, taken together with MMR/MSI status, is a recognized prognostic indicator in colorectal carcinoma. MSI-H/*BRAF* wild type tumors have the best survival outcomes, whereas microsatellite stable

(MSS)/*BRAF* mutant tumors are associated with poor prognosis. MSS/*BRAF* wild type tumors appear to have intermediate outcomes (74,75). Besides its association with *BRAF* mutation in colon cancer, MMR-D status predicts responsiveness to PD-1 inhibitor therapy (76,77). In a phase 3 trial of the PD-1 inhibitor pembrolizumab versus standard of care frontline chemotherapy for patients with MMR-D/MSI-H colorectal carcinoma, pembrolizumab more than doubled progression free survival with a reduction in severe adverse effects as compared to cytotoxic therapy (78). This led to an FDA approval for pembrolizumab as first line treatment of colorectal carcinoma with MMR-D/MSI-H status, irrespective of *BRAF* or other oncogenic driver mutation status (79).

Lung

BRAF exhibits diverse mutations in NSCLC; just under half of reported oncogenic *BRAF* mutations occur at V600E; the remainder are distributed predominantly within exons 11 and 15 and are associated with class 2 or 3 activity (*Figure 2*). The FDA has approved combination dabrafenib and trametinib (RAF and MEK inhibitors) in the first line of therapy for patients with advanced/metastatic *BRAF* V600E-mutated NSCLC (80). This combination is associated with improved response, progression free survival, and overall survival as compared to chemotherapy (81), and a subset of patients experiences durable benefit, with over a third of patients treated in the first line setting surviving more than three years (82). Durable response to this combination therapy may be related to the simplicity of the NSCLC genome, with more complex genetic changes and co-mutations predicting shorter duration of benefit (82). The clinical characteristics of patients with *BRAF* V600E mutations are heterogeneous, including never smokers and former/current smokers; adenocarcinoma is the most common histology observed. In contrast to *EGFR*/*ALK*/*ROS1*-altered lung carcinomas, which tend to show only very limited response to immunotherapies, tumors with *BRAF* mutations, including both V600E and non-V600E mutations, do show response to immune checkpoint inhibitors (83). Combination RAF/MEK inhibitor therapy is not approved for non-V600E *BRAF* mutations in light of preclinical data suggesting limited efficacy of approved BRAF inhibitors in tumors with mutations leading to BRAF dimerization. In NSCLC, class 2 and 3 *BRAF* mutations are more likely to occur in tandem with mutations in *KRAS* and appear to be associated with more aggressive disease (35). Trials of MEK and pan-RAF inhibitors have been attempted in this context but have been limited by drug toxicity (84).

Other rare tumor types

Combined BRAF and MEK inhibitor therapies have been approved for use in *BRAF* V600E mutated anaplastic thyroid carcinoma (85), which represents about 1–2% of thyroid cancers overall and harbors this mutation in nearly 50% of cases. Over two-thirds of *BRAF*-mutated anaplastic thyroid carcinoma patients treated with this regimen respond, with a 12-month overall survival of 80%, dramatically greater than historic 1 year survival rates of 20–40% (86).

The BRAF inhibitor vemurafenib was approved for *BRAF* V600-mutated ECD in 2017, based on results of the VE Basket trial that enrolled 22 adult ECD patients; over half of patients responded and reported an improvement in neurologic symptoms and pain (87,88). Responses to BRAF inhibition with or without MEK inhibition have been reported in other rare tumor types including *BRAF* V600E-mutated ameloblastoma, malignant glomus tumors, cholangiocarcinoma, salivary gland adenocarcinoma, and glioblastoma (89-91).

Diagnostic tools for *BRAF* mutation detection

A wide variety of methodologies have been employed for detection of *BRAF* V600 mutations, and it is important to recognize the strengths and deficiencies of different strategies. Beginning with the approval of vemurafenib therapy for *BRAF* V600-mutated melanoma in 2011, a trend of companion diagnostic approvals began to dictate formally accepted practices for clinical mutation detection. The Roche cobas 4800 BRAF V600 Mutation test was approved as an *in vitro* diagnostic for vemurafenib as monotherapy as well as in combination with cobimetinib (92). This targeted PCR-based assay quickly came under criticism because of its inability to detect less common dinucleotide substitutions leading to V600E/K/R mutations and its suboptimal sensitivity relative to unbiased sequencing based methods (93). At the same time, the highly standardized cobas test appeared to generate fewer invalid results relative to Sanger sequencing and primer-extension/fragment analysis assays (94) and showed superior sensitivity to Sanger sequencing for samples with mutant allele fraction of under 25% (95). A practical downside to companion diagnostic usage in clinical laboratories is the capital expenditure required to acquire

platform-specific instrumentation, creating a barrier to implementation in small laboratories with tighter budgets. Many molecular laboratories already have instruments suited to analysis of the specific clinically relevant mutation and little incentive to purchase another piece of potentially duplicative equipment. Further complicating this space, the FDA more recently approved a different BRAF assay—the therascreen BRAF V600E Rotor-Gene Q PCR kit, requiring dedicated instrumentation—for selection of colorectal carcinoma patients for BRAF and EGFR inhibitor therapy (96). Many molecular diagnosticians will ask why two different FDA-approved platforms should be required to generate information about the same mutational change.

Analyses of over 1000 national proficiency test results between 2011–2015 indicated that acceptable (correct) responses were generated for 96.6% of BRAF results in laboratories relying on laboratory developed tests as compared to 93% for FDA-approved companion diagnostics including the Roche cobas BRAF and bioMerieux THxID BRAF tests; the lower performance was driven by the Roche assay and attributed to its relative insensitivity for detection of the c.1798_1799delGTinsAA (p.V600K) dinucleotide substitution (97). In another proficiency testing analysis examining use of next generation sequencing versus non-NGS practices for mutation detection from 2014–2017, laboratories that employed NGS methodology showed a significantly greater likelihood of achieving a correct result for *BRAF* testing, driven again by the ability to more reliably detect the V600K change (98). Ultimately, evidence for greater cost-effectiveness and shorter overall turnaround time for NGS assays (99) that can deliver information for many more potential targets across tumor types, as well as availability of a number of FDA-approved NGS assays (100), will likely continue to push the field away from reliance on single gene assays.

Immunohistochemistry (IHC)

Mutation-specific BRAF V600E IHC using the VE1 clone is an accepted screening tool for melanoma. Relative to molecular testing, VE1 IHC ranges 85–97% sensitivity and 96-100% specificity, depending on the defined comparator (101,102). The short turnaround time afforded by IHC testing can enable patients to start BRAF inhibitor therapy earlier than may be possible when relying on molecular testing—this can offer clinical benefit to patients with rapidly progressive disease. However, given the less-than-perfect performance characteristics, confirmatory molecular testing is generally recommended (58). Metastatic lesions and pigmented tumors most commonly give rise to IHC—molecular discrepancies (103). Decalcification of metastatic melanoma in bone samples can lead to falsely negative IHC and/or molecular testing results and may be a source of discrepancy between these methods (104,105). VE1 IHC is also insensitive to the BRAF V600K (and other non-V600E mutations); therefore, mutational profiling is required to detect these alterations (103).

For thyroid carcinoma, VE1 IHC has been validated for detection of *BRAF* V600E mutations in tissue and cytology cell block specimens (103,106); however, the performance of the antibody in direct smears and liquid-based preparations, particularly those with obscuring elements, is limited (107,108). The specificity of the VE1 IHC staining is illustrated in a collision tumor including a *BRAF*-wild type lung adenocarcinoma and a metastatic *BRAF* V600E-mutated papillary thyroid carcinoma (*Figure 3*).

BRAF IHC has been proposed as a tool for detection of serrated lesions of the colon (109) as well as to screen colorectal cancers for *BRAF* V600E mutations (110). Early studies of this IHC antibody in cohorts of colon neoplasms gave conflicting results, with very promising results from a laboratory using hybridoma supernatants (109), but evidence of poor sensitivity and specificity for *BRAF*-mutated colon cancers from a variety of groups using commercially available antibodies (111-113). Other groups observed that VE1 IHC and molecular testing could show high concordance but required rigorous optimization of the IHC assay and even so resulted in a substantial number of cases with weak staining requiring confirmation via molecular methods (103,114). Access to molecular assays, including comprehensive next generation sequencing tests that deliver information on a wide variety of potential biomarkers simultaneously, may supersede BRAF IHC, given that it delivers information on only one therapeutically relevant variable. In theory, however, BRAF VE1 IHC combined with MMR protein evaluation can provide most of the necessary information for first or later line treatment decisions in patients with colon adenocarcinoma (see Therapeutic Implications, above).

Detection of *BRAF* V600E mutation in non-small cell lung carcinoma, particularly lung adenocarcinoma, is now an indication for first line therapy with combined BRAF/MEK inhibitors. This clinical indication has driven a renewed interest in use of VE1 IHC for rapid screening/detection of *BRAF* V600E mutations in this tumor type.

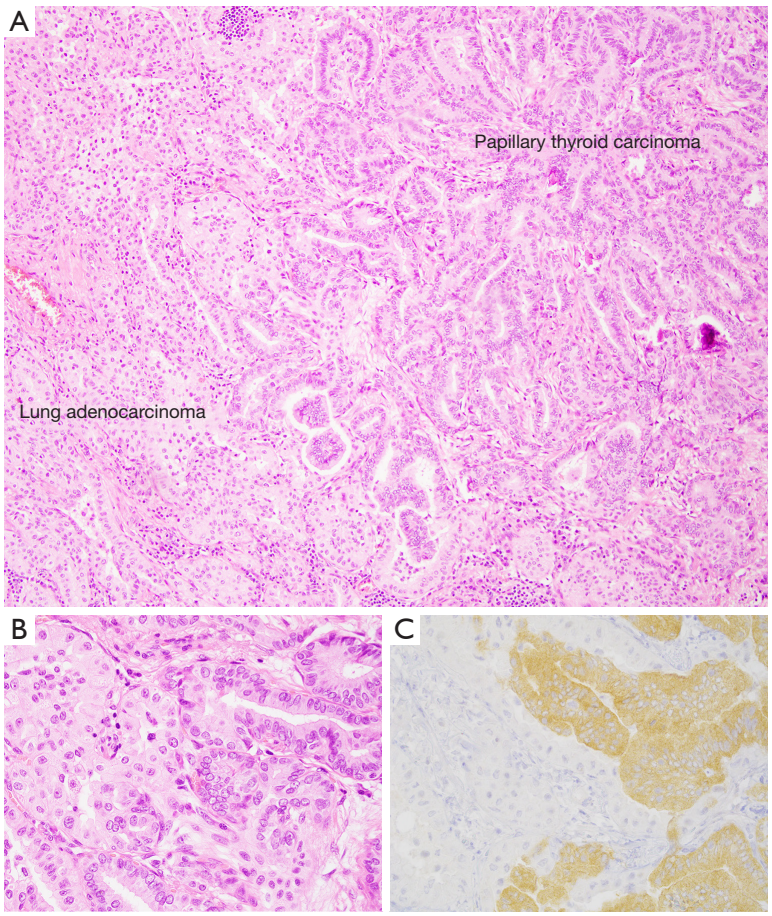

Figure 3 A "collision tumor" comprised of a primary lung adenocarcinoma growing in solid nests and acinar structures, as well as intermingled metastatic papillary thyroid carcinoma (A; HE, ×100); higher power magnification (B; HE, ×400) highlights the similar architecture but distinct cytology; BRAF V600E mutation specific immunohistochemistry (clone VE1) highlights the papillary thyroid carcinoma but is negative in the lung adenocarcinoma (C; ×400).

Mutations occurring at the V600 codon other than V600E are exceptionally rare in NSCLC; therefore, the clinical false negativity issues that impact melanoma testing are not relevant in this tumor type. Given the rarity of *BRAF* V600E mutation in NSCLC, earlier studies had only small numbers of true positive cases (115,116), so despite relatively robust performance characteristics, the data was limited and this testing approach was generally discouraged by clinical testing guidelines (117). A number of more recent studies using larger molecularly defined cohorts have confirmed the utility of VE1 testing in NSCLC, with sensitivities ranging from 96.6–100% and specificities ranging from 98.6–100%. Thus, it may be expected that VE1 IHC can be implemented into routine IHC-based predictive biomarker screening (118,119).

Summary

To summarize, BRAF is a central member of the MAPK signaling pathway, activation of which drives pro-survival and proliferation programs. Critically, activation of BRAF alone appears insufficient to drive malignant tumor behavior, rather, it enables a program of oncogene-driven senescence that manifests as indolent clonal processes such as benign nevi or adenomas arising at various body locations. It appears that a second hit is required for malignant transformation in the setting of a *BRAF* activating event; genes implicated in that transformation step include *CDKN2A*, *PTEN*, and *TP53*. Based on available functional evidence, *BRAF* genomic alterations, which include the well-known point mutations (including at V600), as well as small indels and larger structural variants,

are considered Class 1, 2, or 3, based on their ability to trigger downstream signaling as monomers, dimers, or through RAS dependence. This classification has important implications for the efficacy of BRAF targeted therapies, which appear most potent against those tumors harboring Class 1 mutations.

BRAF is mutated or otherwise altered in tumors arising in essentially every body site in a variety of genomic backgrounds, giving rise to a tremendous diversity of pathologic and clinical neoplastic manifestation. For patients with advanced disease, the efficacy of RAF-targeted therapy is dictated by the context in which the *BRAF* mutation is found, with greatest clinical efficacy coming from combination therapies that target both RAF and other upstream (EGFR) or downstream (MEK/ERK) signals. Given the broad clinical implications for *BRAF* alterations in human neoplasia, accurate and sensitive biomarker testing is essential in practice. An array of testing approaches has been developed and optimized, ranging from targeted PCR-based molecular diagnostics to BRAF V600E mutation-specific IHC to high throughput next generation sequencing. These varied methods all have their strengths and weaknesses, but molecular methods tend to show very similar performance characteristics, irrespective of their status as laboratory tests or FDA-labeled companion diagnostics. Laboratories may choose to employ multiple testing approaches to leverage respective strengths, e.g., turnaround time, sensitivity, or breadth.

Ongoing research into therapies for patients with BRAF-driven malignancies will likely continue to focus on optimizing the combination of targeted inhibitors and will leverage immunotherapy, at least in a subset of BRAF-driven tumors. However, our understanding of the intrinsic and acquired resistance to these therapies is still immature, and in all likelihood analyses of tumors that moves beyond the genome, and into the methylome, proteome, and beyond, will be required to more fully dissect the pathways responsible.

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