Detecting BRAF mutations in a wide array of cancers represents an advance in delivering molecularly targeted therapies to patients with cancer.

**BRAF Mutations: Signaling, Epidemiology, and Clinical Experience in Multiple Malignancies**

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**Background:** Mutations in BRAF were first reported in 2002. Since that time, the molecular basis for oncogenic signaling has been elucidated in multiple malignancies. The development of v-raf murine sarcoma viral oncogene homolog B (BRAF) inhibitors has helped improve clinical outcomes in malignant melanoma and is suggested by case reports in other malignancies.

**Methods:** A review of pertinent articles examining the mechanisms of BRAF signaling in various cancer types and an update on clinical trials of BRAF inhibitions are presented.

**Results:** Clinical response to BRAF inhibition varies by malignancy. In melanoma, single-agent vemurafenib or dabrafenib prolongs overall survival compared with chemotherapy, but both are limited by the development of acquired resistance in many patients. Results of early-phase clinical trials and case reports demonstrate responses in V600E-mutant non–small-cell lung cancer, thyroid cancer, and hairy cell leukemia. However, no significant difference in progression-free survival was seen in colorectal cancer with single-agent vemurafenib. Overcoming resistance to BRAF inhibition with combination therapy is an active area of research.

**Conclusions:** The detection of BRAF mutations represents an advance in delivering molecularly targeted therapies to patients with a variety of cancers. Acquired resistance limits the ability of BRAF inhibitors to produce long-term remissions; however, combining BRAF inhibitors with the mitogen-activated protein kinase pathway and/or other pathway inhibitors represents a promising method to improve long-term outcomes.

**Introduction**

The discovery of mutations in *BRAF*, part of the mitogen-activated protein kinase (MAPK) signaling pathway, heralded a new era of therapeutic options for patients with malignant melanoma, colorectal cancer (CRC), and non–small-cell lung cancer (NSCLC). Additional mutations in *BRAF* have been described in other malignancies as well, including thyroid cancer, hairy cell leukemia (HCL), and multiple myeloma (where they were initially thought to be absent). Significant variation exists in the incidence and epidemiology of *BRAF* mutations across cancers. Mutations in this gene have been found to be universal in...
HCL, in about one-half of patients with melanoma and thyroid cancer, and in about 10\% or less in CRC and NSCLC.\textsuperscript{1,5,6} Although using small molecule inhibitors of v-raf murine sarcoma viral oncogene homolog B (BRAF) in melanoma has produced improved clinical outcomes, their use in CRC has not produced clinical benefit.\textsuperscript{7-11} The V600E mutation results in an amino acid substitution from valine (V) to glutamic acid (E), and it is the most common BRAF mutation detected in human cancer; however, among tumors known to harbor BRAF mutations, lung cancer is notable for a high fraction of non-V600E mutations.\textsuperscript{1,12,13}

In the 11 years since mutations in BRAF were first reported, vemurafenib, dabrafenib, and trametinib have received approval from the US Food and Drug Administration for the treatment of V600-mutated melanoma.\textsuperscript{14,15} This review will examine the current understanding of BRAF cell signaling and will highlight disease-specific epidemiology and clinical experience using BRAF inhibitors across a disparate group of human cancers.

**BRAF Signaling**

Constitutive activation of the MAPK pathway is a common event in many cancers that leads to sustained proliferative signaling.\textsuperscript{16} The MAPK pathway is best defined as the group of kinases comprised of the rapidly accelerated fibroblast (RAF) family of serine/threonine kinases, the MAPK/extracellular-signal-regulated kinase MEK1/2, and terminating with the extracellular signal-regulated kinase (ERK).\textsuperscript{17} Binding of ERK to nuclear protein transcription factors, including the E26 transformation specific (ETS) family, leads to gene expression that promotes cell growth and survival.\textsuperscript{18} In normal conditions, upstream activation of the MAPK pathway occurs most often through ligand binding to receptor tyrosine kinases. For example, binding of the epidermal growth factor family of ligands to the epidermal growth factor receptors (EGFRs) leads to receptor dimerization followed by autophosphorylation and subsequent downstream signaling through both the MAPK pathway and the phosphatidylinositol 3 kinase/protein kinase B/mammalian target of rapamycin (PI3K/Akt/mTOR) pathway.\textsuperscript{19,20} Following receptor dimerization, adaptor proteins undergo phosphorylation that ultimately leads to the activation of the rat sarcoma (RAS) family of GTPases.\textsuperscript{21} Binding of RAS to one of the RAF proteins leads to subsequent downstream MAPK signaling.

The 3 RAS isoforms, HRAS, KRAS, and NRAS, comprise a group of highly conserved GTPases and are the most frequently mutated oncogenes in human cancers.\textsuperscript{22} KRAS mutations are detected in large percentages of CRC, NSCLC, and pancreatic adenocarcinoma, and NRAS is the second most commonly mutated gene in melanoma, occurring in approximately 40\% of cases of BRAF wild-type melanoma.\textsuperscript{22,25} Similar to KRAS, there are 3 RAF isoforms that are serine/threonine kinases, which lead to MEK and ERK phosphorylation when activated via RAS. Under normal conditions, RAS proteins bind to cytosolic RAF dimers, upon which they undergo phosphorylation.\textsuperscript{24} Activated RAF then recruits MEK, ERK, and scaffolding proteins to the cell membrane, thus leading to the phosphorylation of MEK and ERK.\textsuperscript{25,26}

RAF mutations represent another opportunity for malignant cells to sustain MAPK signaling. Mutations in BRAF, first described in 2002,\textsuperscript{1} occur most often at nucleotide 1796, leading to a valine to glutamic acid change at codon 599 (V599E; subsequently renamed to V600E due to a nomenclature change). The V600E mutation leads to a conformational change in the G-loop activation segment of BRAF, rendering it constitutively active and able to bind MEK and ERK as a monomer.\textsuperscript{27} Mutated BRAF results in persistently elevated ERK phosphorylation and target gene transcription. In addition, it is resistant to negative feedback signals that attempt to counterbalance the ERK activation.\textsuperscript{27} The multiple tyrosine kinase signaling pathways within a cell are interconnected and do not exist in isolation. It has been noted that V600E-mutant BRAF activates the mTOR pathway.\textsuperscript{28}

The first report on BRAF mutations described them as being detected in 59\% of melanomas, 18\% of CRCs, 11\% of gliomas, and 4\% of lung adenocarcinomas and ovarian carcinomas.\textsuperscript{1} All mutations occurred in either exon 11 or 15 across all malignancies, and the V600E mutation was the most commonly detected mutation.\textsuperscript{1} In addition to melanoma, CRC, and NSCLC, BRAF mutations have been detected in thyroid cancers, HCL, and multiple myeloma.\textsuperscript{25,56} The wide spectrum of cancers in which BRAF mutations are detected highlights the prominent role MAPK signaling plays in promoting oncogenesis. The following sections will highlight the epidemiology and scientific understanding of BRAF mutations in 5 malignancies: malignant melanoma, CRC, NSCLC, thyroid cancer, and HCL. Clinical experience using small molecule inhibitors to inhibit BRAF signaling are reviewed and summarized in the Table.

**Melanoma**

Malignant melanoma ranks as the fifth and seventh most commonly diagnosed malignancy in men and women, respectively, with an estimated incidence of more than 76,000 persons in the United States in 2013.\textsuperscript{29} Traditionally, metastatic melanoma has carried a dismal prognosis, with 10-year survival rates of less than 10\% as recently as 2009, with a historic 1-year survival rate of 25\%.\textsuperscript{30} However, the discovery of BRAF mutations in melanoma changed the pathologic understanding of the disease, leading to new
treatment options for patients with metastatic disease. Following the discovery of \textit{BRAF} mutations in a large fraction of primary cutaneous melanoma cases, \textit{BRAF} mutations were identified in a similarly high percentage of dysplastic nevi, implicating the \textit{BRAF} mutation as a necessary but insufficient oncogenic driver in early melanoma.\textsuperscript{31} In the metastatic setting, \textit{BRAF} mutations are found in 46\% to 48\% of metastatic biopsy specimens, with \textit{V600E} as the most common mutation (73\%–91\%) followed by \textit{V600K} (7\%–20\%) and, less commonly, \textit{V600D} and \textit{L597R} mutations.\textsuperscript{32–34}

There appear to be differences in mutation type (\textit{V600E} vs non-\textit{V600E}) according to patient age, primary disease site, type of melanoma, and response to \textit{BRAF} inhibition. In a cohort of 302 patients who had melanoma with activating \textit{BRAF} mutations, Bucheit et al\textsuperscript{35} reported a \textit{V600K} mutation rate of 24\%, with statistically significant differences in median age (60.0 vs 44.7 years), male sex, and truncal location compared with patients with the \textit{V600E} mutation. Menzies et al\textsuperscript{34} detected a similar trend between \textit{V600E} and non-\textit{V600E} mutations, with non-\textit{V600E} mutations found in fewer than 20\% of patients younger than 50 years and more than 40\% in patients 70 years of age or older. They also reported a decreasing incidence of \textit{BRAF} mutations by decade of life. A total of 25\% of tumors from patients 70 years of age or older had a mutation, while the tumors of patients younger than 30 years of age almost universally possessed the \textit{BRAF} mutation. \textit{V600E} mutations occur more commonly on intermittently sun-exposed skin and are found most often in superficial spreading melanoma, while non-\textit{V600E} mutations occur more frequently on chronically sun-exposed areas such as the head and neck.\textsuperscript{34,36,37}

Non-\textit{V600E} mutations respond to \textit{BRAF} inhibition in clinical trials; however, retrospective evidence suggests that patients with these mutations have a shorter disease-free interval (defined as the duration of time from primary site diagnosis to metastatic disease) and a trend toward inferior survival rates compared with those who have \textit{V600E}-mutant melanoma.\textsuperscript{7,10,34,35}

Efforts to inhibit mutant \textit{BRAF} in melanoma using small molecule inhibitors began following the characterization of \textit{BRAF} mutations, Based on preclinical data that showed the inhibition of growth among melanoma tumor xenografts, sorafenib entered clinical trials in \textit{BRAF}-mutated melanoma.\textsuperscript{38} Overall, the clinical trial results of sorafenib were disappointing, although initial studies showed some promise in combination with chemotherapy. In a phase 1 trial of sorafenib combined with carboplatin and paclitaxel chemotherapy, patients with melanoma had a longer median progression-free survival (PFS) rate compared with patients who had other tumor types (307 vs 104 days, respectively).\textsuperscript{39} However, a phase 2 trial of sorafenib monotherapy given to 34 patients with stage 4 melanoma revealed a low response rate (2.8\%) and no difference in response rate between \textit{BRAF} mutant and wild type, suggesting that, as single-agent therapy, sorafenib had minimal activity against \textit{BRAF}-mutant melanoma.\textsuperscript{40}

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**Table. — \textit{BRAF} Mutation Prevalence, Clinical Characteristics, and Selected Active Clinical Trials by Cancer Type**

<table>
<thead>
<tr>
<th>Cancer Type</th>
<th>Mutation Frequency and Type</th>
<th>Clinical Characteristics</th>
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| Melanoma             | 46\%–48\%, \textit{V600E} more common than \textit{V600K}; other rare exon 15 mutations reported | \textit{BRAF} \textit{V600E} mutations more common in younger persons and in tumors arising from intermittently sun-exposed skin  
Mutually exclusive with \textit{NRAS} | NCT01726738  
NCT01841463  
NCT01826448  
NCT01616199  
NCT01754376  
NCT01682083 |
| Colorectal           | 7.9\%–15.2\%; predominantly \textit{V600E} | Associated with inferior outcomes compared with \textit{BRAF} wild-type  
Mutually exclusive with \textit{KRAS} and \textit{PIK3CA} | NCT01791309  
NCT01750918  
NCT01793830  
NCT01902173 |
| Thyroid              | 44\% (papillary) and 24\% (anaplastic); predominantly \textit{V600E} | In papillary, associated with increased risk of lymph node invasion and metastasis | NCT01723202  
NCT01709292  
NCT01534897 |
| Non–Small-Cell Lung  | 1.6\%–4.9\% (adenocarcinoma), 4\% (squamous). Approximately equal numbers with \textit{V600E} and non-\textit{V600E} in adenocarcinoma | No difference in clinical outcomes between \textit{V600E} and non-\textit{V600E}  
Non-\textit{V600E} does not respond to \textit{BRAF} inhibitors | NCT01336634  
NCT01514864 |
| Hairy Cell Leukemia  | Approximately 100\%; all \textit{V600E} | Durable complete response reported in 2 patients after short treatment with \textit{BRAF} inhibitors | NCT01711632 |

\textit{BRAF} = \textit{v}-raf murine sarcoma viral oncogene homolog B.
tional, randomized, double-blind, placebo-controlled phase 3 trial of sorafenib or placebo with carboplatin and paclitaxel failed to demonstrate a difference in PFS rate (17.9 weeks with placebo, 17.4 weeks with sorafenib; hazard ratio [HR], 0.91; 99% confidence interval [CI], 0.63–1.31; 2-sided log rank test \( P = .49 \)), though BRAF status was not reported in the baseline characteristics of trial participants.\(^4\) Although additional studies explored combination strategies with sorafenib and either temozolomide or temsirolimus, the development of selective BRAF inhibitors largely supplanted the work to use sorafenib as a therapeutic agent in \(BRAF\)-mutated melanoma.\(^{42-44}\)

Eight years after the discovery and characterization of \(BRAF\) mutations in melanoma, a dose-escalation, extension phase 1 trial of vemurafenib in 81 patients with melanoma was undertaken by Flaherty et al.\(^9\) Of the 32 patients with melanoma who were treated with the recommended phase 2 dose, 24 patients experienced a partial response (PR) and 2 patients had a complete response (CR).\(^9\) In 2011, Chapman et al\(^7\) reported the results of an international, randomized, open-label phase 3 trial comparing vemurafenib with dacarbazine in patients with the V600E mutation. The trial met the prespecified early stopping rule at the time of interim analysis in December 2010, with both overall survival (OS) and PFS rates favoring vemurafenib. Response rates (PR or CR) approached 48% in the vemurafenib-treated cohort compared with 5% in the dacarbazine cohort.\(^7\) Following publication, the US Food and Drug Administration (FDA) approved vemurafenib for the treatment of \(BRAF\)-mutated melanoma, along with a companion diagnostic test, in August 2011.\(^{15}\) Updated OS results from the Chapman et al study were presented in 2012, and showed a continued significant difference in OS rates (13.2 months [95% CI, 12.0–15.0] for vemurafenib and 9.6 months [95% CI, 7.9–11.8] for dacarbazine) and 12-month OS rates of 55% for vemurafenib and 43% for dacarbazine.\(^{45}\)

The BRAF inhibitor dabrafenib has also been studied as a single agent as well as in combination with trametinib, a MEK1/2 inhibitor.\(^{8,10,46}\) In a phase 3 open-labeled, randomized controlled trial comparing dabrafenib with dacarbazine, dabrafenib had significantly improved PFS (5.1 and 2.7 months for dacarbazine; HR, 0.30; 95% CI, 0.18–0.51; \( P < .0001 \)).\(^{10}\)

In addition to the direct inhibition of BRAF, evidence exists to support MEK inhibition in \(BRAF\)-mutant melanoma.\(^{47-49}\) Several clinical trials have examined MEK inhibitors as single agents in \(BRAF\)-mutant melanoma. In a phase 1 study by Falchook et al,\(^{50}\) treatment with trametinib resulted in a 33% overall response rate in patients with melanoma who were treatment naive compared with a 10% response rate in patients with \(BRAF\) wild-type melanoma. A phase 2 trial subsequently compared overall response (CR or PR) in 2 cohorts of patients, ie, those naive to BRAF inhibition (previously treated with chemotherapy or immunotherapy) or patients previously treated with a BRAF inhibitor.\(^{51}\) Interestingly, overall responses were only seen in patients naive to BRAF inhibitors (25% vs 0%), and stable disease was higher in the group naive to BRAF treatment (51% vs. 28%). The results of this trial suggested that the development of acquired resistance following BRAF inhibition also affected response to single-agent MEK inhibition. Therefore, single-agent MEK inhibition following treatment failure by either single-agent BRAF inhibitor is not recommended.

Although the clinical experience involving BRAF and MEK inhibitors as single agents represents a significant advance in the treatment of metastatic melanoma, secondary or acquired resistance to single-agent therapy appears to be universal. Acquired resistance to BRAF inhibitors was predicted by preclinical studies that suggested combination therapies would be needed to treat melanoma.\(^{52-53}\) Unlike the experience derived from the use of small molecule inhibitors in chronic myeloid leukemia, in which progressive disease most often results from kinase domain gatekeeper mutations, Nazarian et al\(^{54}\) reported the absence of acquired mutations in \(BRAF\) among resistant cell lines. Resistance developed through the reactivation of MAPK signaling via upregulated platelet-derived growth factor receptor beta (PDGFR \(\beta\)) and \(NRAS\) mutations in 5 out of 12 patients (mutations in \(NRAS\) were mutually exclusive with increased protein expression of PDGFR \(\beta\)).\(^{54}\) Johannessen et al\(^{55}\) identified a third mechanism by which melanoma expressed \(MAP3K8\) (the gene-encoding cancer Osaka thyroid kinase [COT]/Tpl2), leading to MEK and ERK signaling independent of RAF. In cell-line models, combined RAF and MEK inhibition led to decreased levels of phospho-ERK (p-ERK) and reduced cell growth, suggesting that combined RAF and MEK inhibition may allow cells to circumvent COT-mediated resistance.\(^{55}\) Using serial biopsies obtained as part of a phase 2 clinical study, Trunzer et al\(^{56}\) used immunohistochemistry for p-ERK to demonstrate the reactivation of MAPK signaling at the time of progression. They identified \(NRAS\) mutations in 3 of 13 tumors at progression and \(MEK1\) mutations in 4 of 20 tumors at progression. In all tumors with \(NRAS\) or \(MEK1\) mutations, \(BRAF\) V600E mutation persisted.\(^{56}\)

Combining BRAF inhibitors with MEK inhibitors was a logical next step in the effort to prevent or delay the development of acquired resistance. Work by Paraiso et al\(^{57}\) demonstrated that melanoma cell lines exhibited increased p-ERK signaling prior to the development of BRAF inhibitor resistance, but they also noted that combined treatment with BRAF and MEK inhibitors enhanced apoptosis and prevented the devel-
velopment of resistance in cell lines. In a phase 1/2 study of dabrafenib and trametinib in patients with metastatic melanoma who were naive to BRAF inhibitors, combined treatment with both agents at full doses (dabrafenib 150 mg twice daily and trametinib 2 mg once daily) resulted in improved PFS rates compared with dabrafenib monotherapy (9.4 vs 5.8 months; HR, 0.39; 95% CI, 0.25–0.62; P < .001) and a reduced incidence of cutaneous squamous cell carcinoma and rash in the combination therapy cohort.44 OS data for a phase 2 study comparing dabrafenib alone and dabrafenib plus trametinib were recently reported and showed the OS rate approaching 2 years in the group treated with dabrafenib 150 mg twice daily and trametinib 2 mg daily.58 Two phase 3 trials studying the combination of BRAF and MEK inhibitors have completed accrual, and the final results are anxiously anticipated (NCT01584648, NCT01597908). Contingent upon the successful trial completion and phase 3 study results, in January 2014, the FDA approved combination dabrafenib/trametinib for the treatment of metastatic BRAF-mutant melanoma.44 Based on these results, it is anticipated that combination therapy will become the new standard of care over single-agent BRAF-inhibitor therapy for BRAF-mutant metastatic melanoma.

Wagle et al59 recently reported results from whole exome sequencing and whole transcriptome sequencing of a case series of tumor tissue obtained from 5 patients prior to combined BRAF/MEK treatment and after progression. They detected a MEK2 mutation (MEK2pG609R), a novel BRAF splice variant, and BRAF amplification in tumor tissue at progression in 3 patients but were unable to find a resistance mechanism in 2 patients. MAPK signaling reactivation appears to be a primary driver of clinical resistance to both single-agent therapy and combined BRAF and MEK inhibitor in BRAF-mutated melanoma.54-56,59 The study by Wagle et al59 highlights the importance of obtaining paired biopsy samples from patients enrolled in clinical trials prior to treatment and at the time of disease progression to elucidate mechanisms of acquired resistance and develop more effective therapeutic strategies.

Current clinical trials in BRAF-mutant melanoma study BRAF inhibitors in combination with novel agents and in the adjuvant setting. LCCC 1128 is an open-label phase 2 study of dabrafenib and trametinib that will evaluate tumor tissue of patients with stage 3 or 4 BRAF-mutant melanoma at study entry and at the time of progression to study mechanisms of acquired resistance (NCT01726738). Vemurafenib is the subject of combination trials with P1446A-05, an oral cyclindependent kinase inhibitor (NCT01841463), PLX3397, and oral multikinase inhibitor (NCT01826448), PX-866, an irreversible PI3K inhibitor (NCT01616199), XL888, a HSP90 inhibitor (NCT01657591) and interleukin-2 in patients with metastatic disease who are naive to BRAF-targeted therapy (NCT01754376). A placebo-controlled, randomized, double-blind study comparing dabrafenib and trametinib versus 2 placebos in patients with surgically resected, high-risk, BRAF V600 mutation-positive melanoma is ongoing to study the role of dual BRAF/MEK inhibition in the adjuvant setting (NCT01682083).

Colorectal Adenocarcinoma
Colorectal adenocarcinoma is the third most commonly diagnosed malignancy in both men and women and led to approximately 50,830 deaths in the United States in 2013.29 Mutations in KRAS were discovered in 1983 and are used as a biomarker to predict response to anti-EGFR monoclonal antibodies in patients with metastatic CRC.60,61 Although KRAS mutations are commonly observed in CRC with a frequency approaching 40%, additional mutations in BRAF, PIK3CA, and PTEN have been described.62 Knowledge of the mutational background of CRC has generated significant interest in developing combinatorial therapeutics to target the MAPK pathway.

Notable differences exist in the clinical behavior and pathogenesis of BRAF-mutant CRC when compared with melanoma and other cancers that harbor BRAF mutations. Most cases of colon cancers arise from chromosomal instability and aneuploidy; however, 15% of CRCs develop in the setting of microsatellite instability (MSI), leading to the accumulation of base pair substitutions, frameshift mutations, and small deletions.63 Hereditary nonpolyposis colorectal cancer (HNPPC) is a predisposition syndrome responsible for 3% of CRC and is associated with defective mismatch repair (MMR) machinery leading to MSI.64,65 However, most tumors with MSI arise sporadically and are not associated with HNPPC.64 Such tumors frequently arise from hypermethylation of CpG-rich regions within the promoter region of the MLH1 gene, leading to the CpG island methylator phenotype (CIMP).66 Rajagopalan et al67 were the first to link BRAF status and defective MMR in CRC based on their analysis of 330 cases of CRC. Two years later, Kambara et al68 reported a significant association between BRAF mutations in CIMP-high (20 of 26; 77%), CIMP-low (8 of 44; 18%), and CIMP-negative (0 of 34; 0%) CRCs (P < .0001), as well as between BRAF status and sporadic MSI-high cancers (16 of 17 CIMP-high harbored the V600E mutation compared with 5 of 9 CIMP-low and 0 of 2 CIMP negative; P = .004). Using a novel technique to detect CIMP-positive tumors, Weisenberger et al69 described a highly significant association between CIMP positivity and BRAF mutation and MLH1 methylation. Thus, tumors arising from MSI without genetic predisposition were shown to be highly associated with CIMP and BRAF mutation, defining a unique subset of CRC.
Mutations in *BRAF* were first reported in CRC tumors by Yuen et al\(^70\) with an observed incidence of 5.1% in adenocarcinoma tissue. A population-based study detected *BRAF* mutations in 78 of 513 CRC tumors (15.2%), and studies of *BRAF* mutations in patients with metastatic disease report an incidence of 7.9% to 8.7%.\(^71-73\) Similar to thyroid cancer and melanoma, yet unlike lung adenocarcinoma, *BRAF* mutations almost exclusively affect codon 600.\(^70-72\) In multiple studies *BRAF* mutations are mutually exclusive to *KRAS* or *PIK3CA* mutations.\(^72,74,75\) A significant body of literature supports the observation that *BRAF*-mutated CRC has a more aggressive clinical behavior than *BRAF* wild-type CRC. Early evidence by Ogino et al\(^76\) reported that mutations in this gene were associated with an increased cancer-specific mortality rate (multivariate HR = 1.97; 95% CI, 1.13–3.42) among patients with stages 1 to 4 CRC. *BRAF*-mutant CRC was associated with inferior OS rates among patients with stage 3 resected CRC treated in a large, prospective adjuvant chemotherapy trial, and a meta-analysis of 26 CRC studies revealed a significantly increased risk of overall mortality among patients with a *BRAF* mutation (HR = 2.25; 95% CI, 1.37–2.12).\(^77,78\) Studies have also documented inferior PFS rates in *BRAF*-mutant CRC treated with anti-EGFR antibodies.\(^79,80\)

Clinical experience with *BRAF* inhibition in CRC suggests significant differences in response compared with melanoma. Kopetz et al\(^11\) reported results of vemurafenib in a phase 1 study of 21 patients with metastatic CRC, with 1 confirmed PR out of 19 evalable patients. Several studies have subsequently examined the etiology of intrinsic (primary) resistance to *BRAF* inhibition in patients with CRC. By comparing *BRAF*-mutant CRC and melanoma cell lines, Mao et al\(^81\) reported increased levels of PI3K/Akt activation and lower levels of MEK pathway activation. Inhibition of both the BRAF and PI3K pathways resulted in synergistic growth inhibition in CRC cell lines. The authors also reported that the use of 5-azacytidine (a hypomethylating agent) reduced phospho-Akt expression and produced greater growth inhibition in all CRC cell lines when combined with the BRAF inhibitor vemurafenib than with the BRAF inhibitor alone.\(^81\) Prahallad et al\(^82\) recently demonstrated that *BRAF*-mutant CRC cell lines treated with vemurafenib experienced EGFR feedback activation, and a combined inhibition of EGFR using either cetuximab or gefitinib (a small molecule EGFR inhibitor) with BRAF inhibition produced synergistic growth inhibition in both cell lines and mouse xenograft models.

Based on clinical experience using single-agent BRAF inhibitors in CRC and recent preclinical data, combinatorial therapeutic strategies will be necessary to improve outcomes in patients with *BRAF*-mutant CRC. The Figure summarizes a selection of ongoing efforts to combine pathway inhibitors in CRC as well as melanoma, NSCLC, and thyroid cancer. In CRC, clinical trials targeting both BRAF and EGFR are actively recruiting patients, including a pilot study of vemurafenib and panitumumab (NCT01791309) and an open label, 3-part, phase 1/2 study combining dabrafenib with or without trametinib with panitumumab (NCT01750918). Two combination trials studying dual PI3K pathway and BRAF inhibition are ongoing, including a phase 1/2 study of the BRAF inhibitor encorafenib with or without the PI3K inhibitor BYL719 plus panitumumab (NCT01719380) and a phase 1/2

![Image](image-url)
study combining dabrafenib with the Akt inhibitor GSK214795 (NCT01902173).

Differentiated Thyroid Cancer

More than 60,000 new cases of thyroid malignancies were diagnosed in 2013, many of which were differentiated thyroid tumors in women.29 Thyroid malignancies are commonly categorized according to their aggressiveness. Well-differentiated tumors (papillary and follicular) comprise the majority of new cases each year and have the least aggressive clinical behavior. Intermediate tumors (medullary thyroid carcinoma, Hürthle cell, and poorly differentiated) and undifferentiated tumors (anaplastic thyroid carcinoma [ATC]) make up 10% of newly diagnosed cases each year but have more aggressive clinical behavior.83

Evidence for BRAF mutations in differentiated thyroid cancers was first reported by Kimura et al5 who noted that 28 of their 78 studied patients (35.8%) with papillary thyroid cancer (PTC) possessed the BRAF V600E mutation independent of mutations in RET and RAS. Notably, no BRAF mutations were detected in small cohorts of follicular or Hürthle cell carcinomas.5 Nikifirova et al84 were the first to report that BRAF mutations were restricted to patients with PTC or with poorly differentiated or undifferentiated thyroid cancers arising from previous PTC. A recent compilation of data from 29 studies reported BRAF V600E mutations in 44% of patients with PTC and 24% with ATC.85 Similar to melanoma, the majority of BRAF mutations in PTC or ATC occurs at codon 600, although rare mutations in codons near codon 600 have been described.86 In addition to characterizing BRAF mutations, additional work has demonstrated that genetic alterations in the PI3K/Akt pathway are common in thyroid malignancies, occur with increasing frequency in more aggressive tumors, and are mutually exclusive.87,88

BRAF V600E mutation predicts for a more aggressive clinical course in patients with PTC.89-91 Using multivariate analysis, BRAF mutations in PTC are associated with an increased risk of lymph node invasion and metastasis as well as a more advanced stage of disease at initial surgery.85 In addition, BRAF mutations render tumors less responsive to repeat radioactive iodine treatment in the event of recurrent disease.85,92,93 A large retrospective study consisting of 1,849 patients with PTC found an increased overall mortality rate among patients with the V600E mutation (12.87 deaths per 1,000 person-years; 95% CI, 9.61–17.24) compared with patients not carrying the mutation (2.52 deaths per 1,000 person-years; 95% CI, 1.40–4.55) with an HR of 2.66 (95% CI, 1.30–5.43) after adjusting for age at diagnosis, sex, and medical center.94 However, in the same study, when additional factors associated with worse prognosis, including lymph node metastasis, extrathyroid invasion, and distant metastasis, were included in the model, BRAF mutations were no longer associated with increased mortality.

Preclinical work in thyroid cancer cell lines using MEK1/2 and BRAF inhibitors, along with clinical experience using these drugs in melanoma, has led to clinical trials in patients with thyroid cancer targeting the MAPK and PI3K/Akt pathways.95-97 In a phase 2 study of sorafenib for metastatic thyroid cancer, 6 out of 41 patients with PTC achieved a PR with a median duration of 7.5 months, while none of the 17 patients with a different type of thyroid cancer achieved a PR.98 Among patients with PTC and V600E mutation, 3 out of 9 evaluable patients participating in a phase 1, dose-escalation trial of dabrafenib experienced a PR.8 One case reported highlighted a rapid clinical response to treatment with vemurafenib in a patient with ATC found to have V600E mutation.99 Active clinical trials in thyroid cancers include dabrafenib with or without the MEK inhibitor trametinib (NCT01723202), neoadjuvant vemurafenib in patients with locally advanced thyroid cancer (NCT01709292), and the use of the BRAF inhibitor dabrafenib to resensitize patients with BRAF-mutated thyroid cancer to radioactive iodine (NCT01534897).

Non–Small-Cell Lung Cancer

Lung cancer remains the leading cause of annual cancer-related mortality in the United States.29 Among patients with lung adenocarcinoma, driver mutations have been identified in 62% of patients undergoing testing for at least 1 genomic alteration.100 Mutations in BRAF arise in 1.6% to 4.9% of adenocarcinomas.1,12,13,101,102 Unlike melanoma, PTC, and HCL in which most BRAF mutations are V600E, mutations in lung adenocarcinomas can be separated into either V600E (50%–56.8%) and non-V600E (43.2%–50%).12,13 Although V600E mutations directly phosphorylate MEK, mutations in exon 11 (non-V600E) possess impaired kinase activity and are considered to be mutually exclusive with common driver mutations in NSCLC such as EGFR, KRAS, and the EML4-ALK rearrangement.103 However, one recent analysis detected 1 out of 18 patients with a coexisting V600E mutation and PIK3CA E545K mutation and 2 out of 18 patients with non-V600E mutation and coexisting KRAS mutation.12 In addition to lung adenocarcinoma, recent data from the Cancer Genome Atlas Research Network105 revealed a 4% mutation rate in squamous cell carcinoma of the lung.

Clinical predictors of BRAF mutations in lung adenocarcinomas are controversial. Paik et al104 reported a statistically significant association between the presence of BRAF mutations and current or previous smoking status, while a larger study by Cardarella et al12
reported no significant association between smoking status and BRAF mutations. No difference in PFS was seen after first-line platinum doublet chemotherapy between patients with V600E mutation compared with patients without a driver mutation (EGFR, KRAS, and BRAF wild-type and EML4-ALK nontranslocated). In addition, no difference in PFS was detected between patients with V600E and non-V600E mutations. Nonetheless, differences in response to BRAF inhibition with vemurafenib have been reported based on V600E mutation status (objective response in 1 patient with V600E mutation and primary progressive disease in a patient with non-V600E mutation). Two patients with NSCLC and V600E mutation have also experienced treatment response to dabrafenib, yet another patient with Y472C BRAF mutation experienced a prolonged remission after treatment with dasatinib.

Another mechanism to inhibit downstream activation of ERK in patients with both V600E and non-V600E mutations is through MEK inhibition, which was the subject of a completed phase 1 study using selumetinib, a MEK1/2 inhibitor, in nonmelanoma, BRAF-mutated solid tumors (NCT00888134). Active clinical trials in patients with NSCLC and both V600E and non-V600E mutant BRAF are ongoing. Dabrafenib is the subject of an ongoing international phase 2 trial in V600E-mutant NSCLC (NCT01356634). Interim results were recently presented and revealed an overall response rate of 54% (7 PRs of 13 patients evaluable for response). Dasatinib is currently the subject of an ongoing phase 2 trial in patients with non-V600E inactivating BRAF mutations (NCT01514864).

**Hairy Cell Leukemia**

HCL is a rare clonal disorder of B cells characterized by progressive splenomegaly, pancytopenia, and the absence of peripheral lymphadenopathy. Its annual incidence is estimated to be 3.3 persons per 1 million person-years in the United States. The underlying genomic etiology of HCL remained elusive until a landmark paper by Tiacci et al. Another mechanism to inhibit downstream activation of ERK in patients with both V600E and non-V600E mutations is through MEK inhibition, which was the subject of a completed phase 1 study using selumetinib, a MEK1/2 inhibitor, in nonmelanoma, BRAF-mutated solid tumors (NCT00888134). Active clinical trials in patients with NSCLC and both V600E and non-V600E mutant BRAF are ongoing. Dabrafenib is the subject of an ongoing international phase 2 trial in V600E-mutant NSCLC (NCT01356634). Interim results were recently presented and revealed an overall response rate of 54% (7 PRs of 13 patients evaluable for response). Dasatinib is currently the subject of an ongoing phase 2 trial in patients with non-V600E inactivating BRAF mutations (NCT01514864).

When patients require treatment for HCL, a single course of continuous infusion cladribine over 7 days induces CRs in more than 90% of patients with an OS rate of 96% at 48 months. After 7 years of follow-up in the same cohort of patients, the median duration of first response was 98 months, with 37% of patients experiencing relapsed disease. In patients with relapsed disease, re-treatment with cladribine induced complete remission in the majority of patients. Thus, while cladribine is a highly effective treatment for HCL, eventual relapse is common. After the discovery of the V600E mutation, Dietrich et al. offered off-label vemurafenib to a patient with refractory HCL, massive splenomegaly, and cytopenias. The patient was treated for 56 days after dose escalation to a maximum of 1440 mg/day and experienced significant reduction in spleen size (24.8 × 8.3 cm pretreatment to 14 × 5 cm at day 16) and hematological CR at 43 days. Six months after completing vemurafenib treatment, the patient remained in complete remission. Another patient with refractory HCL was treated with vemurafenib 960 mg orally twice daily for 3 days and was recently reported to have a normalization of platelets, white blood cells, and neutrophils at 5 weeks; this normalization persisted for 4 months after treatment discontinuation. The optimal dosing and duration of treatment using BRAF inhibitors is unknown for patients with treatment refractory HCL; however, a multicenter, phase 2 study of vemurafenib in treatment refractory HCL is ongoing (NCT01711632).

**Conclusion**

The characterization and discovery of BRAF mutations in both epithelial and hematological malignancies illustrates the promise of personalized medicine in oncology. The detection of BRAF mutations in a variety of malignancies is still ongoing, and it is leading to rapid drug development across a wide range of cancers. Although we have seen significant improvements in patient outcomes within the last 4 years, particularly in melanoma, more research is needed to understand the mechanisms of intrinsic and acquired resistance in BRAF inhibitors. Applying whole exome sequencing and improving the capabilities of bioinformatics will contribute to continued gains in the development of combinatorial therapeutic strategies against mutated BRAF and the effects of its downstream signaling.

**References**