Molecular Pathways: Response and Resistance to BRAF and MEK Inhibitors in BRAF\textsuperscript{V600E} Tumors

Meghna Das Thakur and Darrin D. Stuart

Abstract

The RAS–RAF–MEK (MAP–ERK kinase)–ERK (extracellular signal–regulated kinase) pathway plays a central role in driving proliferation, survival, and metastasis signals in tumor cells, and the prevalence of oncogenic mutations in RAS and BRAF and upstream nodes makes this pathway the focus of significant oncology drug development. This focus has been justified by the recent success of BRAF and MEK inhibitors in prolonging the lives of patients with BRAF\textsuperscript{V600E/K}-mutant melanoma. Although it is disappointing that cures are relatively rare, this should not detract from the value of these agents to patients with cancer and the opportunity they provide in allowing us to gain a deeper understanding of drug response and resistance. These insights have already provided the basis for the evaluation of alternative dosing regimens and combination therapies in patients with melanoma. Clin Cancer Res; 20(5); 1074–80. ©2013 AACR.

Background

The RAS–RAF–MEK (MAP–ERK kinase)–ERK (extracellular signal–regulated kinase) pathway controls cell growth, differentiation, survival, and migration in normal tissues. Signals from cell surface receptors are transmitted through RAS–GTP to the RAF–MEK–ERK kinase cascade to intracellular substrates in the cytoplasm and nucleus. The RAF family of serine/threonine kinases consists of three family members: ARAF, BRAF, and CRAF. All three family members share similar structural motifs, with two conserved regions in the N-terminus (CR1 and CR2) and one in the C-terminus, which encodes the kinase domain (CR3). Although ARAF and CRAF are ubiquitously expressed, BRAF is expressed in approximately 50% of human melanomas, through oncogenic mutations such as BRAF\textsuperscript{V600E}, which is expressed in approximately 50% of human melanomas, 33% to 60% of thyroid tumors, and a lower proportion of

and, unlike the RAF and MEK kinases, have a wide range of cytoplasmic and nuclear substrates (reviewed in ref. 5). The scope of physiologic responses to RAF–MEK–ERK pathway activation is partly dictated by the magnitude and the duration of the signal. For example, pathway stimulation by different growth factor receptors leads to differences in the kinetics of pathway activation, which result in differences in phenotypic response (e.g., proliferation vs. differentiation; ref. 6). Furthermore, the proliferative response to RAF activation has been shown to follow a bell-shaped, rather than sigmoidal dose–response curve, with moderate levels of BRAF or CRAF activation inducing proliferation and high levels of RAF activation associated with a lack of induction of proliferation (7). These effects may not be shared equally between the two ERK isoforms as phenotypic differences have been observed in response to ERK1 versus ERK2 activation (8, 9). Therefore, fine-tuning of the RAF–MEK–ERK is achieved, at least partially by the architecture of the pathway with three modules and multiple isoforms existing at each node.

Sturm and colleagues suggest that the pathway architecture enables high signaling rates and amplification, with the negative loops providing rich dynamic properties such as oscillations and switch-like properties similar to a negative feedback amplifier (10). Negative feedback occurs at multiple levels and the transcriptional output of the pathway includes the dual-specificity phosphatase (DUSP) family of ERK phosphatases as well as the SPRY proteins that are negative regulators of RAS signaling. These qualities dictate the response to stimuli and this model provides a basis for understanding signaling in the context of normal physiologic processes, as well as in cancer in which the pathway is often dysregulated through oncogenic mutations such as BRAF\textsuperscript{V600E}, which is expressed in approximately 50% of human melanomas, 33% to 60% of thyroid tumors, and a lower proportion of
ovarian, colorectal, and lung carcinomas (11). Figure 1A depicts a simplified model in which RAF–MEK–ERK signaling in BRAFV600E cells functions independent of upstream RAS activation and is not subject to regulation by feedback that occurs at nodes upstream (12). In such a model cells expressing BRAFV600E rely on the DUSP family of ERK phosphatases to regulate phospho-ERK levels.

Clinical–Translational Advances

Allosteric MEK inhibitors represent the first pharmacologic inhibitors of the RAS–RAF–MEK–ERK pathway, with CI-1040 being the first to be tested in human clinical trials (13). Although the human efficacy of CI-1040 was likely limited due to poor drug-like properties and a lack of potency, this inhibitor provided preclinical proof-of-
concept that targeting MEK can result in antitumor activity in preclinical models (14). This work led to the development of more potent allosteric inhibitors with improved properties, such as PD0325901 and ARRY142886/ AZD6244, as well as a host of others that have demonstrated therapeutic efficacy in human clinical trials (reviewed in ref. 15). The most advanced MEK inhibitor, trametinib, was recently approved for the treatment of metastatic melanoma expressing BRAFV600E/K in a phase III trial in patients with metastatic melanoma whose tumors express BRAFV600E/K trametinib treatment resulted in a 22% response rate and 4.8 months median progression-free survival, which compared favorably with chemotherapy (8% and 1.5 months; ref. 16).

The discovery that mutant BRAF is an oncogenic driver led to a significant investment in the development of BRAF kinase inhibitors. SB590885 was the first RAF inhibitor to demonstrate preferential inhibition of the RAF–MEK–ERK pathway in tumor cells expression BRAFV600E and this profile was suggested to provide a "genetic therapeutic index" (17). PLX4720 also demonstrated differential pharmacologic of BRAFV600E/K tumors versus BRAF wild-type tumors in vivo and the structural analog, vemurafenib (PLX4032), was approved in 2011 for the treatment of patients with metastatic melanoma whose tumors express the BRAFV600E/K mutation (18, 19). In a phase III trial in patients with metastatic melanoma whose tumors express BRAFV600E/K, vemurafenib treatment resulted in a 48% response rate and 5.3 months median progression-free survival, which compared favorably with dacarbazine (5% and 1.6 months; ref. 20). Other selective RAF inhibitors are at various stages of clinical development such as dabrafenib (approved by the U.S. Food and Drug Administration in 2013; ref. 21) and LGX818 (phase III; ref. 22). A model for the pharmacologic inhibition of BRAFV600E is depicted in Fig. 1B, in which the pathway is effectively silenced by a BRAF inhibitor. It is important to note that inhibition also leads to loss of negative feedback, which could be critical to the emergence of resistance as discussed in the next section (23).

An interesting aspect to the pharmacologic profile of these BRAF inhibitors is that they paradoxically activate the RAF–MEK–ERK pathway in cells expressing wild-type BRAF (24–26). The lack of inhibition spares normal tissues some of the toxicities caused by pathway inhibition with MEK inhibitors, but seems to cause other toxicities (especially skin) as a consequence of pathway activation (27–29). This is supported by the observation that skin toxicities decrease to a significant investment in the development of BRAF inhibitors is that they paradoxically activate the RAF–MEK–ERK pathway with often very dramatic initial tumor regression followed by relapse within a few months, highlights the need to understand the mechanisms of resistance. In the following section, we will explore the mechanisms and potential approaches to overcome acquired resistance, as well as intrinsic resistance in melanoma.

Melanoma cell lines have been used extensively to study BRAF and MEK inhibitor resistance and in fact, a significant proportion of BRAFV600E mutated melanoma cell lines are intrinsically resistant to BRAF and MEK inhibitors (32, 34). Understanding the mechanisms of intrinsic resistance in these lines could provide insight into mechanisms of acquired resistance. For instance, cell lines with genetic alterations downstream of BRAFV600E, such as amplified cyclin D1 are resistant to BRAF inhibition because they evade cell-cycle arrest (35). Genetic alterations upstream of BRAFV600E such as NF1 loss can also confer intrinsic resistance to BRAF inhibition by activation of RAS and signaling through CRAF (36). Melanoma cell lines with PTEN deletion have an impaired apoptotic response due to an inability to upregulate BIM upon BRAF or MEK inhibition (37). Receptor tyrosine kinase (RTK) signaling through the PI3K pathway has also been shown to cause intrinsic resistance to BRAF and MEK inhibition. MEK inhibitor insensitive BRAFV600E/K melanoma cell lines upregulate insulin-like growth factor receptor 1 (IGFR1)–AKT pathway signaling and these cells can be sensitized by a combination of AZD6244 with IGFR1, AKT or mTORC1/2 inhibitors (38). Other BRAFV600E tumor types such as colorectal and thyroid tend to be somewhat intrinsically resistant to BRAF and MEK inhibition. In these tumors BRAFV600E dependence is not absolute and transient pathway inhibition leads to relief of feedback inhibition of RTK signaling and rapid reactivation of the pathway (39, 40). Therefore, RAF–MEK–ERK signaling in BRAFV600E/K tumors is more heterogeneous than depicted in Fig. 1A and B and seems to differ across tumor types.

Acquired resistance is characterized by initial period of tumor response, followed by relapse and this has been noted for several targeted agents besides BRAF inhibitors. For example, BCR–ABL inhibitors in chronic myelogenous leukemia (CML), KIT inhibitors in gastrointestinal stromal tumors, EGFR inhibitors in lung cancer and smoothened inhibitors in medulloblastoma (41–43). Analysis of the resistance mechanisms in each of these examples show that acquired drug resistance can arise from the acquisition of a secondary mutation in the kinase being targeted. The mutations occur at “gatekeeper” residues of the ATP-binding pocket in the kinase, preventing the drug from binding and inhibiting the kinase activity. The T790M mutation and T315I mutation in EGFR and BCR–ABL, respectively, are gatekeeper mutations. Preclinical studies identified T529 as the gatekeeper site in BRAFV600E that confers resistance to BRAF inhibitors (44). However, this mutation has never been observed as a mechanism of acquired resistance to
BRAF inhibitors in cell lines or patient biopsies. Instead, a diverse array of resistance mechanisms have been published, which in most cases lead to the reactivation of ERK in the presence of the BRAF inhibitor (Fig. 1C).

The major ERK reactivating mechanisms of resistance discovered to date involve alterations in RAS, RAF, and MEK. Activating mutations in NRAS, such as NRASQ61K, have been shown to drive resistance to vemurafenib through activation of CRAF leading to reactivation of MEK and ERK (45, 46). The flexible switching of signaling between the RAF isoforms has been shown in other cases to drive resistance to BRAF inhibitors, for example, activation or overexpression of CRAF can re-activate p-ERK independent of BRAFV600E (47, 48). In addition the genomic amplification of BRAFT1799A or the upregulation of RAF protein levels can also mediate resistance to vemurafenib with cross-resistance to MEK inhibition as well (49, 50). Expression of a 61-kDa splice variant of BRAFV600E, which lacks the RAS-binding domain, leads to enhanced dimerization resulting in resistance to vemurafenib (51). MEK mutations have also been identified in the context of BRAF inhibitor resistance. MEK1 (C121S) confers increased kinase activity in vitro and overexpression of this mutant protein induced cross-resistance to both MEK (AZD6244) and BRAF (PLX4720) inhibitors (52). Two additional MEK mutations, MEK1 (P124L) and MEK1 (Q56P), identified from a random mutagenesis screen promote resistance to MEK inhibition and cross-resistance to BRAF inhibitor PLX4720 (53). However, exposing BRAF-mutant melanoma cells containing either of the two MEK mutations to a MEK plus BRAF inhibitor combination (AZD6244 and PLX4720) prevented emergence of resistant clones. In summary, resistance to vemurafenib frequently occurs through reactivation of ERK as a result of genetic changes to RAS, RAF, and MEK.

Parallel survival pathways may also drive acquired resistance to BRAF and MEK inhibitors in BRAFV600E melanoma. IGF1R has been found to be constitutively activated with a simultaneous increase in PI3K/AKT signaling in the resistant cells and combinations of PI3K and MEK inhibitors or IGF1R and MEK inhibitors reversed the resistance (47, 48). Other studies show that vemurafenib induces FGF2 secretion, which leads to the upregulation and activation of STAT3–PAX3 signaling pathway, which in turn drives resistance to vemurafenib (54). Similar studies show that hepatocyte growth factor/c-MET and fibroblast growth factor (FGF)/FGFR3 confer resistance to BRAF inhibition via RAF–MEK–ERK pathway reactivation (55, 56).

Resistance Can Lead to a Fitness Deficit

The expression of mutant NRAS and BRAFV600E are normally mutually exclusive (11, 57, 58), but as described above mutant NRAS is found to reactivate the RAF–MEK–ERK pathway in BRAFV600E tumors with acquired resistance to BRAF inhibitors. Such ERK activating resistance mechanisms seem to be deleterious to resistant tumor cells in the absence of BRAF inhibitor treatment. For example, vemurafenib-resistant tumor cells expressing p61-BRAFV600E or over-expressed BRAFV600E suffer a fitness deficit in the absence of drug (49). This was demonstrated directly by expressing a BRAFV600E–estrogen receptor fusion protein and treating cells with 4-hydroxy-tamoxifen, which induced dose-dependent activation of the BRAFV600E kinase activity, increased phospho-ERK, and a decrease in melanoma cell proliferation (49). These observations are consistent with previous studies that demonstrated when activated NRAS is expressed in cells expressing BRAFV600E, the proliferation rate slows as the result of cells accumulating in G0–G1, and cells show characteristics of senescence (59). Whether this phenomenon will occur in the context of resistance mechanisms that do not involve ERK reactivation (i.e., ERK remains inhibited) is unclear.

These data are consistent with the concepts described in the first section that describe the phenotypic effects associated with differential kinetics of RAF–MEK–ERK pathway activation. In this case, melanoma cells are dependent on a precise level of BRAFV600E—MEK–ERK pathway activation, such that too little, in response to pathway inhibition, or too much, in response to elevated flux through the pathway is deleterious to cell proliferation. Resistant tumor cells seem to suffer a selective disadvantage over drug-sensitive tumor cells in the absence vemurafenib treatment. This observation led to the design of a simple and effective intermittent dosing schedule that was found to delay or prevent the onset of drug-resistant disease in patient-derived melanoma xenografts (49).

Anecdotal evidence already exists for the successful application of intermittent dosing of vemurafenib in patients with melanoma (60). As discussed above, vemurafenib induces paradoxical activation of the RAF–MEK–ERK pathway in cells expressing wild-type BRAF and a recent case study describes the induction of a previously undetected NRAS-mutant CML in a patient with melanoma treated with vemurafenib. Treatment with vemurafenib caused the patient’s white-cell counts to spike leading to rapid progressive CML. Cessation of vemurafenib treatment led to decreased white-cell and monocyte counts; however, treatment had to be reintiated to control the growth of the melanoma tumors. To keep both diseases under control, the patient was maintained on an intermittent schedule of vemurafenib with the dose schedule determined by closely monitoring the white-cell counts. This patient remained on this schedule for more than 80 weeks (Paul Chapman, personal communication). In another example, 2 patients who previously progressed on treatment with the BRAF inhibitor dabrafenib or with a BRAF and MEK inhibitor combination (dabrafenib plus trametinib), following a treatment-free period were successfully rechallenged with vemurafenib or dabrafenib plus trametinib, respectively, and both patients experienced tumor regression (61). Although these case studies provide evidence that support intermittent dosing, a thorough testing of the efficacy,
feasibility, and optimization of the dosing regimen in patients is still necessary. Such testing is now underway in a phase II clinical trial evaluating intermittent dosing with the BRAF inhibitor LGX818 in patients with BRAF\(^{V600E}\) -mutant metastatic melanoma (www.clinicaltrials.gov).

Combination therapy represents a more traditional approach to preventing resistance. As described above, the efficacy of a BRAF plus MEK inhibitor combination seems to be superior to single-agent BRAF inhibitor (30). Targeting two nodes provides a more robust inhibition of the pathway and presumably delays or prevents the emergence of resistance through reactivation of downstream ERK. Given the number of potential resistance mechanisms described above, there are multiple BRAF inhibitor combinations that could be investigated and evaluating each of these clinically becomes a logistical challenge. One innovative approach to tackling this issue has recently been initiated in a phase II study with patients with melanoma who progress on the BRAF inhibitor LGX818. Resistant tumors will be biopsied and compared with a pretreatment biopsy in an attempt to identify the mechanism of resistance in each tumor. On the basis of the results, a second targeted agent from a list of MEK, CDK4/6, FGFR, PI3K, and c-MET inhibitors will be added to the treatment regimen (www.clinicaltrials.gov). Also, given the recent encouraging results with immunotherapy in melanoma, combination trials are now underway evaluating vemurafenib with anti-PD-1/PD-L1 and CTLA4 antibodies (reviewed in ref. 62).

Conclusions

The impressive clinical efficacy observed with BRAF inhibitors in patients with BRAF\(^{V600E}\) melanoma exemplifies the success of oncogene-targeted cancer therapy, but as with other targeted therapies, the duration of clinical benefit is limited by the emergence of drug resistance disease. Improving the durability of response to these agents will require a deep understanding of both the genetics and the intracellular signaling pathways in drug-sensitive and -resistant tumor cells. These studies require a significant investment in translational approaches using both preclinical and clinical tumor samples. Recent studies using such translational models suggest that vemurafenib-resistant tumors suffer a fitness deficit in the absence of drug treatment and intermittent dosing schedules upfront could prevent or delay the emergence of resistant disease. Such an approach should be considered along with combination therapy to improve the efficacy of BRAF inhibitors in melanoma. In the future, it may be possible to rationally design intermittent treatment regimens in which drug combinations are cycled on and off together or separately based on a deeper understanding of RAF–MEK–ERK signal transduction and crosstalk with other pathways in treatment naïve and resistant tumors.

Disclosure of Potential Conflicts of Interest

M. Das Thakur is employed as a Research Investigator II for Novartis Institutes for BioMedical Research. D.D. Stuart is employed as a Senior Investigator II for and has ownership interest (including patents) in Novartis Institutes for BioMedical Research.

Authors’ Contributions

Conception and design: M. Das Thakur, D.D. Stuart
Development of methodology: M. Das Thakur, D.D. Stuart
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): M. Das Thakur, D.D. Stuart
Writing, review, and/or revision of the manuscript: M. Das Thakur, D.D. Stuart

Received September 22, 2013; revised November 25, 2013; accepted December 2, 2013; published OnlineFirst December 18, 2013.

References


Clinical Cancer Research

Molecular Pathways: Response and Resistance to BRAF and MEK Inhibitors in BRAF V600E Tumors

Meghna Das Thakur and Darrin D. Stuart


Updated version
Access the most recent version of this article at:
doi:10.1158/1078-0432.CCR-13-0103

Cited articles
This article cites 62 articles, 23 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/20/5/1074.full.html#ref-list-1

Citing articles
This article has been cited by 4 HighWire-hosted articles. Access the articles at:
/content/20/5/1074.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.