Clinical experience of MEK inhibitors in cancer therapy

Ding Wang a, Scott A. Boerner a, James D. Winkler b, Patricia M. LoRusso a,⁎

a Department of Internal Medicine, Karmanos Cancer Institute, Wayne State University, 4th Floor HWCRC, 4100 John R, Detroit, MI 48201, USA
b Array BioPharma, Boulder, CO 80301, USA

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Abstract
Finding new therapies to assist in the treatment of cancer is a major challenge of clinical research. Small molecules that inhibit different molecular targets at the different levels of the MAPK pathway have been developed. Several MEK inhibitors have been examined in early-phase clinical trials and the current state of clinical results using these therapies is presented here.
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1. Mitogen-activated protein kinase (MAPK) pathway

Proliferation, differentiation, and cell death are coordinated processes that help maintain homeostasis among the diverse cell types in higher organisms. Growth factors play a key role in the control of these processes and exert this function by triggering signal transduction cascades upon binding to their cognate membrane receptors. Given the importance of homeostasis and the multiple components involved, it is not surprising that any component of these regulatory cascades, from the growth factor to the final effector, may become unregulated and contribute to transformation and tumorigenesis.

Through years of study, the Ras-mitogen activated protein kinase (MAPK) pathway has emerged as the central piece of a signaling network regulating cell growth and survival. The MAPK pathway acts by transferring growth-promoting signals from the cell surface or cytoplasm to the nucleus through a sequential protein kinase cascade that regulates a wide array of substrates, including transcription factors, cytoskeletal elements, and other protein kinases. These signals have been demonstrated to contribute to a myriad of cellular functions, including cell division, proliferation, growth, differentiation, movement and cell death.

The MAPK pathway has been widely observed to be dysregulated in various human malignancies. Constitutive activation of the MAPK pathway has been demonstrated to contribute to malignant transformation of mammalian cells, and has been associated with an aggressive neoplastic phenotype, including uncontrolled cell proliferation and resistance to apoptosis. A wide spectrum of inhibitors against the components of this pathway have been sought after, discovered and investigated, both in vitro and in vivo, and they have demonstrated anticancer effects by suppressing tumor growth, limiting cancer invasion and/or inducing apoptosis of cancer cells.

2. RAS and BRAF mutations in human cancers

Constitutive activation of the MAPK pathway may contribute to cancer cell resistance to chemotherapy in many human malignancies, including pancreatic, colon, lung, ovary, breast, thyroid and kidney cancers [1–3]. Thus, identifying mutations in critical genes of the MAPK pathway which result in abnormal activity has been a high priority of researchers.

RAS proteins were some of the first proteins to be identified as having a role in the regulation of cell growth [4]. Human tumors frequently express RAS proteins that are constitutively activated by point mutations; approximately 20% of all tumors have an activating mutation in at least one RAS gene [5]. Aberrant activation of RAS proteins contributes significantly to the malignant phenotype by deregulating tumor cell growth in the tumor, apoptosis, invasiveness, and angiogenesis [6]. In order to be biologically active, RAS proteins require post-translational modification. Enzymes performing this modifica-
BRAF mutations occur at a high frequency in melanomas, and to a lesser extent in thyroid, ovarian, colon, lung, and other tumor types [9–13]. Most of these BRAF mutations are located in the kinase domain, leading to elevated kinase activity and transforming activity [13]. The identification of BRAF as an oncogene led to renewed interest in the discovery and development of pharmacologic inhibitors to target BRAF proteins. One of these, BAY 43-9006 (Sorafenib), was initially developed as a targeted specific BRAF inhibitor and entered into Phase I and Phase II testing, but was later found to inhibit VEGF and PDGF receptors on blood vessel cells [14]. While several pharmaceutical companies are now developing agents that specifically target BRAF, clinical trials of these inhibitors have yet to be performed.

Mutations in RAS and BRAF typically demonstrate mutual exclusivity in tumors; therefore, either mutation might exert its oncogenic activity through common downstream proteins such as the mitogen-activated protein kinase kinase (MEK) and the extracellular signal-related kinase (ERK), and these enzymes may be better exploited as drug targets. Solit et al. examined the effects of BRAF mutations on the activity of the downstream MEK and ERK kinase cascade [15]. The authors used small-molecule MEK inhibitors in cells with RAS or BRAF mutations and found that tumors with BRAF mutations demonstrated enhanced sensitivity to MEK inhibition when compared with wild-type cells and cells harboring various RAS mutations. In addition, following treatment with MEK inhibitors, growth of tumors in BRAF-mutant xenografts was completely suppressed whereas RAS-mutant tumors were only partially inhibited. The finding that tumors containing mutations in BRAF are much more dependent on MEK inhibition than tumors with mutant RAS has implications for drug development; it is possible that MEK inhibitors have a selectivity that could potentially be exploited in the treatment of BRAF-mutation-dependent cancers.

3. MEK inhibitors

Numerous small molecules that inhibit different molecular targets, at the different levels of the MAPK pathway, have been discovered [16–20]. Some of these have recently entered human clinical trials to evaluate their safety, toxicities, and to assess their activity against various cancer types. This targeted therapy includes the clinical development of drugs that specifically inhibit MEK. MEK inhibitors represent the first selective inhibitors of MAPK pathway activation to enter the clinic. Several MEK inhibitors have been examined in early-phase clinical trials and the current state of clinical results using these therapies is presented here (Table 1).

### 3.1. PD098059 and U0126

The first MEK inhibitor to be disclosed was PD 098059 [2-(2′-amino-3′-methoxyphenyl)-oxanaphthalen-4-one] [17]. It has been mostly used in cell systems to study MEK inhibition in order to further delineate the role of the MAPK pathway in carcinogenesis [17,21–25]. Similarly, U0126, a second MEK inhibitor with more potency than PD 098059, has been mostly used as an in vitro laboratory reagent [16,26,27]. These particular inhibitors are non-competitive with ATP and act on the MAPK cascade by preventing the activation of MEK and not by inhibiting MEK activity directly [28]. In an in vitro assay, none of these compounds significantly inhibited the activity of a large panel of protein kinases, including ERK1, c-Jun N-terminal kinase (JNK) 1, and p38 MAP kinases [28]. Unfortunately, PD98059 has not been shown to be sufficiently soluble nor sufficiently bioavailable to be conducive to clinical testing (Sebolt-Leopold, J.S., personal communication). Due to this limitation, PD98059 only has activity in vitro. The only in vivo activity reported for U0126 was by intraperitoneal administration [29]. Based on the lack of reported oral activity for U0126, it presumably suffers from the same limitations as PD98059.

### 3.2. CI-1040 (PD184352)

Due to the attractive anticancer potential of MEK inhibitors, continued efforts have been invested in their discovery and development. CI-1040 (PD 184352) was the first MEK inhibitor reported to inhibit tumor growth in vivo [18,19]. In mice with colon carcinomas of both mouse and human origin, tumor growth was inhibited by as much as 80% after treatment with CI-1040 [18]. Reported toxicity was minimal, and efficacy correlated with a reduction in the levels of phosphorylated ERK (pERK) in excised tumors.

Based on such preclinical activity, CI-1040 became the first MEK inhibitor moved into a clinical trial [30]. This initial phase

<table>
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<tr>
<th>Agent</th>
<th>Phase</th>
<th>Adverse events</th>
<th>Clinical activity</th>
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<tbody>
<tr>
<td>CI-1040 (PD 184352)</td>
<td>I</td>
<td>Diarrhea, nausea, asthenia, and rash</td>
<td>Sixty-six patients evaluable for response. One patient with pancreatic cancer achieved PR. Nineteen additional patients with a variety of solid tumors achieved SD.</td>
</tr>
<tr>
<td>PD 0325901</td>
<td>II</td>
<td>Diarrhea, nausea, asthenia, and rash</td>
<td>No response documented. Eight patients with a variety of solid tumors achieved SD.</td>
</tr>
<tr>
<td>ARRY-142886 (AZD6244)</td>
<td>I</td>
<td>Rash, diarrhea, visual disturbance, nausea, edema, pruritis, anemia, and dyspepsia</td>
<td>Twenty-seven patients evaluable for response. Two patients with melanoma achieved PR. Eight additional patients with a variety of solid tumors achieved SD.</td>
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<tr>
<td></td>
<td></td>
<td>Rash, diarrhea, nausea, fatigue, peripheral edema, vomiting, change in taste, and blurred vision</td>
<td>Of 57 patients, 39 completed 1 cycle, 19 achieved SD, 9 of which were &gt; 5 months.</td>
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I study was undertaken in patients with advanced solid tumors with the purpose of defining the toxicity, pharmacokinetics, pharmacodynamics, maximum tolerated dose (MTD) and initial clinical activity of CI-1040. Similar to PD 098059 and U0126, the mechanism of MEK 1/2 inhibition is non-competitive with respect to ATP, as the compound binds to a unique allosteric site, thus ensuring its strict specificity against its target.

As the first ever human trial with this compound, extensive efforts were made to determine an acceptable treatment schedule. CI-1040 was initially administered once daily from 100 mg/day all the way up to 1600 mg/day. To achieve a maximal pharmacokinetic (AUC) exposure within the MTD, multiple daily dosing of CI-1040, including 500 mg bid and tid, were also tested. In addition, the effect of food intake on drug absorption was also studied in this trial. In order to assess MEK inhibition, levels of ERK were evaluated from both peripheral blood mononuclear cells (PBMCs) and through biopsies of specimens by quantitative immunohistochemistry.

A total of 77 patients received at least one dose of CI-1040 and were assessable for toxicity. The most frequent tumors types enrolled included colorectal (n=25), non-small-cell lung (n=10), pancreatic (n=6), melanoma (n=6), and kidney (n=5). The most common toxicities were generally grade 1 or 2 in severity and included diarrhea, asthenia, rash, nausea, and vomiting. There were no drug-related grade 4 events. Administration with food increased oral absorption of CI-1040 by three- to five-fold, and continuous dosing of 800 mg bid with food was determined to be safe for phase II testing. Increasing the dosing frequency from once daily to bid resulted in a substantial increase in daily exposure (AUC) at steady-state, with the tid regimen providing minimal increased exposure over bid at steady state. Sixty-six patients were assessable for response. One patient with pancreatic cancer achieved a partial response (PR) lasting 12 months, and 19 additional patients, with a variety of solid tumors, achieved stable disease (SD) lasting a median of 5.5 months (range, 4 to 17 months). Phosphorylated ERK levels were measured in tumor samples by quantitative immunohistochemistry and were found to be inhibited by an average of 71% (range, 46% to 100%), indicating promising on-target activity.

On the basis of the encouraging phase I results, a phase II, multicenter, parallel arm study was carried out in patients with advanced breast cancer, colon cancer, non-small-cell lung cancer (NSCLC), and pancreatic cancer [31]. Due to the poor metabolic stability and bioavailability of the drug as demonstrated by the Phase I study, patients received oral CI-1040 continuously at a high dose of 800 mg bid. Expression of pERK, pAkt, and Ki-67 were assessed in archived tumor specimens by quantitative immunohistochemistry.

A total of 67 patients with breast (n=14), colon (n=20), NSCLC (n=18), and pancreatic (n=15) cancer received at least one dose of CI-1040. Treatment was well tolerated, with 81% of patients experiencing toxicities of grade 2 or less severity. Only 13 patients (19%) experienced toxicities of grade 3 severity. Among the most common toxicities (≥ 10% of patients) observed in this trial, in descending order of incidence, were diarrhea, nausea, asthenia, rash, edema, vomiting, abdominal pain, anorexia and facial edema. These toxicities were generally mild or moderate in severity. A serial measurement of left ventricular ejection fraction (LVEF) after three cycles of treatment was performed in 28 patients. Seven of them experienced asymptomatic decreases of LVEF ≥ 10%. An additional patient developed a symptomatic decrease of LVEF from 60% at baseline to 15% at a time when the patient had complications of sepsis in the intensive-care setting. At this time, it remains unclear whether the decrease of LVEF observed in some of the patients was a result of MEK inhibition from CI-1040 therapy or was due to cardiac deconditioning resulting from their progressive deteriorating nutritional status and advanced malignancies.

In contrast to the phase I study, no complete or partial responses were observed. A mild association (P<.055) between baseline pERK expression in archived tumor specimens and SD was observed. Stable disease, lasting a median of 4.4 months (range, 4 to 18 months), was confirmed in eight patients (one breast, two colon, two pancreas, and three NSCLC patients). While CI-1040 was generally well tolerated, its antitumor activity, metabolic stability, and bioavailability were considered insufficient to warrant further development in the four tumor types tested. Development of CI-1040 was terminated in favor of developing a more potent and biopharmaceutically superior compound.

3.3. PD 0325901

A second-generation oral MEK inhibitor, compound PD 0325901, was subsequently developed. Relatively minor changes distinguish the chemical structure of PD 0325901 from that of CI-1040. The cyclopropylmethoxy group of CI-1040 was replaced with a (R)-dihydroxy-propoxy group and the 2-chloro substituent of CI-1040 was replaced with a 2-flouro group on the second aromatic ring (Fig. 1). Nevertheless, these minor structural changes imparted significant increases in potency with PD 0325901. In pre-clinical testing, potency for target inhibition by PD 0325901 (pERK suppression) was increased >90-fold relative to CI-1040, and pre-clinical efficacy (the dose necessary to achieve a 70% complete response (CR) in the murine C26 cellular assay) was increased >30-fold. This increase in potency, together with improved bioavailability, resulted in a predicted dose in human subjects of approximately 15 mg/day compared to the CI-1040 dose of 1600 mg/day, a greater than 2 log difference. PD 0325901 has an IC_{50} value of 1 nM against MEK1/2 and has been demonstrated to inhibit tumor growth in six out of seven xenograft models tested [19]. These preclinical findings of significantly improved pharmacologic and pharmaceutical properties of PD 0325901 were determined to hold promise for the use of the compound as a therapeutic agent.

The first-in-human trial of PD 0325901 employed an open-label, dose-escalating design [32,33]. Several biopsies were a requirement of this study. Patients with any of four tumor types, including breast, colon, nonsmall cell lung cancer (NSCLC), or melanoma, were considered for enrollment. At the time of initial reporting, two-thirds of the patients enrolled in this study had
melanoma, perhaps reflecting the accessibility of tumor tissue for biopsy in these patients. Patients with breast, lung and colon cancers accounted for 17%, 12% and 5% of enrollment, respectively.

Initial patients received a 21-day course of oral PD 0325901 (qd or bid) every 4 weeks. Eventually, the regimen was changed to continuous daily dosing. Doses ranged from 1 mg qd to >20 mg bid. The pharmacokinetics of PD 0325901 and its metabolite PD 0315209 were assessed on Day 1 (Cycles 1 and 2) and on Day 21. The effect of food on the pharmacokinetics of PD 0325901 was evaluated in a limited number of patients (Day 1 of Cycles 1 and 2). Pharmacodynamic markers of MEK1/2 activity (pERK) and cell proliferation (Ki67) were assessed by quantitative immunohistochemistry in tumor biopsies obtained at baseline and on Day 15 (2–4 h after dosing) [32,33].

After confirming the safety from once daily dosing, dose escalation continued with bid dosing at 1, 2, 4, 8, 15, 20, and 30 mg and eventually switched to continuous dosing. From 35 evaluable patients, the most common adverse effects (observed from ≥10% of patients) were rash (49%), diarrhea (49%), fatigue (34%), visual disturbance (34%), nausea (29%), edema (29%), pruritus (14%), anemia (11%) and dyspepsia (11%). Dose-limiting toxicities were grade 3 rash in three patients, cardiac adverse events presenting as congestive heart failure and syncope in two patients, and a combination of anemia, diarrhea, and mucositis in one patient. A neurologic adverse effect presenting as transient cognitive impairment (confusion, hallucination) was observed in 3 patients. CT and/or MRI scans of the brain were negative and symptoms and signs resolved after discontinuation of the MEK inhibitor therapy. Onset of rash occurred within 1–2 weeks at all dose levels. The frequency of and, to a lesser extent, the severity of rash generally increased with the dose of PD 0325901. There were no drug-related adverse events of Grade 4 severity. At the time of reporting, dose-finding was ongoing to identify a recommended phase II dose and schedule.

Nineteen of 35 patients had tumor tissues available for evaluation of pERK inhibition. Phosphorylated ERK suppression was demonstrated at all dose levels and in all tumor types, including melanoma, breast, colon, and lung. Preliminary anticancer activity has also been evaluated from 27 assessable patients. Two partial responses were observed in melanoma patients, while 8 patients (5 melanoma, 2 NSCLC and 1 colon cancer) achieved stable disease lasting 3–7 months (Unpublished data).

3.4. ARRY-142886 (AZD6244)

ARRY-142886 (AZD6244), another novel and highly-selective oral MEK inhibitor, is currently under study in clinical trials. ARRY-142886 is a benzimidazole with reported nanomolar activity against the purified MEK1 enzyme (Fig. 2) [34]. It is non-competitive with respect to ATP and is highly selective for MEK1/2 compared to a panel of other tyrosine and serine/threonine protein kinases [34]. In cell-based assays, MEK1/2, which is measured by ERK1/2 phosphorylation levels, is inhibited with an IC50 value of ∼10 nM [34–36]. Antiproliferative effects of ARRY-142886 have been observed in cell lines harboring Ras and B-Raf mutations [36]. It has also demonstrated potent activity in a variety of human tumor xenograft models, including colon, pancreas, breast, non-small-cell lung carcinomas, and melanoma [37].

A first-in-human dose-ranging study (Part A) of ARRY-142886 (AZD6244) was reported by Chow et al. at the AACR-
arry-142886 showed a mean inhibition of 84% compared to pre-dose levels reported at the EORTC meeting in Nov 2006, showing that tumor ERK levels are examined at the plateau compartment, to assess the accuracy of the PBMC data as a representation of the tumor profile may not be obtained, unless biopsies are obtained from the examination of MEK inhibition from tumor biopsies performed –32].

It has been reported previously that much information can be obtained from the examination of pERK in PBMCs; this is because PBMCs can be obtained at multiple time points after drug dosing and can be examined in patients at multiple doses, which allows investigators to obtain pERK data at different drug concentrations [38]. This allows for a thorough understanding of the dose–response relationship for inhibition of the target in the blood compartment. In contrast, data on inhibition of pERK in tumors is more difficult to obtain. Tissue samples are often obtained at only one or two doses, at one time point after a dose (typically Cmax) for comparison to a pre-dose level, and can suffer more from sample variation, especially if obtained by a fine needle biopsy. Typically, these data are viewed as confirmatory of the results from the blood compartment, to assess the accuracy of the PBMC data as a surrogate. For example, data on ARRY-142886 was reported at the EORTC meeting in Nov 2006, showing that tumor pERK showed a mean inhibition of 84% compared to pre-dose levels in patients receiving 100 mg BID [39]. This result confirmed that the inhibition of pERK seen in PBMCs at that dose and time translated well into an effect within the tumor. Thus, PBMCs represent an essential surrogate for MEK inhibition within the tumor. Though a mild association is seen between baseline pERK levels in archived tumor samples and subsequent stable disease in those patients treated with a MEK inhibitor, pERK inhibition in either PBMCs or in tumor tissues from patients receiving MEK inhibitor therapy has not correlated with clinical benefit. Therefore, the presence of activated ERK, as well as the percentage of ERK inhibition, may not be sufficient in themselves as a guide to the anticancer effects of MEK inhibition.

Based on available pre-clinical and clinical data, we have speculated on a few possible factors that may influence the potential therapeutic outcomes of MEK inhibition in cancer patients: (1) The tumor pERK levels are examined at the specified time points, and these data may reflect ERK activation at that time, but may not differentiate between short-lived mitogen-activation and sustained constitutive MAPK pathway activation. Thus, the extent of MEK and pERK inhibition observed in any one patient may or may not predict the ultimate therapeutic response. It is possible that a more accurate assessment of MEK inhibition may be obtained from an examination of pERK inhibition from tumor biopsies performed after reaching a steady-state drug level. A technical limitation lies within tumor sampling itself; tumor heterogeneity is likely to represent a key challenge in the attempt to quantitate the true ERK activation status of a given tumor. Unless biopsies are obtained from several different regions of a tumor, a true representation of the tumor profile may not be obtained,
resulting in a false reading. (2) All three MEK inhibitors that have entered into clinical trials are non-competitive MEK inhibitors with respect to ATP. This non-competitive characteristic may, in part, contribute to the agent’s potency in cells, due to the fact that they are not affected by the high intracellular ATP concentration. Alternatively, Ohren et al. demonstrated that the high potency of MEK inhibitors, such as CI-1040, is due to their very high affinity for a unique binding pocket adjacent to the ATP-binding site that induces a conformational change which locks unphosphorylated MEK into a closed, catalytically inactive form [40]. Thus, they may have efficacy at lower exposures in vivo. In addition, their action at an allosteric site makes these molecules extremely selective for MEK versus other kinases. Because of this selectivity it will be interesting to see if these molecules turn out to be better tolerated than other, ATP-competitive kinase inhibitors. (3) Constitutive activation of the MAPK pathway can frequently lead to crosstalk with other signal transduction pathways, which may provide mechanisms of escape for cancer cells from MEK inhibition. For example, the connection between the MAPK and phosphatidylinositol 3-kinase (PI3K) pathway is a significant factor; RAS serves as a primary effector for both RAF and PI3K and thus activated MAPK signaling logically goes hand in hand with increased flux through the PI3K pathway as reflected by concurrent increases in phosphorylated AKT levels [41]. Recent evidence from Smalley et al. showed that multiple signaling pathways may need to be targeted for maximal therapeutic efficiency [42]. Therefore, in certain genetic contexts, MEK inhibitors might need to be used in combination with other targeted therapies in order to maximize their effectiveness. Inhibition of multiple signal transduction pathways may synergize the anticancer activity of each individual small molecule inhibitor. However, multi-signal pathway blockades may generate intolerable side effects that limit or attenuate any therapeutic benefits arising from such a combination. (4) Our understanding of the biology of the response to MEK inhibitors is incomplete. Clearly BRAF mutations can activate the pathway, but not all cell lines with BRAF mutations are sensitive to MEK inhibitors. Some cells with normal RAF, especially those with N-Ras mutations, are also sensitive to MEK inhibitors, but again this is not a universal rule. We need to understand more about the roles of other mutations, such as p-53, in cancer cells and of the apoptotic machinery in general, and their roles in determining the apoptotic responses to MEK inhibitors. (5) There is growing evidence in the clinic that patients treated with kinase inhibitors can develop mutations that can infer resistance. The best examples are the T315I mutations in BCR-ABL in patients treated with imatinib [43] and the T790M mutations in EGFR that confer resistance to gefitinib and erlotinib [44]. Preclinically, mutant screening from a MEK cDNA yeast library revealed several amino acids that, when mutated, either hindered or completely abolished PD 184352-mediated inhibition of MEK kinase activity [45]. Recently, the first identified instance of naturally occurring mutations in MEK1 and MEK2 were reported, in patients with cardio-facio-cutaneous (CFC) syndrome [46]. The discovery that germline mutations in MEK are associated with a specific developmental syndrome may lead to even more importance being placed on the development of MEK inhibitor therapy.

5. Future directions

As researchers continue to study the use of MEK inhibitors in clinical trials, several questions remain unanswered. Foremost among them are these: What will be the clinical utility of MEK inhibitors, and in what cancer patients, and with what regimens and co-therapies?

As to which cancer patients to target, one possibility is that the analysis of archival or fresh tumor may disclose a correlation between patient response and genetic and/or biochemical aberrations. As mentioned above, the target that will receive the most attention initially is mutated BRAF. Identification of relevant biomarkers may allow the pre-selection of patient populations most likely to derive the greatest clinical benefit. While BRAF mutations remain an intriguing possibility, the current information has not allowed us to draw any conclusions at this time.

In addition to genetic markers, it will be important to focus efforts on the development of other early markers of response. However, while the identification of early markers of response will be helpful, it must be remembered that the most measurable responses are tumor stabilization and/or shrinkage. At this time, biomarkers/predictive markers appear too premature to be the hinge that drives therapeutic programs utilizing MEK inhibitors forward.

Once safety of these agents has been confirmed in humans, we must design rational combination regimens with agents that inhibit other targets in order to maximize response and prevent resistance. Rational combinations need to take into account many factors, among which are the activation of other pathways that could compensate for inhibition of MEK, the effects of MEK inhibitors on cell cycle, and the high proportion of patients with stable disease. Indeed, long-term trials will need to be done to understand the relationship between stable disease with these compounds and any resulting increases in survival of the patients.

References


