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BRAF inhibitors in clinical oncology

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Abstract

Activating mutations of the BRAF oncogene are present in approximately 5-10% of all human malignancies and lead to constitutive activation of the mitogen activated protein kinase (MAPK) pathway. The introduction of BRAF inhibitors has greatly improved the short term prospects of some patients with these tumors, but the tumors tend to become resistant to therapy with time by activating alternative signaling pathways. Consequently, combination strategies with drugs that block not only the primary mutated BRAF kinase but also the alternative pathways implicated in development of resistance may represent a better strategy for improving survival in patients with tumors harboring BRAF mutations.

Introduction

The identification of mutations that drive signaling pathways critical to tumor growth and survival has led to a new taxonomy in oncology in which cancers are classified according to the molecular aberration present. One pathway of particular interest is the MAPK pathway, which is estimated to be dysregulated in approximately 50% of all human malignancies [1,2]. RAF is one kinase along this signaling cascade that, once activated, phosphorylates MEK [3,4], which in turn triggers activation of MAPK and leads to downstream promotion of cell growth [5].

Activating mutations of the BRAF oncogene are present in approximately 5-10% of all human malignancies [2] and lead to constitutive activation of the MAPK pathway [6,7]. The most common mutation of BRAF is a valine-to-glutamic acid substitution at codon 600 (V600E), which has been implicated as the driving factor in subpopulations of patients with melanoma, colorectal cancer, papillary thyroid cancer, non-small cell lung cancer (NSCLC), and ovarian cancer [2,8-16]. The introduction of inhibitors of activated BRAF represents an important therapeutic approach in recent oncological care. This review will discuss the successes and early challenges of BRAF inhibitors in clinical oncology.

BRAF inhibitors in melanoma

BRAF V600E mutations are found in approximately 50% of all cutaneous melanomas [2,17]. Understanding the importance of this mutation in the oncologic behavior of melanoma has led to the development of vemurafenib, an inhibitor of the kinase domain of mutated BRAF that, *in vitro*, blocks signaling of the MAPK pathway and decreases melanoma cell proliferation [18-20]. A randomized phase III trial that compared vemurafenib to dacarbazine, then a standard first-line treatment for stage IV disease, in 675 patients with previously untreated metastatic melanoma showed an improvement in overall survival rate at 6 months (84% versus 64%) for those given the former therapy [21]. Patients receiving the BRAF inhibitor were 63% more likely to be alive at the time of interim analysis (hazard ratio 0.37, 95% confidence interval 0.26-0.55, $p < 0.001$), with an associated improvement in median progression-free survival from 1.6 months to 5.3 months. The development of keratoacanthomas and cutaneous squamous cell carcinomas, the most common grade III toxicity occurring in approximately 25% of trial participants, appeared within 2-3 months of treatment initiation, but can be readily excised without the need for dose modification of vemurafenib. This side effect is thought to be caused by the paradoxical activation of wildtype RAF

kinases to BRAF inhibitor therapy [22]. More recently, results were published of another phase III trial comparing the BRAF inhibitor dabrafenib (a newer-generation, reversible kinase inhibitor of V600E-mutant BRAF with a higher affinity than the wildtype enzyme for mutant BRAF) to dacarbazine [22]. Here again, progression-free survival was increased in the arm receiving the BRAF inhibitor (5.1 months versus 2.7 months, respectively, $p < 0.001$), but grade III toxicities were likewise uncommon in this group, at only 4%.

Despite the initial successes of vemurafenib, tumors inevitably develop resistance and circumvent BRAF inhibition, a theme unfortunately common with the use of single-agent targeted therapeutic agents. Resistance to vemurafenib seemingly occurs because of alternative activation of the MAPK pathway despite BRAF inhibition, and multiple mechanisms driving activation of this signaling pathway have been described [23-26]. To that end, given the oncologic 'addiction' of melanoma cells to this pathway [27], a phase I/II trial of dabrafenib and trametinib (a selective MEK inhibitor) was recently completed in previously untreated patients with metastatic melanoma [28]. Here, those receiving the combination of both drugs showed a 3.6 month improvement in progression-free survival relative to those treated with dabrafenib alone (9.4 months versus 5.8 months, $p < 0.001$), a finding suggesting that dual blockade at multiple steps of this pathway may delay the onset of resistance. Phase III trials are currently ongoing, and it will be interesting to see whether or not dual inhibitor therapy will replace BRAF inhibitor monotherapy as the standard of care for patients with BRAF-mutant metastatic melanoma.

BRAF inhibitors in colorectal cancer

Approximately 10% of all patients with colorectal cancer have tumors with an activating BRAF mutation, with the V600E mutation being the most common. BRAF-mutant tumors in colorectal cancer are associated with older age, female gender, right-sided primary colon tumors, gene hypermethylation, and microsatellite instability [29,30]. Responses to systemic chemotherapy are considered poor in BRAF-mutant tumors [31-34], and so it is not surprising that overall survival is especially grim in this subpopulation [35,36], with one recent retrospective review reporting a 10.4 months versus 34.7 month overall survival among patients with BRAF-mutant and BRAF wildtype metastatic colorectal cancer, respectively [37].

A phase IB study of vemurafenib reported a response rate in only 5% (one partial response, no complete responses) among twenty patients with metastatic colorectal cancer evaluated for radiographic response. Median progression-free survival was only 3.7 months in this study [38]. Several patients did demonstrate a mixed response pattern in their various tumor sites, suggesting that BRAF inhibitors may serve as the backbone for additional therapy as more of the underlying biology of this disease is uncovered. Likewise, the prospect of translating the successes of concomitant BRAF/MEK inhibition noted in the BRAF-mutant metastatic melanoma seems less promising for colorectal cancer. Initial data presented at the 2012 ASCO Annual Convention reported a response rate of only 5% (1 partial response among 20 patients evaluated, 10 with stable disease) in patients with BRAF-mutated metastatic colorectal cancer treated with dabrafenib and trametinib together [39].

Interestingly, the reason for resistance to therapy in BRAF-mutated colorectal cancer appears to be continued activation of critical signaling pathways. Two groups independently reported that blockade of BRAF causes rapid feedback activation of epidermal growth factor receptor (EGFR) [40,41], which, upon phosphorylation triggers sustained MAPK signaling and cell proliferation via activation of RAS and CRAF. *In vitro*, blocking EGFR activity with cetuximab, a monoclonal antibody to EGFR, restores sensitivity to vemurafenib. Clinical trials of the combination of vemurafenib and cetuximab in metastatic BRAF-mutated colorectal cancer are currently underway. Additionally, resistance to BRAF inhibition may also develop by activation of other pathways. Recently, our group has shown that, in cell lines, colorectal cancer demonstrates higher levels of phosphatidylinositol-3 kinase (PI3K)/Akt signaling (implicated in anti-apoptotic behavior) than melanoma and that BRAF-mutated colorectal cells display less sensitivity *in vitro* to vemurafenib when concomitant PTEN or PI3K mutations are present [42]. In mice xenografts, treatment with vemurafenib and a PI3K inhibitor lead to greater inhibition of tumor growth than vemurafenib alone, a finding suggesting that targeting compensatory pathways may provide an improved approach to treating patients whose tumors are driven by activating BRAF mutations.

BRAF mutations in papillary thyroid cancer

Activating BRAF mutations occur in approximately 45-50% of all papillary thyroid cancers [43] and are associated with extrathyroidal extension, lymph node metastases, and an overall poorer prognosis than BRAF wildtype tumors [44-46]. However, even though the seminal phase 1 study with vemurafenib showed a complete or partial response in all three patients with papillary thyroid cancer [47], to date, no clinical trials have been reported detailing the effects of BRAF inhibitor

therapies on patients specifically with BRAF-mutant papillary thyroid tumors. Phase II trials are currently enrolling patients with metastatic papillary thyroid cancer for treatment with vemurafenib, and with locally advanced disease using vemurafenib as a neoadjuvant approach with which to improve surgical resectability.

BRAF mutations in non-small cell lung cancer

BRAF mutations have been reported to occur in 3% of lung adenocarcinomas [16,48]. Essentially all patients harboring these mutations are active or former tobacco smokers [48-50]. Even though V600E substitutions are the most common among BRAF mutations, one series reported a 39% prevalence of G469A substitutions in lung cancer [48]. Whether or not V600E-specific kinase inhibitors will exhibit clinical improvements in patients with a spectrum of different BRAF point mutations has yet to be determined, although clinical trials are currently underway (see Table 1 below).

Inhibitors in clinical development

Following the results of the aforementioned phase III trial, vemurafenib was approved by the U.S. Food and Drug Administration in 2011 for use in patients with BRAF-mutant metastatic melanoma. The use of vemurafenib outside this context is otherwise limited to participants in clinical trials, although several trials investigating other BRAF inhibitors have recently been completed (results pending) or are actively accruing enrollment.

Future directions

Although the significance of an activating BRAF mutation is now well appreciated in clinical oncology, treating these tumors with targeted therapies has proven a challenge. Regardless of initial response to BRAF inhibitors, tumors eventually develop resistance to these agents, due to activation of various alternative signaling pathways that perpetuate cell proliferation and survival. One recent commentary proposed that the onset of drug resistance in tumors may be delayed with multi-drug regimens targeting multiple oncogenic substrates, akin to the approach seen in four-drug therapies for tuberculosis and in antiretroviral therapy with HIV [51]. Indeed, using combination strategies with drugs that block not only the primary mutated BRAF kinase but also the pathways most commonly implicated in development of resistance may represent a better strategy for improving survival outcomes in patients with tumors harboring BRAF mutations.

Table 1. BRAF inhibitors in development

BRAF inhibitor	Company	Phase	Patients included	Study description
BMS-908662 [52]	Bristol-Myers Squibb	I/II trial completed	Advanced or metastatic colorectal cancer with BRAF or KRAS mutations	To identify the maximum tolerated dose of BMS-908662 in combination with cetuximab; to evaluate tumor response with the BRAF inhibitor alone or in combination with cetuximab
LGX818 [53]	Novartis	I/II	Advanced solid tumors with V600 BRAF mutations	To determine the maximum tolerated dose and the efficacy of LGX818 in combination with a MEK inhibitor
PLX3603 [54]	Hofmann-LaRoche	I	Advanced solid tumors with BRAF mutations	To evaluate the safety, tolerability, and pharmacokinetics of PLX3603
RAF265 [55]	Novartis	Ib	1. Advanced solid tumors with BRAF V600 mutations 2. Advanced solid tumors with RAS mutations	To determine the maximum tolerated dose safety and tolerability of RAF265 in combination with the MEK inhibitor MEK162; to determine initial anti-tumor efficacy in two separate patient populations
RO5185426 [56]	Hofmann-LaRoche	II	Unresectable or metastatic papillary thyroid cancer harboring a BRAF mutation and resistant to radioactive iodine therapy	To study the safety and efficacy of RO5185426 as a single agent therapy
GSK2118436 [57]	GlaxoSmithKline	II	Advanced stage/metastatic NSCLC with a V600E BRAF mutation that progressed after platinum chemotherapy	To assess the efficacy, safety, and tolerability of GSK2118436 as a single-agent therapy

Abbreviations

EGFR, epidermal growth factor receptor; MAPK, mitogen-activated protein kinase; NSCLC, non-small cell lung cancers; PI3K, phosphatidylinositol-3 kinase.

Disclosures

The authors declare that they have no disclosures.

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