

Novel Targets in Solid Tumors: MEK Inhibitors

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Abstract: MAPK/ERK kinase (MEK) inhibitors constitute a promising class of novel targeted therapies. Although some of the initial clinical results with these compounds were disappointing, newer agents with more advantageous pharmacokinetic and pharmacodynamic properties have entered clinical trials. Partial responses have been seen in patients with advanced melanoma and pancreatic cancer, along with prolonged stable disease in several tumor types. Rash, diarrhea, edema, and visual disturbances have been common toxicities. With the recent finding that cell lines with B-raf mutations are exquisitely sensitive to these compounds, the possibility of genetic-based patient selection and individualized therapy has been raised. Whether these predictions will be borne out in clinical testing remains to be seen.

Though significant incremental progress has been made in the treatment of some solid tumors in the last few years, most remain incurable and new agents are clearly needed. Advances in the understanding of cancer biology have identified molecular targets for novel drugs. MAPK/ERK kinase (MEK) is such a target with inhibitors in clinical development after several decades of preclinical work. Progress in the understanding of how MEK functions has led to second-generation compounds with more promise for clinical development. Emblematic of this progress is the incorporation of pharmacodynamic studies on both tumor and normal surrogate tissues (such as peripheral blood mononuclear cells [PBMCs]) to show proof of mechanism and proof of principle, which can be critical in the drug development process. Phase I studies of MEK inhibitors have shown acceptable toxicity profiles at doses that appear to inhibit the target. Both responses and prolonged stable disease have been seen in early safety-oriented trials, and efficacy studies are planned or underway.

MAPK/ERK Pathway

Numerous critical growth factors and cytokines transduce their signals from the cell membrane to the nucleus via protein kinase networks called signal transduction pathways, which have become major targets for anticancer drugs (Figure 1). An important example is the mitogen-activated protein kinase (MAPK), or extracellular signal-regulated protein kinase (ERK) pathway.¹ This pathway has

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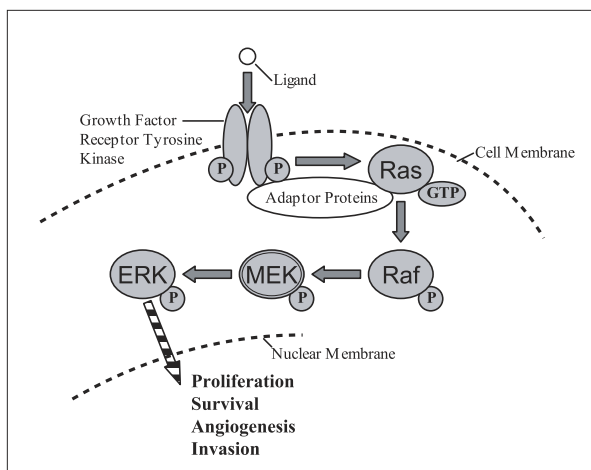


Figure 1. Schematic of ERK pathway.

Note: After binding of the ligand, growth factor receptor tyrosine kinases (eg, epidermal growth factor receptor) become activated and induce the binding of adaptor proteins. Ras is activated to its guanine triphosphate (GTP)-bound form, and in turn activates the kinase activity of RAF, which then phosphorylates MEK. Activated MEK then activates mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK). ERK acts on multiple downstream kinases and transcription factors, leading to changes in gene regulation which ultimately results in cell proliferation, survival, angiogenesis, and invasion.

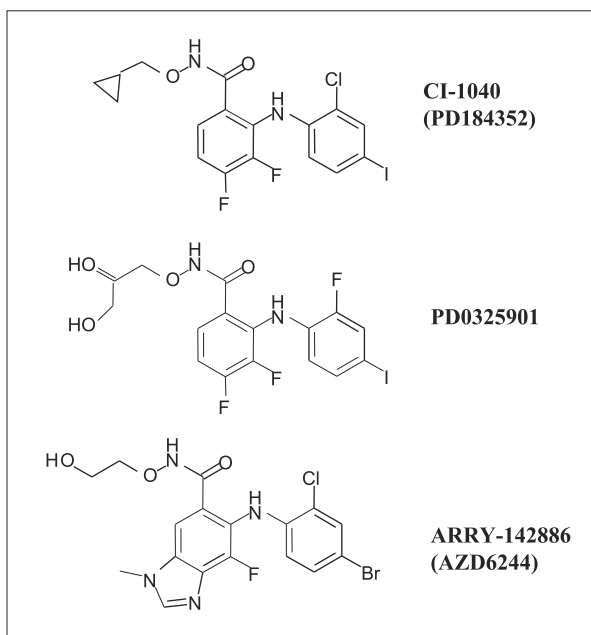


Figure 2. Chemical structure of selected MEK inhibitors.

been shown to be constitutively activated in a number of human cancers. Activation of the pathway causes gene expression changes and changes in cell proliferation, survival, and differentiation.²

When extracellular growth factors such as the epidermal growth factor (EGF) bind to receptors (such as EGF

receptor [EGFR]), conformational changes are induced in the receptor, which lead to autophosphorylation, receptor dimerization, and recruitment of proteins such as Ras at the inner surface of the cell membrane.³ Ras stimulates Raf activation, which in turn phosphorylates MEK, which then activates ERK. At each step in the pathway, phosphorylation of the next signaling member is required for activation and downstream phosphorylation of the next protein kinase. ERK coordinates responses to extracellular signals by regulating gene expression, cytoskeletal rearrangements, and metabolism, as well as cell proliferation, differentiation, and apoptosis.

Multiple lines of evidence indicate that the MAPK pathway is important in human cancer. Inappropriate Ras activation is associated with nearly a third of all human cancers.⁴ One of the raf paralogs, B-raf, is mutated in many cancers, including malignant melanoma (27–70%), papillary thyroid cancer (36–53%), ovarian cancer (30%), and colorectal cancer (5–22%), and the mutations are frequently gain-of-function substitutions that result in constitutive activity.⁵ ERK is elevated in nearly 50% of breast cancers and is associated with a poor prognosis.^{6–8} Over one third of tumor cell lines have constitutive activation of the ERK pathway.⁹

MEK occupies a central role in the MAPK pathway. Expression of constitutively active forms of MEK leads to transformation of cell lines.^{10,11} MEK kinases have dual kinase activity, with phosphorylation of both serine/ threonine and tyrosine. There are two MEK homologs, MEK1 and MEK2, which are 80% amino-acid identical, have very similar three-dimensional structures, and are similarly targeted by known inhibitors.^{12,13} Both kinases are highly specific, with no known substrates aside from ERK.¹⁴ Despite these similarities, there are a few differences between the MEK homologs. MEK2 is approximately seven times more catalytically active than MEK1,¹⁵ yet MEK2 knock-out mice are fully viable, whereas MEK1 knock-out is embryonically lethal.^{16,17} The general interpretation of these findings is that MEK1 is able to compensate for the absence of MEK2.¹ Constitutively active MEK1 expression in transgenic mouse models has been shown to have substantial effects, such as increased cell numbers and size in every tissue tested, including cardiac myocytes, chondrocytes, and skin.¹⁸

It is interesting to note that most of the known MEK inhibitors are noncompetitive (ie, they do not bind with the adenosine triphosphate [ATP]-binding site of the kinase).¹⁹ Structural analysis of MEK demonstrates a unique inhibitor binding site adjacent to the ATP site.¹³ This property is thought to explain the selectivity of the MEK inhibitors, because although the ATP-binding pocket is highly conserved among different human protein kinases, the pocket bound by MEK inhibitors is

Table 1. Selected MEK Inhibitors Undergoing Clinical Testing

Drug	IC ₅₀	Phase	N	Doses Tested	RD	DLTs	Common Toxicities (>10%)	Clinical Response	PD Markers
CI-1040 (PD184352)	2 μM	Phase I ²⁵	77	100–1600 mg QD; 800 mg TID	800 mg BID*	Fatigue	Diarrhea, fatigue, rash, N/V	1 PR (pancreas) 19 SD (28%)	post-Rx tumor pERK suppression shown (IHC; n=10)
		Phase II ²⁶	67	800 mg BID*	–	–	Diarrhea, N/V, fatigue, rash, edema, abdominal pain, anorexia	8 SD	Baseline archived tumor pERK expression (IHC)
PD0325901	<1 nM	Phase I ²⁸	41	1–30 mg BID	15–20 mg BID	Rash, syncope	Rash, diarrhea, fatigue, visual changes, N/V, edema, pruritis, anemia, dyspepsia	2 PR (melanoma) 8 SD	Post-Rx tumor pERK suppression shown (IHC, n=13)
ARRY-142886 (AZD6244)	10 nM	Phase I ³¹	23	50–300 mg BID	200 mg BID	Hypoxia, rash, diarrhea	Rash, diarrhea, N/V, fatigue, edema, altered taste, blurred vision	4 SD [†]	Post-Rx PBMC pERK (TPA-stimulated; flow cytometry)

* Administered with food.

† Four subjects remained on study for more than four 28-day cycles.

DLTs = dose-limiting toxicities; IC₅₀ = concentration to inhibit tumor cell growth by 50%; IHC = immunohistochemistry; N/V = nausea/vomiting; PBMC = peripheral blood mononuclear cells; PD = pharmacodynamic; pERK = phosphorylated (activated) ERK; PR = partial response; RD = recommended dose for phase II testing; SD = stable disease; TPA = 12-O-tetradecanoylphorbol-13-acetate.

unique. Binding of an inhibitor is thought to stabilize the inactive conformation of the kinase. This interaction is believed to explain the high degree of specificity of the MEK inhibitors compared to other kinase inhibitors with competitive activity.

MEK Inhibitor Compounds

Several MEK inhibitors have been identified (Table 1 and Figure 2). PD98059 was the first specific MEK inhibitor and has been a useful tool in studying the MEK/ERK pathway.^{20,21} In cell line studies, PD98059 treatment has been shown to reverse the malignant phenotype of mutated ras.²¹ Another highly specific, non-ATP-competitive inhibitor used in preclinical studies is UO126, which is more potent than PD98059.^{22,23} Both compounds, however, have limited in vivo activity in blocking phosphorylation of ERK and thus were not further developed.²⁴

CI-1040

CI-1040 (PD184352, Pfizer) is an oral MEK inhibitor with promising preclinical activity that led to its clinical development. In in vitro studies of a breast cancer cell

line, MDA-MB-231, CI-1040 at a concentration of only 1 μmol/L inhibited ERK1 and ERK2 phosphorylation by 99% and 92%, respectively, and also inhibited growth in soft agar.²⁵ Animal studies showed promising activity, particularly in xenografted pancreas, colon, and breast cancer cell lines. Inhibition of ERK phosphorylation was reversible, lasting for at least 6 hours and returning to baseline at 24 hours. CI-1040 exhibited antitumor activity in xenografts with high baseline levels of phosphorylated ERK (pERK), and activity was also correlated with inhibition of pERK in tumor tissue.

Based on these promising data, CI-1040 underwent phase I testing in 77 patients with advanced solid tumors (including lymphoma).²⁵ In addition to the usual safety, toxicity, and pharmacokinetic components, biomarker studies were performed including activation of ERK in PBMCs as well as tumor biopsies in selected cases. Doses ranged from 100 mg to 1,600 mg and the tested schedules included two, three, and four times daily for 3 of every 4 weeks, or continuous. The mean terminal half-life was found to be 21 hours for the parent compound, but concentrations of an acid metabolite, PD0184264 (with reduced cellular antitumor activity²⁵), were found to be

30-fold greater. After a plateau in drug concentrations was seen at 1,600 mg/day, divided dose schedules were tested. The 800 mg twice daily oral dose level was carried forward to phase II testing after it was found that thrice-daily dosing did not appreciably increase drug concentrations. A high-fat meal increased the area under the curve and maximum plasma concentration by 3- to 5-fold, so CI-1040 was subsequently administered with food.

In the phase I study, CI-1040 was found to be well-tolerated with no grade 4 (Common Toxicity Criteria v2.0) toxicities and only a limited number of grade 3 toxicities. The majority of toxicities (98%) were grade 1/2 and included diarrhea (43%), fatigue (30%), rash (18%), and nausea/vomiting (16%). Fatigue led to dose interruptions and/or reductions. Antitumor activity was seen in 1 pancreatic cancer patient who had a partial response (PR) lasting 12 months. Nineteen (25%) subjects had stable disease for 3 months, and this observation was commonly associated with symptomatic benefit. As for the biomarker studies, a dose-dependent inhibition of phorbol myristate acetate stimulation of ERK activation in PBMCs was seen at approximately 30 ng/mL. Ten tumor biopsies were analyzed and showed 46–100% inhibition of pERK.

On the basis of these results, a multicenter, parallel-arm phase II study of CI-1040 was performed in patients with advanced breast, colon, pancreatic, and non-small cell lung (NSCL) cancers.²⁶ This trial essentially comprised four simultaneous Simon two-stage phase II design studies in each tumor type, with early stoppage after 13 patients if the criteria of at least one partial response (PR) or complete response (CR), or four clinical benefit responses (CBRs = CR + PR + stable disease), were not met. If these criteria were satisfied, then stage 2 would open, for a total of 30 patients of each tumor type. These criteria were calculated based on a null response rate of 5%, and a positive response rate of 20%, or 40% CBR. Due to a lack of responses, the trial was closed after the first stage in all arms.

Sixty-seven patients were enrolled, with some arms having more than 13 patients due to rapid accrual: 14 breast, 20 colorectal, 18 NSCL, and 15 pancreatic cancer patients. The majority (90%) had an Eastern Cooperative Oncology Group performance status of 0–1, and 75% had received either one or no prior chemotherapy regimens. As with the phase I trial, CI-1040 was relatively well-tolerated, with 19% experiencing grade 3 toxicities and no patients having grade 4 toxicities. The toxicities included diarrhea, nausea, fatigue, rash, edema, abdominal pain, anorexia, and facial edema. Eight patients experienced disease stabilization at 3 months (1 breast, 2 colon, 2 pancreas, and 3 NSCL cancers). No patients had a CR or PR. Pharmacokinetic results were

very similar to those in the phase I trial. As for biomarker studies, no tumor biopsies or PBMCs were obtained for post-treatment analysis. Instead, immunohistochemical staining with quantitative analysis was performed on archival tumor specimens. It was found that pERK was elevated in most tumor types and a nearly significant relationship was seen between pERK and the possibility of stable disease ($P < .055$) on logistic regression. Based on these results, it appears that CI-1040 will not be further developed in these tumor types.

PD0325901

A second-generation oral MEK inhibitor, PD0325901 (Pfizer), is structurally very similar to CI-1040 but has markedly improved properties, including 50-fold more potency against MEK, better bioavailability, and longer MEK suppression (24 hours instead of 6–8 hours for CI-1040). Anticancer activity has been demonstrated against a range of cancer cell lines.²⁷ A multicenter phase I clinical and pharmacokinetic study of PD0325901 has been performed in 41 patients with melanoma (n=27) or breast (n=7), NSCL (n=5), and colon (n=2) cancers.^{28,29} After a single cohort at 1 mg orally daily, the dosing was changed to twice daily, ranging from 1 to 30 mg, with continuous dosing at the 15 mg and 20 mg twice daily levels. Rash, elevated liver tests, and syncope were dose-limiting, and diarrhea, fatigue, visual disturbances, nausea, and edema were seen in nearly one third of the subjects. The rash was acneiform and appeared similar to that seen with EGFR inhibitors. Visual disturbances included halos, spots, and decreased acuity. Two subjects experienced congestive heart failure (CHF), 1 with a history of CHF and 1 new-onset. Pharmacokinetic studies indicated a dose-dependent increase in exposure, with doses of 15 mg or higher exhibiting a prolonged plasma concentration above 50 ng/mL. The 50% inhibitory concentration (IC_{50}) for susceptible cell lines ranged from 5 to 53 ng/mL.

PD0325901 exhibited modest antitumor activity against this fairly heavily pretreated patient population. Two melanoma patients had PRs, both at the 20 mg twice daily dose level. Eight subjects at various dose levels experienced stable disease lasting 3–7 months: 5 with melanoma, 2 with NSCL cancer, and 1 with colon cancer. This compound is currently undergoing phase II testing in these tumor types.

Importantly, tumor biopsies were obtained prior to treatment and 15 days post-treatment in the phase I study with PD0325901 and stained for pERK, the activated form of the kinase that is the target of PD0325901. In subjects with melanoma, over 80% inhibition compared to baseline was seen at the lowest dose level (1 mg daily), and other tumor types showed similarly strong inhibition

at various dose levels as well. However, even subjects at the highest dose levels (20 and 30 mg twice daily) did not demonstrate any inhibition of the target as assessed by immunohistochemistry.

ARRY-142886

ARRY-142886 (AZD6244; Array BioPharma/AstraZeneca) is another potent, highly specific MEK inhibitor with nanomolar activity against purified MEK and antitumor activity against a wide range of cell lines in mouse models.³⁰ Antitumor activity has been shown to be correlated with decreased pERK levels in xenografted tumors. ARRY-142886 has undergone phase I testing in a recently reported trial of 23 patients with a variety of tumor types.³¹

In the phase I study, subjects were given an oral liquid dosage of ARRY-142886 for 28 consecutive days at doses ranging from 50 mg to 300 mg twice daily. Twenty-three patients with melanoma (n=7) or colorectal (n=3), breast (n=2), bronchoalveolar carcinoma (n=2), and multiple other solid tumor types were enrolled. Dose-limiting toxicities included hypoxia (n=1), rash (n=2), and diarrhea (n=1). Nausea, fatigue, peripheral edema, altered taste, and blurred vision were also seen. Exposure appeared to be dose-related, and the mean half-life was 10 hours at the recommended phase II dose of 200 mg twice daily. TPA (12-O-tetradecanoylphorbol-13-acetate)-induced ERK phosphorylation in PBMCs was maximally inhibited by 65–80%, correlated with plasma concentrations of ARRY-142886, and decreased in a time-dependent manner. The best response reported was stable disease, with 4 subjects (3 with melanoma, 1 with NSCL cancer) remaining on study for four 28-day cycles, and 2 subjects with melanoma on study for 8 and 10 cycles. A second part of the phase I study is ongoing, with multiple biomarkers assessed at both 100 mg and 200 mg twice daily.

Future Directions

Recently, integrated genetic and pharmacologic analysis has shown that B-raf (but not N-ras) mutations predict sensitivity to MEK inhibitors.³² In these experiments, cell lines harboring the most common B-raf mutation (V600E) were exquisitely sensitive to CI-1040, with IC₅₀ values of 0.024–0.11 μM. Cell lines with wild-type B-raf, or either wild-type or mutant N-ras, were not sensitive despite showing effective inhibition of pERK. These results were recapitulated in xenograft experiments. B-raf mutant cells thus seem to be critically dependent on MEK-ERK, making them very sensitive to MEK inhibitors. The authors called for clinical trials with MEK inhibitors in which patients are stratified based on B-raf mutation status. This is akin to stratifying subjects with EGFR mutations to treatment with erlotinib (Tarceva, Genentech/OSI),

and holds the promise of individually tailored therapy based on genetic analysis of tumors. However, as MEK is downstream of multiple growth factor pathways, patient selection could be difficult.

Combination strategies with MEK inhibitors have shown preclinical promise. One of the original MEK inhibitors, PD98059, was found to have synergistic effects on breast cancer cell lines when combined with the EGFR inhibitor gefitinib (Iressa, AstraZeneca)³³ as well as additive effects with tamoxifen.³⁴ In melanoma cell lines, PD98059 and phosphatidylinositol 3-kinase inhibitors have shown additive activity.³⁵ In NSCL cancer cell lines, synergism between CI-1040 and mTOR (mammalian target of rapamycin) inhibitors has been demonstrated.³⁶ Successful combination strategies with conventional cytotoxic chemotherapy agents such as docetaxel (Taxotere, Sanofi-Aventis) have also been shown preclinically in xenograft models, demonstrating both antiproliferative and antiangiogenic effects.³⁷ Indeed, antiangiogenic properties of MEK inhibitors have been demonstrated in multiple preclinical models³⁷⁻³⁹ and may be found to represent an important element of clinical antitumor activity in the future.

Preclinical studies with MEK inhibitors have also been performed in multiple other tumor types, such as hepatocellular carcinoma⁴⁰ as well as hematologic malignancies. Both PD98059 and CI-1040 directly impair leukemia cell growth,⁴¹ and CI-1040 has been shown to markedly increase apoptosis of human leukemia cells when combined with imatinib (Gleevec, Novartis) or the checkpoint abrogator UCN-01.^{42,43} The clinical relevance of these findings remains unknown.

Finally, another avenue for future exploration with these compounds is the mechanism of toxicities. Side effects such as rash and diarrhea are commonly seen with small molecule tyrosine kinase inhibitors of the EGFR pathway such as erlotinib, and therefore their emergence during phase I/II clinical testing of MEK inhibitors was not surprising. However, there are a few unique toxicities with MEK inhibitors, such as edema and visual disturbances. It is unknown whether these clinical findings are due to MEK inhibition itself, which seems more likely as they have been seen across multiple compounds in the class, or perhaps some off-target effect. Further experience and testing of these compounds may help elucidate these issues in the future.

Conclusions

Despite impressive preclinical results, the clinical activity for the lead MEK inhibitor compound, CI-1040, was disappointing. The reasons behind the lack of success are unknown, but many questions were raised by the results. It is unclear whether objective tumor response is the best outcome for this drug class, or whether some sort of

randomized design (such as randomized discontinuation) should have been chosen to evaluate stable disease.⁴⁴ The optimal degree of MEK signaling inhibition, with over 90% suppression seen in only 3 of 10 biopsy patients, is also unclear. The increased potency of the second-generation MEK inhibitors may solve this issue, but the optimal amount of target suppression is unknown. Another issue in the development of such signal transduction inhibitors is patient selection. Screening patients for activating B-raf mutations (in a “pharmacodiagnostic” manner), so that only patients who have the best chance of responding based on what is known about these compounds are included, is an attractive approach. The economic viability of such testing as a drug development strategy is also in question. Finally, the significant multilayering and cross-talk that are present in many signal transduction pathways such as MAPK/ERK presents their own set of challenges. Whether these agents will be successful alone, or will require combination strategies, remains to be seen.

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