

Advances in the Development of Cancer Therapeutics Directed against the RAS-Mitogen-Activated Protein Kinase Pathway

Judith S. Sebolt-Leopold

Abstract Among mammalian mitogen-activated protein kinase (MAPK) signaling cascades, the extracellular signal-related kinase (ERK) pathway has received the most attention in the oncology drug discovery arena. By virtue of its central role in promoting proliferation, survival, and metastasis, this pathway directly affects both the formation and progression of human tumors. The identification of non-ATP-competitive inhibitors of the MAPK kinase MAPK/ERK kinase (MEK) resulted in the first demonstration that the ERK pathway could be effectively shut down in a highly selective fashion. Subsequent discovery of the oncogenic nature of B-raf kinase led to the escalation of drug discovery efforts revolving around MEK and RAF. The emergence of multiple drug candidates targeting these downstream kinases provides us with the means for validating the importance of the RAS-RAF-MEK-ERK signaling cascade in human tumors. This article highlights the lessons learned in the clinical evaluation of MAPK pathway inhibitors as anticancer agents and the complexities surrounding optimization of their therapeutic potential in light of the challenges posed by genetic heterogeneity within patient populations.

Background

It has been well established that tumorigenesis in humans proceeds in a multistep fashion as genetic alterations in normal cells drive them toward a progressively more malignant state. Hanahan and Weinberg (1) suggest that most, if not all, cancers acquire the same functional capabilities, including autonomy from growth factor signaling. Much attention has focused on the RAS-RAF-mitogen-activated protein kinase (MAPK)/extracellular signal-related kinase (ERK) kinase (MEK)-ERK cascade (Fig. 1), hereafter referred to as the RAS-MAPK pathway, as a prototypical signal transduction pathway that is aberrantly activated in many neoplasms. Structural alterations in the upstream GTPase RAS occur in ~25% of human cancers and confer them with the ability to relay mitogenic signals in a ligand-independent manner, thereby obviating the need for ligand activation of growth factor receptors that occurs in normal cells. In addition, this pathway contributes to enhanced survival of tumor cells while also facilitating their metastatic spread to distant organs. Therefore, the integral role of the RAS-RAF-MEK-ERK pathway in mediating multiple hallmarks of cancer has spurred intense explorations into its amenability for pharmacologic intervention (2–4).

When contemplating how best to cripple this pathway, several key proteins are reasonable candidates for consider-

ation. Most efforts to design small-molecule inhibitors of RAS-MAPK signaling have focused on the major protein players (i.e., RAS, RAF, MEK, and ERK). We may see more attention devoted in the future, however, to several scaffolding proteins and endogenous inhibitors that also come into play with respect to pathway dynamics (5). Recent advances in the development of genome-wide RNA interference libraries have enabled screening that may potentially identify other novel regulators of MAPK signaling amenable to pharmacologic intervention (6, 7).

Is RAS druggable? Attempts to target RAS by perturbing its interaction with either SOS or GRB2 have not yielded viable drug development candidates largely because of the inherent difficulties of disrupting protein-protein interactions with drug-like molecules. Several drug discovery programs have also been devoted to finding inhibitors of farnesyltransferase as a means to prevent the membrane localization of RAS. Despite the successful identification of several chemical leads that effectively inhibited this prenylation enzyme, however, tumor cells proved generally to be impervious to their action. *K-ras*, which is the most frequently mutated *ras* isoform, is also a substrate for geranylgeranyltransferase and can therefore be prenylated by an alternative route. Disappointing clinical data with farnesyltransferase inhibitors have led to the evaluation of other posttranslational targets involved in RAS localization [i.e., the endoprotease Rce1 and isoprenylcysteine carboxylmethyltransferase (Icmt)]. Icmt carries out methylation of the cysteine carboxylate generated from the Rce1 catalyzed proteolysis of the -AAX residues from the CAAX motif found in RAS. RAS-induced transformation has been shown to be blocked by inactivation of either Rce1 or Icmt (8, 9). Novel inhibitors of Icmt have subsequently been identified that are currently undergoing further evaluation (10, 11). One of these agents, cysmethynil, has been shown to effectively block anchorage-independent growth of colon tumor cells, an effect that could be reversed by overexpression of Icmt (10). While we await further data on the promise of these inhibitors, attention has

Authors' Affiliation: Oncovera Therapeutics, Ann Arbor, Michigan
Received 2/7/08; accepted 2/7/08.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: Judith S. Sebolt-Leopold, Oncovera Therapeutics, 3975 Red Hawk, Ann Arbor, MI 48103. Phone: 734-646-8726; Fax: 734-821-1066; E-mail: judith.leopold@comcast.net.

© 2008 American Association for Cancer Research.
doi:10.1158/1078-0432.CCR-08-0333

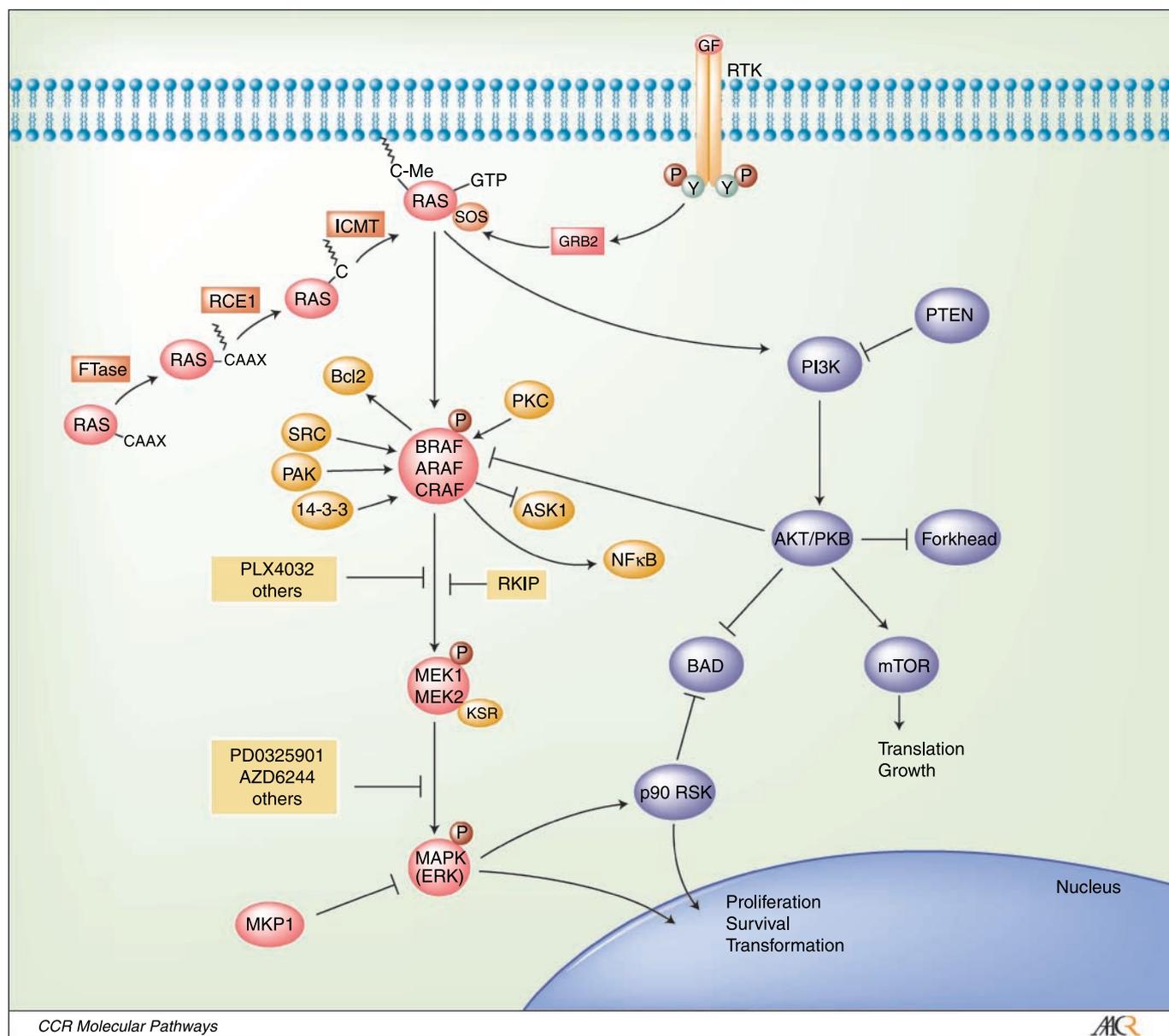


Fig. 1. The RAS/MAPK signaling pathway. RAS plays a central role in mediating both proliferation and survival signaling through the MAPK and PI3K pathways. Shown here is a general program for RAS activation. The RAS/MAPK module is a critical component of a complex signaling network, comprehensively reviewed elsewhere (4). Scaffolding proteins, including kinase suppressor of RAS (KSR) shown here, are important for activation efficiency and spatial regulation of signaling through this pathway. In addition to RAS, which is the most frequently mutated oncogene in human tumors, BRAF and PI3K have been found to be frequently mutated in a wide range of malignancies. Drug discovery programs targeting the MAPK pathway have largely focused on BRAF and MEK.

shifted to clinical candidates that target the downstream kinases RAF and MEK. The recent emergence of highly selective kinase inhibitors that effectively block MAPK phosphorylation should prove highly valuable for validating the overall attractiveness of the RAS-MAPK pathway for future cancer drug development.

RAF-targeted agents. Therapeutic strategies targeting RAF have received increased momentum since publication of the seminal study in 2002 by Davies et al. (12) reporting a high incidence of *B-raf* mutations in human cancers. *B-raf* mutations occur at a particularly high frequency in melanomas (30-60%) and at a significant frequency in a variety of other tumor types, including thyroid (30-50%), ovarian (30%), and colorectal (5-20%) cancers (13). The most prevalent *B-raf* mutation is the

substitution of a glutamic acid residue for the valine moiety at codon 599 (V599E), which occurs in the activation loop of the kinase domain resulting in constitutive kinase activity. The use of geldanamycin analogues to indirectly target RAF by inhibiting chaperone proteins, thereby resulting in the destabilization and subsequent degradation of RAF, represents one therapeutic approach to intervention of MAPK signaling. Although the activity of these agents is impressive, their efficacy likely results from their broader pleiotropic effects (14). Several other drug discovery programs are focusing on the development of small-molecule inhibitors of RAF kinase activity. Whereas the RAF serine/threonine family, composed of A-RAF, B-RAF, and C-RAF (RAF-1), shares a common architecture consisting of

three conserved regions, the isoforms vary in their cell-specific expression and possess unique regulatory functions (15). RAF regulation is complex, with many questions remaining unanswered. Emerging data, however, have made a compelling case for BRAF serving as the primary MEK kinase *in vivo* based on MEK-binding variables and relative levels of basal MEK kinase activity (16, 17). In contrast to the high incidence of *B-raf* mutations in human tumors, *C-raf* mutations are rare due to its low basal kinase activity (18). Data showing the involvement of CRAF in melanoma cell proliferation, however, suggest that pan-specific RAF agents would be more efficacious against melanomas than BRAF-specific drugs (19, 20). Several RAF kinase inhibitors have now been reported and exhibit varying selectivity profiles (21–23). BAY 43-9006 (sorafenib), an ATP-competitive inhibitor of CRAF kinase, has received the most attention based on its clinical activity and subsequent regulatory approval (24). Sorafenib, however, failed to elicit clinical activity against melanoma and its efficacy against renal tumors is likely due to its multitargeted mechanistic nature, as it has been shown to inhibit vascular endothelial growth factor receptor 2, platelet-derived growth factor receptor, FLT-3, and c-Kit. CHIR-265 is also a dual vascular endothelial growth factor receptor/RAF kinase inhibitor currently undergoing clinical evaluation. With respect to the identification of inhibitors that are selective against mutant (V600E) BRAF tumors, PLX4032 has emerged as a first-in-class agent (25). Several other RAF inhibitors are also nearing the clinical testing stage. Clinical testing is pivotal for addressing the relative merits and liabilities of targeting RAF kinase as opposed to targets further downstream, most notably MEK, a target that has received much attention in recent years. The ability of RAF-1 to block apoptosis by multiple MEK-independent pathways suggests that the spectrum of activity for RAF inhibitors will differ from that observed for MEK inhibitors. It will be interesting to see these questions play out in clinical testing.

Exquisite selectivity of MEK inhibitors. The MEK homologues MEK1 and MEK2 are dual specificity kinases, uniquely sharing the consensus kinase motifs of both serine/threonine as well as tyrosine kinases. They exhibit a high degree of stringency in their ability to phosphorylate their ERK substrates. Both MEK isoforms sequentially phosphorylate ERK1 and ERK2 at two sites, initially Tyr¹⁸⁵ followed by Thr¹⁸³ (26). No catalytic substrates for MEK proteins have been identified other than ERK1 and ERK2 (27). Such tight selectivity in conjunction with dual specificity kinase capability is consistent with these proteins occupying a strategic position in the RAS-MAPK pathway where they serve to integrate signals into the pathway. Comparison of the biological attributes of MEK1 and MEK2 discloses key differences between the two homologues. MEK1 is more highly up-regulated in *ras*-transformed cells, whereas MEK2 has never been detected in a RAS/RAF/MEK ternary complex (28). Studies done with mutant mouse lines deficient in MEK1 and MEK2 would seem to suggest that MEK1 can compensate for MEK2 but not vice versa (29, 30). The high sequence homology between the two isoforms (80% identical) suggests that it is unlikely that highly specific inhibition of only one isoform could easily be achieved.

PD98059 proved to be the first of several highly selective small-molecule inhibitors of MEK that have now been reported

(31). The high degree of selectivity was also observed for U0126, a MEK inhibitor with increased potency relative to PD98059 (32). The pharmaceutical limitations of both PD98059 and U0126 compromised their utility as *in vivo* anticancer agents. They have served, however, as extremely useful tools in academic research and have provided insight into the importance of MAPK signaling in human tumors. CI-1040 emerged as the first MEK inhibitor capable of eliciting oral anticancer activity in preclinical tumor models (33). As discussed later in this review, CI-1040 was also the first MEK inhibitor to be tested in patients. All of these compounds in addition to more recent clinical candidates, PD0325901 and AZD6244, inhibit MEK in a non-ATP-competitive manner consistent with their exquisite selectivity. The development of CI-1040-like analogues in the PD0325901 class enabled solving the three-dimensional structure of human MEK1, consequently shedding light on the basis for such specificity. X-ray structures of human MEK1 and MEK2 were determined as ternary complexes with MgATP and the CI-1040-related analogue PD318088 (34). Consequently, Ohren and his colleagues discovered that MEK contains a unique inhibitor-binding pocket adjacent to the MgATP-binding site. Binding of CI-1040 and structurally related MEK inhibitors into this hydrophobic pocket induces conformational changes in unphosphorylated MEK that locks the kinase into a closed but catalytically inactive form. This finding is consistent with the noncompetitive kinetics of inhibition observed for this class of inhibitors and explains the basis for their high degree of selectivity, as this binding pocket occurs in a region without known sequence homology to other kinases. The reader is referred elsewhere for a comprehensive report of novel inhibitors of both RAF and MEK that have been described in the literature (35).

Clinical-Translational Advances

CI-1040—lessons learned. CI-1040 was advanced into clinical trials based on a promising preclinical profile that included oral efficacy as measured by significant tumor regressions against a broad spectrum of human xenografts (33). Despite low bioavailability in preclinical species, it was hoped that sufficient exposure could be achieved in cancer patients to address the question of whether MEK inhibition would prove to be safe and efficacious. Because MEK was an unproven drug target, concerns existed at the time that agents that interfered with MAPK signaling might also interfere with immune signaling. These concerns were based in part on the *Erk1*^{-/-} mouse knockout data pointing to a 2-fold reduction in mature thymocyte count (36). Furthermore, *Mek1*^{-/-} fibroblasts were reported to be defective in fibronectin-mediated migration despite normal activated levels of MEK2 and ERK, pointing to a further role for MEK1 beyond ERK activation, which, if impaired, might prove to be deleterious (29). Phase 1 trials with CI-1040, however, indicated a well-tolerated safety profile at drug levels resulting in ~70% inhibition of MAPK activation in tumor biopsies (37). Roughly 60% of the patients treated with CI-1040 experienced adverse events that were generally mild in nature (i.e., grade 1 or 2 severity). As might be expected for agents of this mechanistic class, common toxicities included diarrhea and skin rash. Phase 1 clinical testing of CI-1040 (77 patients) also produced encouraging signs of clinical

activity as evidenced by one partial remission in a pancreatic cancer patient, who also experienced significant symptomatic improvement in pain, anorexia, and fatigue. Disease stabilization lasting 4 to 17 months was reported in 19 additional patients with a variety of tumors, including breast, colon, and non-small cell lung cancer. Phase 2 testing of this agent against advanced colorectal, breast, non-small cell lung, and pancreatic cancer, however, disclosed insufficient clinical activity to warrant its further development (38). Despite its disappointing lack of clinical efficacy, the encouraging safety profile of CI-1040 provided the impetus to search for more potent analogues that would more robustly shut down the MAPK pathway. There were several pharmaceutical properties of CI-1040 that compromised its clinical profile. CI-1040 trials were marked by the need to use extremely high doses (800 mg twice daily), a finding that reflected not only poor bioavailability of this agent but also its high propensity for metabolism to a carboxylic acid derivative. In plasma, a 30-fold higher C_{max} was observed for the metabolite compared with parent compound (37).

Entry of second-generation MEK inhibitors—PD0325901 and AZD6244. PD0325901, a structural analogue of CI-1040, represents a significant advance in target potency as reflected by its ability to inhibit both purified MEK as well as cellular activation of MAPK at concentrations in the low nanomolar range (2). Importantly, the improved target potency of PD0325901 is accompanied by other key attributes, including metabolic stability and high bioavailability. Consequently, this compound exhibits longer-lasting biological effects at significantly reduced doses compared with CI-1040. Based on its improved potency, a projected human dosage of 15 mg/d was estimated before the initiation of clinical trials with this agent (39). The benzimidazole AZD6244 (ARRY-142886) is also highly selective for MEK with activity reported against *in vitro* and *in vivo* tumor models (40, 41). The spectrum of activity of this compound against a panel of tumor xenografts is comparable with that of PD0325901 and encompasses tumors of pancreatic, colon, breast, lung, and skin origin (41). Response to AZD6244, which is ~10-fold less potent than PD0325901, was found to be tumor specific and ranged from antiproliferative effects to the induction of apoptosis and differentiation (42). PD0325901 and AZD6244 have both been advanced into clinical development by Pfizer and AstraZeneca/Array BioPharma, respectively, and, based on potency considerations, have been touted to have the potential to ultimately provide validation for the utility of MEK inhibitors as anticancer drugs.

Phase 1 trials with PD0325901 have shown that this agent is capable of suppressing phosphorylation of MAPK in tumors by >90% at doses as low as 1 mg (43). Importantly, this agent has also shown early signs of clinical activity as reflected by the incidence of three partial remissions in melanoma patients (44). The toxicities associated with PD0325901, however, seem to be more severe than those observed for its clinical predecessor CI-1040. The first-in-human trial of PD0325901 used an open-label, dose-escalating design (43, 45). From 35 evaluable patients, the most commonly observed adverse effects were rash, diarrhea, fatigue, and visual disturbances occurring at an incidence rate of 49%, 49%, 34%, and 34%, respectively (39). Visual disturbances, which also occurred with lower frequency and shorter duration in earlier trials with CI-1040, were reported at doses of PD0325901 \geq 15 mg twice daily and took the form of colored spots or halos (43). Extended phase 1

evaluation resulted in three patients also experiencing a retinal vein occlusion, a toxicity that presented late (after 13-38 weeks of therapy; ref. 44). Blurred vision was also reported during phase 1 evaluation of AZD6244, however, on lowering the dose, this toxicity was not noted and the maximum tolerated dose was established at 100 mg twice daily (46, 47). The relative difference in clinical doses used in trials with PD0325901 and AZD6244 are consistent with potency differences observed during preclinical testing of these two agents. During expanded phase 1 testing of AZD6244 aimed at further exploration of its safety and tolerability, no objective responses were reported, whereas the overall incidence of stable disease was 49% (39, 47). Initial results from a randomized phase 2 study comparing AZD6244 monotherapy with the alkylating agent temozolamide for first-line treatment of melanoma showed no apparent difference in efficacy between the two agents using progression-free survival as the primary end point (48). While we await disclosure of the clinical data, several other MEK-targeted agents are beginning to enter clinical trials.

The Path Forward

Clearly, PD0325901 and AZD6244 represent great progress in attempts to develop agents that will effectively impair signaling through the MAPK pathway. We still have, however, more questions than we have answers. Is the ocular toxicity observed with PD0325901 mechanism or chemotype related? This question is not easily addressed by comparing side effects with currently available inhibitors based on their strong structural similarity. Although subtle structural changes can impart significant differences in biological attributes, it is probably not coincidental that the severity of ocular toxicity has increased in concert with increased potency against the target molecule MEK. Thus, we are likely dealing with a mechanism-based toxicity that will need to be adequately managed as we move forward with the evaluation of these agents. If, in fact, the vision-based side effects are a consequence of blocking the MAPK pathway, then we might expect to see comparable adverse events with highly selective RAF kinase inhibitors. As RAF inhibitors enter the clinical arena, clinicians and study sponsors should be prepared for this possibility as trials are designed. In the meantime, future development strategies with extremely potent MEK inhibitors such as PD0325901 will need to take into account the predisposing factors for retinopathy that put certain patients at higher risk. The clinical oncology field will also anxiously await evaluation of other non-ATP-competitive MEK inhibitors (undisclosed chemical structures) that were recently reported (49, 50). One of these compounds, XL518, has been shown to have reduced pharmacodynamic activity in brain tissue compared with PD0325901 (49).

A current challenge facing development of both RAF and MEK inhibitors is selection of the appropriate patient population for clinical trial evaluation. We should not be quick to discount the value of preclinical models in guiding us here. Human genetic data on the incidence of *B-raf* mutations in a large fraction of melanoma patients are consistent with the striking activity of PD0325901 observed against a panel of *B-raf*-mutated xenografts (51). Solit and colleagues also reported partial inhibition in certain *ras*-mutated tumors with

some *N-ras* melanoma lines responding to treatment with this MEK inhibitor. It is particularly noteworthy that the three partial remissions reported in the phase 1 clinical trial of PD0325901 were all mutated in either *B-raf* (two cases) or *N-ras* (one case; ref. 52). Although the presence of either mutation does not necessarily ensure a favorable outcome with MEK-targeted monotherapy, early clinical data do not support treatment of patients who are wild-type with respect to *ras* and *raf*. The significant incidence of *raf* mutations in lung, colorectal, and thyroid cancers makes these reasonable patient populations to evaluate as well as melanoma populations. It is critical that we tackle the logistical complexities associated with accruing patients from multiple disease-based clinics into a single trial where patients share a common genetic marker(s) as opposed to tumor origin. Although it can be said that this imperative applies to most if not all signal transduction agents, it should be relatively straightforward in the case of MAPK pathway inhibitors where testing for *ras* and *raf* mutations can be carried out expediently in archival specimens if biopsy material is unavailable.

More aggressive use of surrogate markers of response that will guide us earlier in determining patient outcome also warrants attention. For some patients, clinical benefit from signal transduction inhibitors may take the form of cytostasis and not tumor regressions. The effects of MEK inhibitors on melanomas, in particular, are often cytostatic and show little evidence of apoptosis (53, 54). The use of noninvasive [¹⁸F]fluorothymidine positron emission tomography imaging of tumors for assessing early biological response to MEK inhibition may have potential utility in clinical trials based on encouraging preclinical data (53). With respect to biochemical markers of response, caution should be taken to not overinterpret phosphorylated ERK suppression data in posttreatment biopsies. Whereas phosphorylated ERK is a valid marker for assessing MEK inhibition, lowered phosphorylated ERK levels does not necessarily mean that the tumor is sufficiently dependent on the MAPK pathway to be impaired in any meaningful way by this treatment approach. Tumors that prove to be

refractory to MEK inhibitor treatment have been shown to have lowered phosphorylated ERK levels (51). Ki67 has been proposed as a more reliable marker for the antiproliferative effects of MEK-directed agents (54).

Finally, we are faced with the challenge of how best to optimize the use of these agents against tumors that harbor multiple genetic defects. MAPK pathway inhibitors will likely find applicability across a wider range of tumors as other targeted agents become available for rational combination regimens. RAS signals through multiple effectors, not just RAF. Consequently, activation by RAS of the phosphatidylinositol 3-kinase (PI3K)/AKT survival signaling pathway may erode in some tumors the therapeutic gain derived from shutting off MAPK activation. It has been shown experimentally that the coexistence of an activating PI3K mutation reduces a KRAS-mutated tumor's dependence on MEK/ERK signaling (55). Agents targeting upstream as well as downstream targets in the PI3K pathway, including PI3K, AKT, and mammalian target of rapamycin, are logical candidates to combine with MEK and RAF inhibitors. The role of tumor necrosis factor- α in preventing apoptosis when BRAF signaling is blocked in melanoma cells also suggests that combination of RAF or MEK inhibitors with agents targeting tumor necrosis factor- α could prove beneficial in this particular patient population (56). It will be relatively straightforward to select the appropriate agents for combination based on mechanistic considerations. More daunting, but absolutely critical, is the task of preselecting the appropriate patient population to match to that combination by fully understanding the genomics of individual patient's tumors. We have made significant advances in our knowledge of how to make highly potent and selective MAPK pathway inhibitors. We are now on the verge of knowing how best to use them where it really matters—in cancer patients.

Disclosure of Potential Conflicts of Interest

J.S. Sebolt-Leopold is a former employee and present stockholder of Pfizer Global Research and Development.

References

- Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell* 2000;100:57–70.
- Sebolt-Leopold JS, Herrera R. Targeting the mitogen-activated protein kinase cascade to treat cancer. *Nature Rev Cancer* 2004;4:937–47.
- Sebolt-Leopold JS. MEK inhibitors: a therapeutic approach to targeting the Ras-MAP kinase pathway in tumors. *Current Pharm Design* 2004;10:1907–14.
- Roberts PJ, Der CJ. Targeting the Raf-MEK-ERK mitogen-activated protein kinase cascade for the treatment of cancer. *Oncogene* 2007;26:3291–310.
- Kolch W. Coordinating ERK/MAPK signaling through scaffolds and inhibitors. *Nat Rev Mol Cell Biol* 2005;6:827–37.
- Friedman A, Perrimon N. A functional RNAi screen for regulators of receptor tyrosine kinase and ERK signaling. *Nature* 2006;444:230–4.
- Friedman A, Perrimon N. High-throughput approaches to dissecting MAPK signaling pathways. *Methods* 2006;40:262–71.
- Bergo MO, Ambroziak P, Gregory C, et al. Absence of the CAAX endoprotease Rce1: effects on cell growth and transformation. *Mol Cell Biol* 2002;22:171–81.
- Bergo MO, Gavino BJ, Hong C, et al. Inactivation of *lcm1* inhibits transformation by oncogenic K-Ras and B-Raf. *J Clin Invest* 2004;113:539–50.
- Winter-Vann AM, Baron RA, Wong W, et al. A small-molecule inhibitor of isoprenylcysteine carboxyl methyltransferase with antitumor activity in cancer cells. *Proc Natl Acad Sci U S A* 2005;102:4336–41.
- Anderson JL, Henriksen BS, Gibbs RA, Hrycyna CA. The isoprenoid substrate specificity of isoprenylcysteine carboxylmethyltransferase. *J Biol Chem* 2005;280:29454–61.
- Davies H, Bignell GR, Cox C, et al. Mutations of the BRAF gene in human cancer. *Nature* 2002;417:949–54.
- Wellbrock C, Karasides M, Marais R. The RAF proteins take centre stage. *Nat Rev Mol Cell Biol* 2004;5:875–85.
- Maloney A, Workman P. HSP90 as a new therapeutic target for cancer therapy: the story unfolds. *Expert Opin Biol Ther* 2002;2:3–24.
- Hagemann C, Rapp UR. Isotype-specific functions of Raf kinases. *Exp Cell Res* 1999;253:34–46.
- Papin C, Denouel A, Calothy G, Eychene A. Identification of signaling proteins interacting with B-Raf in the yeast two-hybrid system. *Oncogene* 1996;12:2213–21.
- Marais R, Light Y, Paterson HF, et al. Differential regulation of Raf-1, A-Raf, and B-Raf by oncogenic ras and tyrosine kinases. *J Biol Chem* 1997;272:4348–83.
- Emuss V, Garnett M, Mason C, et al. Mutations of C-RAF are rare in human cancer because C-RAF has a low basal kinase activity compared with B-RAF. *Cancer Res* 2005;65:9719–26.
- Gray-Schopfer VC, da Rocha Dias S, Marais R. The role of B-RAF in melanoma. *Cancer Metastasis Rev* 2005;24:165–83.
- Gray-Schopfer V, Wellbrock C, Marais R. Melanoma biology and new targeted therapy. *Nature* 2007;445:851–7.
- Bollag G, Freeman S, Lyons JF, Post LE. Raf pathway inhibitors in oncology. *Curr Opin in Invest Drugs* 2003;4:1436–41.
- Beeram M, Patnaik A, Rowinsky EK. Raf: a strategic target for therapeutic development against cancer. *J Clin Oncol* 2005;23:6771–90.
- King AJ, Patrick DR, Batorsky RS, et al. Demonstration of a genetic therapeutic index for tumors

- expressing oncogenic BRAF by the kinase inhibitor SB-590885. *Cancer Res* 2006;66:11100–5.
24. Wilhelm S, Carter C, Lynch M, et al. Discovery and development of sorafenib: a multikinase inhibitor for treating cancer. *Nat Rev Drug Disc* 2006;5:835–44.
 25. Su F, Yang H, Higgins B, et al. Plx 4032, a selective B-Raf^{V600E} inhibitor, has potent anti-tumor activity in B-Raf^{V600E}-bearing colorectal xenografts and shows additive effect with other chemoagents. *Mol Cancer Ther* 2007;6:3417s [abstr A252].
 26. Haystead TA, Dent P, Wu J, et al. Ordered phosphorylation of p42mapk by MAP kinase kinase. *FEBS Lett* 1992;306:17–22.
 27. Seger R, Ahn NG, Posada J, et al. Purification and characterization of mitogen-activated protein kinase activator from epidermal growth factor-stimulated A431 cells. *J Biol Chem* 1992;267:14373–81.
 28. Jelinek T, Catling AD, Reuter CW, et al. RAS and RAF-1 form a signaling complex with MEK-1 but not MEK-2. *Mol Cell Biol* 1994;14:8212–8.
 29. Giroux S, Tremblay M, Bernard D, et al. Embryonic death of Mek-1 deficient mice reveals a role for this kinase in angiogenesis in the labyrinthine region of the placenta. *Curr Biol* 1999;9:369–72.
 30. Belanger LF, Roy S, Tremblay M, et al. Mek2 is dispensable for mouse growth and development. *Mol Cell Biol* 2003;23:4778–87.
 31. Dudley DT, Pang L, Decker SJ, et al. A synthetic inhibitor of the mitogen-activated protein kinase cascade. *Proc Natl Acad Sci U S A* 1995;92:7686–9.
 32. Ahn NG, Nahreini TS, Tolwinski NS, Resing KA. Pharmacological inhibitors of MKK1 and MKK2. *Methods Enzymol* 2001;332:417–31.
 33. Sebolt-Leopold JS, Dudley DT, Herrera R, et al. Blockade of the MAP kinase pathway suppresses growth of colon tumors *in vivo*. *Nature* 1999;5:810–6.
 34. Ohren JF, Chen H, Pavlovsky A, et al. Structures of human MAP kinase kinase 1 (MEK1) and MEK2 describe novel noncompetitive kinase inhibition. *Nat Struct Mol Biol* 2004;11:1192–7.
 35. Wang J, Wilcoxon KM, Nomoto K, Wu S. Recent advances of MEK inhibitors and their clinical progress. *Curr Topics in Med Chem* 2007;7:1364–78.
 36. Pages G, Girard A, Jeannoton O, et al. Defective thymocyte maturation in p44MAPkinase (Erk1) knockout mice. *Science* 1999;286:1374–7.
 37. Lorusso PM, Adjei AA, Varterasian M, et al. Phase I and pharmacodynamic study of the oral MEK inhibitor CI-1040 in patients with advanced malignancies. *J Clin Oncol* 2005;23:5281–93.
 38. Rinehart J, Adjei AA, LoRusso PM, et al. Multi-center phase II study of the oral MEK inhibitor, CI-1040 in patients with advanced non-small-cell lung, breast, colon, and pancreatic cancer. *J Clin Oncol* 2004;22:4456–62.
 39. Wang D, Boerner SA, Winkler JD, LoRusso PM. Clinical experience of MEK inhibitors in cancer therapy. *Biochim Biophys Acta* 2007;1773:1248–55.
 40. Yeh TC, Marsh V, Bernat BA, et al. Biological characterization of ARRY-142886 (AZD6244), a potent, highly selective mitogen-activated protein kinase kinase 1/2 inhibitor. *Clin Cancer Res* 2007;13:1576–83.
 41. Lee P, Wallace E, Yeh T, et al. ARRY-142886, a potent and selective MEK inhibitor. III. Efficacy in murine xenograft models correlates with decreased ERK phosphorylation [abstract 897]. Proceedings of the 95th Annual AACR Meeting.
 42. Davies BR, Logie A, McKay JS, et al. AZD6244 (ARRY-142886), a potent inhibitor of mitogen-activated protein kinase/extracellular signal-regulated kinase kinase 1/2 kinases: mechanism of action *in vivo*, pharmacokinetic/pharmacodynamic relationship, and potential for combination in preclinical models. *Mol Cancer Ther* 2007;6:2209–19.
 43. LoRusso PA, Krishnamurthi S, Rinehart JJ, et al. A phase 1-2 clinical study of a second generation oral MEK inhibitor, PD 0325901 in patients with advanced cancer. *J Clin Oncol* [abstract] 2005;23:3006.
 44. LoRusso PA, Krishnamurthi SS, Rinehart JJ, et al. Clinical aspects of a phase I study of PD-0325901, a selective oral MEK inhibitor, in patients with advanced cancer. *Mol Cancer Ther* 2007;6:3649s [abstr B113].
 45. Menon SS, Whitfield LR, Sadis S, et al. Pharmacokinetics (PK) and pharmacodynamics (PD) of PD 0325901, a second generation MEK inhibitor after multiple oral doses of PD 0325901 to advanced cancer patients. *J Clin Oncol* [abstract] 2005;23:3006.
 46. Chow LQM, Eckhardt SG, Reid JM, et al. A first in human dose-ranging study to assess the pharmacokinetics, pharmacodynamics, and toxicities of the MEK inhibitor, ARRY-142886 (AZD6244) in patients with advanced solid malignancies. AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapeutics 2005.
 47. Adjei AA, Cohen RB, Franklin WA, et al. Phase I pharmacokinetic and pharmacodynamic study of the MEK inhibitor AZD6244 (ARRY-142886). AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapeutics 2006.
 48. Available from: <http://www.arraybiopharma.com>; press release 12/19/07.
 49. Johnston S. XL518, a potent, selective orally bioavailable MEK1 inhibitor, down-regulates the RAS/RAF/MEK/ERK pathway *in vivo*, resulting in tumor growth inhibition and regression in preclinical models. *Mol Cancer Ther* 2007;6:3595s [abstr C209].
 50. Weingarten P, Hamatake R, Gunawan S, et al. RDEA119: a potent and highly selective MEK inhibitor for the treatment of cancer. *Mol Cancer Ther* 2007;6:3472s [abstr B120].
 51. Solit DB, Garraway LA, Pratilas CA, et al. BRAF mutation predicts sensitivity to MEK inhibition. *Nature* 2006;439:358–62.
 52. Tan W, DePrimo S, Krishnamurthi SS, et al. Pharmacokinetic (PK) and pharmacodynamic (PD) results of a phase I study of PD-0325901, a second generation oral MEK inhibitor, in patients with advanced cancer. *Mol Cancer Ther* 2007;6:3648s [abstr B109].
 53. Solit DB, Santos E, Pratilas CA, et al. FLT PET as a non-invasive marker of response to MEK inhibition. *Cancer Res* 2007;67:11463–9.
 54. Smalley KS, Contractor R, Haass NK, et al. Ki67 expression levels are a better marker of reduced melanoma growth following MEK inhibitor treatment than phospho-ERK levels. *Br J Cancer* 2007;96:445–9.
 55. Halliловic E, She QB, Qing Y, et al. Coexistent PI3K mutation in human tumors is associated with decreased dependency on mutant KRAS and MEK/ERK signaling for transformation. AACR Meeting Abstracts 2008;49:4938.
 56. Gray-Schopfer VC, Karasarides M, Hayward R, Marais R. Tumor necrosis factor- α blocks apoptosis in melanoma cells when BRAF signaling is inhibited. *Cancer Res* 2007;67:122–9.

Clinical Cancer Research

Advances in the Development of Cancer Therapeutics Directed against the RAS-Mitogen-Activated Protein Kinase Pathway

Judith S. Sebolt-Leopold

Clin Cancer Res 2008;14:3651-3656.

Updated version Access the most recent version of this article at:
<http://clincancerres.aacrjournals.org/content/14/12/3651>

Cited articles This article cites 51 articles, 17 of which you can access for free at:
<http://clincancerres.aacrjournals.org/content/14/12/3651.full#ref-list-1>

Citing articles This article has been cited by 28 HighWire-hosted articles. Access the articles at:
<http://clincancerres.aacrjournals.org/content/14/12/3651.full#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, use this link
<http://clincancerres.aacrjournals.org/content/14/12/3651>.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.