Background

The mitogen-activated protein kinase (MAPK) cascade is a key intracellular signaling pathway that regulates diverse cellular functions including cell proliferation, cell cycle regulation, cell survival, angiogenesis, and cell migration (Fig. 1). The cascade includes a diverse group of members, but is generally described as a linear signaling pathway initiated by receptor tyrosine kinases at the cell surface and culminates in the regulation of gene transcription in the nucleus directed by the extracellular signal–regulated kinase (Erk). Although conceptually linear, considerable cross talk occurs between the Ras/Raf/MAPK/Erk pathway and other MAPK pathways as well as many other signaling cascades. The pivotal role of the Ras/Raf/MAPK/Erk MAPK pathway in multiple cellular functions underlies the importance of the cascade in oncogenesis and growth of transformed cells. As such, the MAPK pathway has been a focus of intense investigation for therapeutic targeting.

Classic activation of the MAPK cascade occurs following ligand binding to a receptor tyrosine kinase at the cell surface, but a vast array of other receptors have the ability to activate the cascade as well, such as integrins, serpentine receptors, heterotrimeric G-proteins, and cytokine receptors (1–6). Many receptor tyrosine kinases are capable of initiating MAPK signaling including receptors important in cancer biology, such as the human epidermal growth factor receptor family, plate-derived growth factor receptors, vascular endothelial growth factor receptors, and c-Kit. The epidermal growth factor receptor (EGFR) pathway serves as a relevant model for examining the activation and targets of MAPK signaling. Epidermal growth factor binding to EGFR activates the intracellular receptor tyrosine kinase function of EGFR and results in autophosphorylation of the receptor (7). Adaptor proteins such as Grb2 associate with the phosphorylated intracellular domain that recruits guanine nucleotide exchange factors like SOS-1 or CDC25 to the cell membrane (8). The guanine nucleotide exchange factor is now capable of interacting with Ras proteins at the cell membrane to promote a conformational change and the exchange of GDP for GTP bound to Ras. Multiple Ras isoforms have been described, including K-Ras, N-Ras, and H-Ras. Termination of Ras activation occurs on hydrolysis of the GTP to GDP, but Ras proteins have intrinsically low GTPase activity. Thus, the GTPase activity is stimulated by GTPase-activating proteins such as NF-1 GTPase-activating protein/neurofibromin and p120 GTPase-activating protein (9) thereby preventing prolonged Ras stimulated signaling.

Ras activation is the first step in activation of the MAPK cascade. Following Ras activation, Raf (A-Raf, B-Raf, or Raf-1) is recruited to the cell membrane through binding to Ras and activated in a complex process involving phosphorylation and multiple cofactors that is not completely understood. Raf proteins directly activate MEK1 and MEK2 via phosphorylation of multiple serine residues. MEK1 and MEK2 are themselves tyrosine and threonine/serine dual-specificity kinases that subsequently phosphorylate threonine and tyrosine residues in Erk1 and Erk2 resulting in activation. Although MEK1/2 have no known targets besides Erk proteins, Erk has multiple targets including Elk-1, c-Ets1, c-Ets2, p90RSK1, MNK1, MNK2, and TOB (10–12). The cellular functions of Erk are diverse and include regulation of cell proliferation, survival, mitosis, and migration.

A significant role for the MAPK in cancer biology has been well established. The Ras proteins were initially identified as the transforming component of oncogenic viruses for K-Ras and H-Ras, whereas N-Ras was identified as the transforming component of a neuroblastoma (13). Additional support for the importance of the MAPK pathway in oncogenesis comes from the prevalence of activating mutations among family members in multiple cancer types. Ras mutations are found in up to 30% of all cancers and are particularly common in pancreatic cancers (90%) and colon cancers (50%; ref. 14). B-RAF mutations have a more narrow distribution, but are prevalent in a few specific malignancies including melanoma (63%),
papillary thyroid cancers (45%), and low-grade ovarian cancers (36%; refs. 15–17). Despite the absence of MEK and Erk mutations in human cancers, there is ample evidence that these proteins also have transforming capacity. Based on the well-defined role for the MAPK pathway in human cancers, therapeutic targeting has been an area of intense investigations. Development of Small-Molecule MEK Inhibitors

Recently, blocking MAPK via small-molecule MEK inhibitors has come to the forefront as an exciting approach in cancer therapeutics. PD 098059 was the first specific MEK inhibitor described. It was identified by screening a compound library for inhibitors with an assay that measured phosphorylation of an Erk target protein in the presence of both MEK1 and Erk (18). The compound inhibited MEK with an IC50 ≈ 10 μmol/L but had no inhibitory effects when tested against a panel of 18 other serine/threonine kinases (19). A second MEK inhibitor, U0126, was also identified by screening a compound library using an assay designed to find an inhibitor that could antagonize activator protein-1–driven transcription without blocking the transcription of glucocorticoid response elements (20). U0126 was identified in this screen because the constituents of activator protein-1, c-Fos and c-Jun, are transcriptionally regulated by Erk. U0126 inhibited MEK1 and MEK2 with IC50 ≈ 5 to 7 nmol/L, and similar to PD 098059, had little effect on a panel of other kinases. Inhibition was noncompetitive with respect to the MEK substrates ATP and Erk. Both PD 098059 and U0126 have shown in vitro antiproliferative effects on transformed cell lines (19, 20). Although U0126 and PD 098059 have been extremely useful in the in vitro study of MAPK signaling, they have not been pursued in clinical development because of poor pharmacologic characteristics.

CI-1040 (PD 184352) was the first small-molecule MEK inhibitor that proceeded to clinical testing. CI-1040 was developed based on compounds and structures identified during the screening that led to the identification of PD 098059, but had improved potency and selectivity (21). CI-1040 selectively inhibited MEK1 in a noncompetitive manner with respect to ATP with an in vitro IC50 of 17 nmol/L and also inhibited MEK in cell-based assays and reduced human colon cancer xenograft growth, thus providing the first glimpse of anticancer activity for this class of drug (21). The noncompetitive inhibition suggested an allosteric inhibitory site; this was confirmed by finding a MEK inhibitor binding pocket adjacent to the ATP binding site (22). The unique binding pocket identified in MEK1 and MEK2 also suggests a mechanism that explains the high degree of specificity found for all of the MEK inhibitors.
Two additional high-potency MEK inhibitors have been developed that have advanced to the clinic. PD 0325901 is a second-generation analogue of CI-1040 with an IC_{50} of 1 nmol/L and is significantly more potent than CI-1040 in vivo, with a single oral dose providing >50% target inhibition at 24 h (23). Preclinical antitumor activity has been shown in a variety of human tumor xenograft models. AZD6244 (ARRY-142886) is another second-generation MEK inhibitor based on the structure of CI-1040 that also has improved pharmacologic properties (24). When tested against purified constitutively active MEK1, the IC_{50} for inhibition by AZD6244 was ~14 nmol/L. There were no significant inhibitory effects when tested against a broad range of serine/threonine kinases and the inhibition was noncompetitive with respect to ATP. Preclinical antitumor activity has been shown in a variety of human tumor xenograft models with evidence of excellent target inhibition (24, 25). Based on their high potency, oral bioavailability, and preclinical evidence of antitumor activity and target inhibition, PD 0325901 and AZD6244 represent the best candidate drugs to determine the efficacy MEK inhibition for treating human cancers.

**Clinical Advances**

Phase I testing of CI-1040 was done on a total of 77 patients with metastatic or locally advanced solid tumors with oral dosages ranging from 100 mg daily to 800 mg twice daily (26). Initial testing found that bioavailability using once-daily dosing on an empty stomach plateaued at a dose of 800 mg with a maximum plasma concentration (C_{max}) of 134 ng/mL. Using twice-daily administration, the C_{max} improved to 229 ng/mL, and absorption was not altered significantly whether the drug was taken on an empty stomach or with food. Overall, CI-1040 was well tolerated in this patient population. Forty-six (59.6%) patients experienced one or more drug-related toxicities, but only 2% were grade 3, including elevated liver function tests, atrial fibrillation, allergic reaction, cardiomyopathy, and asthenia. The most common drug-related toxicities included diarrhea, asthenia, acneiform rash, nausea, and vomiting. The recommended dose for future study was 800 mg orally twice daily.

CI-1040 displayed encouraging antitumor activity in this phase I trial. Of the 66 patients who were assessable for response, 1 patient had a partial response (pancreatic cancer) and 19 (28%) patients achieved stable disease with a median duration of 5.5 months. Importantly, target inhibition by CI-1040 was shown in patient samples both by immunohistochemistry in pretreatment and posttreatment tumor biopsies and by an in vitro assay in peripheral blood mononuclear cells. With serum concentrations >100 ng/mL, CI-1040 blocked phosphorylation of a number of mitogen-activated protein kinase (MAPK) pathways and therefore target inhibition by monitoring phosphorylated Erk suppression.

As a result of the findings in the phase I study, CI-1040 advanced to phase II testing in a multicenter, open-label trial in patients with metastatic or inoperable breast cancer, colon cancer, non–small-cell lung cancer, or pancreatic cancer (27). The study was designed to independently screen for single-agent response to CI-1040 in each tumor type (stage I) using a design that would advance enrollment (stage II) based on two coprimary end points, either objective response or clinical benefit response. For each tumor type, 13 patients were planned for evaluation in stage I and 30 patients in stage II. The study enrolled 67 patients, including 14 patients with breast cancer, 20 with colon cancer, 18 with non–small-cell lung cancer, and 15 with pancreatic cancer. Grade 3 adverse events occurred in 13 (19%) patients and included diarrhea, fatique, vomiting, and anorexia. There were no grade 4 adverse events. The study did not meet predetermined end points to progress to stage II for any tumor type.

Based on the phase II results, clinical development of CI-1040 was stopped, and a second-generation agent, PD 0325901, with higher potency and improved pharmacodynamic properties was introduced into the clinic (28–30). Advanced patients with breast cancer, colon cancer, non–small-cell lung cancer, or melanoma were treated in an open-label, dose-escalating design with doses ranging from 1 mg daily to 30 mg twice daily. Patients were initially treated using an intermittent dosing schedule that was subsequently changed to continuous dosing. Drug-related adverse events were similar to those reported for CI-1040, including rash, fatigue, nausea, diarrhea, and vomiting. Visual changes including blurred vision and haloes were reported in five patients receiving >15 mg twice daily. Dose-limiting toxicities reported included rash (3 patients), cardiac events (2 patients), and anemia/diarrhea/mucositis (1 patient).

Tumor biopsies from 19 of 35 patients were evaluated for target effect by monitoring phosphorylated Erk suppression. At doses >2 mg twice daily, phosphorylated Erk was suppressed by >84% compared with baseline expression (28). Steady-state plasma concentrations of PD 0325901 averaged >270 ng/mL at doses >15 mg twice daily, which would be predicted to result in near maximal phospho-Erk suppression based on xenograft studies. Encouraging evidence of antitumor activity was observed in this phase I trial with 2 partial responses in melanoma patients and an additional 8 patients (5 melanoma, 2 non–small-cell lung cancer, and 1 colon cancer) achieving stable disease that lasted for 3 to 7 months (30). Development of this compound, however, has been stopped because of toxicity.

The final MEK inhibitor to undergo clinical testing is AZD6244, which has been investigated in an open-label, multicenter two-part phase I trial in patients with advanced solid malignancies. Part A was designed to determine the maximum tolerated dose and to examine the safety, pharmacokinetic profile, and biological activity (31). Twenty-three patients were treated with escalating doses of AZD6244 orally, ranging from 50 mg twice daily to 300 mg twice daily. Common toxicities in this trial were similar to previous toxicities associated with MEK inhibitors and included rash, fatigue, edema, hypothyroidism, increased liver function tests, and diarrhea. The mean minimum plasma concentration in patients receiving continuous 200 mg twice daily dosing was 290 nmol/L, which exceeded the IC_{50} for MEK inhibition in vitro by ~50-fold. No objective responses were identified in part A, but four patients had prolonged stable disease.

Part B of this study was designed to confirm a sustainable dose for long-term therapy and to assess target modulation in tumor tissue. In part B, adverse events were similar to part A,
and the dose for future phase II studies was defined as 100 mg twice daily. Although there were no objective responses, stable disease was observed in 14 of 31 (45%) patients who were assessable for response. Nine of these patients had stable disease lasting longer than 5 months (6 melanoma, 1 breast cancer, 1 non–small-cell lung cancer, and 1 thyroid cancer). Pretreatment and posttreatment tumor biopsies obtained from 17 patients showed a mean 83% reduction in nuclear phospho-Erk staining. These results shows AZD6244-induced target modulation within tumor tissue. Based on these results, multiple phase II studies are ongoing.

In addition to the drugs described above, there are three other MEK inhibitors including a backup compound to AZD6244 that have entered the clinic.

Conclusions

Extensive preclinical data support the importance of the MAPK signaling pathway in cancer biology and its potential as a therapeutic target in human cancers. Although many strategies have been developed to suppress MAPK activity, small-molecule MEK inhibitors represent the most specific and effective strategy tested to date. Overall, data from 236 patients treated with CI-1040, PD 0325901, or AZD6244 have been reported (26–31). In general, MEK inhibitors seem to be well tolerated with rash, edema, and transient blurred vision emerging as class effects. Importantly, target suppression has been shown for each compound in tumor tissue and peripheral blood mononuclear cells, and achievable plasma concentrations correspond to levels sufficient to inhibit MEK in vitro. Although data are primarily from phase I trials, there is preliminary evidence of clinical activity, four partial responses have been observed, and stable disease has been reported in 53 of 236 (22%) patients. Future phase II studies will aim to further define the clinical utility and role of the small-molecule MEK inhibitors.

Although the preclinical data are extensive, the objective response rate in these studies has been modest, as described above. Several factors may contribute to this modest response rate. First, although target suppression has been shown in tumor tissue, the relative level of suppression in tumors needed to produce cytotoxicity is not clear. Second, the most commonly mutated members of the MAPK pathway (Ras and Raf) have additional targets besides MEK, and it is likely that alternative pathways retain the ability to compensate for the effects of the MEK inhibitors. Third, cancer most often results from dysregulation of multiple signaling pathways, and inhibition of only a single pathway may not be sufficient to promote apoptosis or growth arrest. Finally, although MAPK is activated in many tumor cells, its function may not be necessary for tumor growth and survival in a number of tumors.

Targeted therapies such as small-molecule MEK inhibitors are likely to benefit only a subset of patients as single agents. Efforts to identify predictive molecular markers of response have been successful for some targeted therapies. The identification of EGFR mutations that predict for response to EGFR inhibitors is one notable example (32). To date, no clear association has been made between either baseline activated Erk levels or magnitude of phosphorylated Erk suppression and clinical benefit in patients treated with MEK inhibitors. Recent in vitro studies have identified a specific BRAF mutation, V600E, that predicts for sensitivity to the MEK inhibitor CI-1040 (33). Similar results have also been shown using AZD6244 (34), but the mechanism responsible for sensitivity in cell lines harboring this mutation has not been elucidated. BRAF V600E is an activating mutation that is found at high frequency in specific malignancies including melanoma and thyroid cancers. Suppression of BRAF V600E expression in melanoma xenografts or a metastatic model induced tumor regression or growth inhibition (35), whereas suppression of BRAF V600E in papillary thyroid carcinoma cell lines reduced proliferation, transformation, and tumorigenicity (36). These results suggest that identification of the BRAF V600E in patient tumors may represent a viable biomarker to predict clinical responsiveness to MEK inhibitors.

Clinical studies done with MEK inhibitors have examined only single agent activity. Given the excellent safety profile of these drugs, it is likely that combinations including either cytotoxic agents or other targeted therapies will be tested in the future. Preclinical data support the development of MEK inhibitor combinations. CI-1040 sensitizes human lung tumor heterotransplants to paclitaxel (37), and AZD6244 enhanced the antitumor activity of both docetaxel and irinotecan in human colon cancer xenografts (38). Synergistic or additive cytotoxicity has been observed between MEK inhibitors and targeted therapies including EGFR inhibitors (38), tamoxifen (39), phosphatidylinositol 3-kinase inhibitors (40), and histone deacetylase inhibitors (41). Presently, a phase II study of AZD6244 in combination with capcetabine is ongoing in patients with metastatic or locally advanced pancreatic cancer. Future studies including MEK inhibitor combinations are likely and may show the true utility of MEK inhibitors in cancer treatment given the modest benefits seen to date in single agent studies.

The clinical development of MEK inhibitors rests on extensive preclinical and laboratory-based studies that support the MAPK pathway as a viable target for cancer therapy. Small-molecule MEK inhibitors have proved to be well tolerated in early studies and reports of clinical activity are encouraging. Future studies will aim to further define the clinical utility of this exciting novel class of therapeutics.

References

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Advances in Targeting the Ras/Raf/MEK/Erk Mitogen-Activated Protein Kinase Cascade with MEK Inhibitors for Cancer Therapy

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