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MEK1/2 Inhibitors: Molecular Activity and Resistance Mechanisms

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Abstract

Aberrant activation of the three-layered protein kinase cascade, Raf/MEK/ERK, is often detected in human cancer, which is mainly attributed to the oncogenic alterations of *RAF*, or its upstream activators *RAS* or cell surface receptor tyrosine kinases. Deregulated activity of the Raf/MEK/ERK pathway drives uncontrolled tumor cell proliferation and survival, thus providing a rational therapeutic target for the treatment of many cancers. While Raf, MEK1/2, and ERK1/2 are equally important targets for the design of therapeutic small molecular weight inhibitors, the effort to develop MEK1/2-specific inhibitors has been greatly successful. Particularly, MEK1/2 have been relatively advantageous for the design of highly selective ATP-noncompetitive inhibitors. Indeed, a plethora of highly selective and potent MEK1/2 inhibitors are now available and many of those inhibitors have been evaluated for their therapeutic potential. Herein, we review different MEK1/2 inhibitors that have been studied for their inhibitory mechanisms and therapeutic potential in cancer. Some of the key structural features of MEK1/2 that are important for the efficacy of these inhibitors are also discussed. In addition, we discuss current challenges and future prospective in using these advanced MEK1/2 inhibitors for cancer therapy.

Introduction

Although the first mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) was discovered in mammalian cells only a few decades ago [1–3], the significance of MAPK/ERK-mediated signal transduction has been rapidly established in a number of biological contexts spanning from early development to various diseases with tremendous implication in cancer. MAPK/ERK serves as the key effector of a three-layered

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kinase cascade called the Raf/MEK/ERK pathway, which relays various signals transmitted from cell surface receptors to cytosolic and nuclear targets. The ubiquitously expressed Ser/Thr kinases ERK1 and ERK2 (collectively referred to as ERK1/2) are specific effectors of the Raf/MEK/ERK pathway that also consists of the Ser/Thr kinase Raf (i.e., A-Raf, B-Raf, or C-Raf/Raf-1) and the dual-specificity kinases MEK1 and its homologue MEK2 [4]. Upon activation, Raf phosphorylates MEK1/2, which in turn sequentially phosphorylate Tyr and Thr on the activation loop of their only substrates, ERK1/2. ERK1/2 then activate/inactivate many proteins to mediate diverse cellular processes [5, 6] (Fig. 1A). The Raf/MEK/ERK pathway is controlled by a complex network of regulators, including the small GTPase Ras and Rap, phosphatases, scaffolds, and other kinases, which affect the magnitude, duration, and compartmentalization of the pathway activity [4, 7–9]. The Raf/MEK/ERK pathway plays pivotal roles in regulating cell survival, cell cycle progression and differentiation, and its deregulated activity is a central signature of many epithelial cancers [reviewed in [10–13]].

Aberrant activation of the Raf/MEK/ERK pathway is mainly driven by mutations in *BRAF* or its upstream activator, *RAS* (i.e., *KRAS*, *HRAS*, or *NRAS*), although other genetic and epigenetic alterations also drive the pathway. For example, mutations in *BRAF*, mainly affecting the Val600 codon (i.e., Val600 to Glu, Lys, or Arg), are detected at high frequencies in different cancers, including melanoma (50–70%), papillary thyroid cancers (40%), hairy cell leukemia (100%), Langerhans cell histiocytosis (57%), low-grade ovarian carcinomas (>30%), colorectal cancers (8%), non-small cell lung cancers (2–3%), and multiple myeloma (4%) [14–21]. *RAS* mutations, mainly affecting Gly12 or Glu61, are among the most often detected genetic alterations in human cancers, including the malignancies of pancreas (63%), colon (36%), biliary tract (33%), skin (27%), small intestine (20%), lung (19%), ovary (18%), salivary gland (18%), urinary tract (18%), cervix (17%), endometrium (16%), upper aero-digestive tract (16%), prostate (15%), hematopoietic cells/lymphoid (15%), and thyroid (14%) [22]. Mutations in *BRAF* and *RAS* are mutually exclusive in cancer, which suggests that activation of the MEK/ERK cascade is a critical process in mediating Ras- or Raf-driven carcinogenesis [12, 21, 23–25].

MEK1/2 is a key therapeutic target in cancer

Although MEK1 and MEK2 are rarely mutated in cancer, expression of constitutively active forms of their mutants (i.e., MEK1-ΔN3/S218E/S222D and MEK2-ΔN4/S222D/S226D) was sufficient to induce oncogenic transformation of normal cells [26, 27]. This demonstrates the pivotal roles of MEK1/2 in malignant transformation, rationalizing therapeutic targeting of upregulated MEK1/2 activity in cancer. In addition, there are unique characteristics of MEK1 and MEK2 that might support the advantage of therapeutic development of MEK1/2 inhibition. First, MEK1/2 have very narrow substrate specificity, thus MEK1/2 inhibition specifically shuts off ERK1/2 signaling without directly affecting other signaling pathways. Second, MEK1/2 have a unique structural advantage for the design of highly selective ATP-noncompetitive inhibitors, which induce conformational changes that lock MEK1/2 into a catalytically inactive state [28, 29]. ATP-noncompetitive inhibitors are advantageous over ATP-competitive inhibitors in that they have relatively low chances to induce the undesired adverse effects associated with inadvertent inhibition of the highly conserved ATP-binding

pockets in protein kinases and they avoid the challenge of competing with ATP that are abundantly present (usually at mM ranges) in cells [28]. Development of the ATP-noncompetitive inhibitors has been particularly successful with MEK1/2, and some of the MEK1/2 inhibitors have shown therapeutic efficacy in cancers when tested in a clinical setting. Some of the key features of MEK1/2 structure that are important for their inhibition are highlighted below.

Structural basis for MEK1/2 activation and catalysis

MEK1 and MEK2 were first identified in 1991[30, 31]. MEK1 (45 kDa) and MEK2 (46 kDa) are encoded by *MAP2K1* and *MAP2K2* respectively, among the seven *MAP2K* genes in humans, exhibiting 85% peptide sequence homology with 86% identity being detected in their catalytic domains. The typical MEK1/2 secondary structures feature the N-terminal ~70 amino acid residues, the protein kinase domain (~290 amino acids), and the C-terminal ~30 amino acid residues (Fig. 2). The N-terminal sequence consists of the "D-domain" that interacts with the "common docking site" of ERK1/2, the nuclear export sequence (NES), and the inhibitory segment, whereas the role of C-terminal residues are less known although it has been previously demonstrated to affect cytoplasmic localization of MEK1 [27, 32–37] (Fig. 2). Tertiary structures of MEK1/2 revealed by X-ray crystallography are also highly homologous although the N- and C-termini of MEK2 are more disordered than those of MEK1 [28]. These tertiary structures consist of a small N-terminal lobe and a large C-terminal lobe, which contain several α-helices and β-strands that are conserved in most protein kinases [38] (Fig. 3A).

The key structural features in the small lobe include a conserved five-stranded antiparallel β-sheet (β1-β5), the αC-helix, and the glycine-rich (Gly-Xaa-Gly-Xaa-Xaa-Gly) loop (also referred to as "P-loop"), which are important for binding and positioning ATP for catalysis (Fig. 3A). The key structural features in the large lobe include six conserved α-helixes (αD-αI) and four short conserved β-strands (β6-β9) that contain most of the catalytic residues required for catalysis and for interaction with the substrate, ERK1/2 [39]. MEK1/2 also have a unique prolin-rich insert (Fig. 2), which is not present in other MEK family members. This structurally flexible region facilitates efficient activation of ERK1/2, although it does not promote Raf-MEK interaction [40].

The catalytic site of MEK1/2 is formed in the deep cleft located between the small and large lobes (Fig. 3A). The β1 and β2 strands in the small lobe harbor the adenine ring of ATP and the proximal glycine-rich loop positions γ-phosphate of ATP for kinase reaction. Three conserved amino acids, known as the K/D/D (Lys/Asp/Asp) motif, are the key to subsequent phosphoryl transfer reaction [41]. Briefly, a lysine (Lys97/101 of MEK1/2) in the β3 strand couples α- and β-phosphates of ATP, which facilitates the formation of a salt-bridge between the lysine and a conserved glutamate (Glu114/118 in MEK1/2) near the center of αC-helix in the small lobe. The presence of this bond indicates a kinase in a catalytically active state, and vice versa [41]. A base in the catalytic loop (Asp190/194 of MEK1/2), which is present between the β6- and β7-strands, abstracts the proton from the hydroxyl group of tyrosine or threonine in the activation loop of EKR1/2, facilitating the nucleophilic attack of oxygen on the γ-phosphate of ATP. For coordination of these α-, β- and γ-phosphates of

ATP, two Mg^{2+} ions are bound by an aspartate (Asp208/212 of MEK1/2) in the activation loop, which begins and ends with the DFG (Asp-Phe-Gly) and APE (Ala-Pro-Glu, Ser-Pro-Glu in MEK1/2) sequences that are conserved in kinases [41].

The active site in the cleft opens and closes during the catalytic cycle. Whereas the open form allows the access of ATP and the release of ADP, the closed form aligns the key residues in catalytically active positions. These dynamic movements are driven by a few conserved segments that undergo significant conformational changes. Whereas the αC-helix is the key regulatory segment in the small lobe, the activation loop in the large lobe dramatically changes its inactive versus active orientations in response to Raf-mediated phosphorylation of the two serine residues (i.e., Ser218/222 for MEK1 and Ser222/226 for MEK2) in this segment [42]. In the inactive conformation, the aspartate side chain (Asp208/212 of MEK1/2) of the conserved DFG sequence in the activation segment faces away from the active site, which is called the “DFG-out” conformation [43, 44]. In contrast, this aspartate side chain faces into the active site in the active conformation, which is called the “DFG-in” conformation. In the inactive (DFG-out) conformation, the activation loop blocks the ERK1/2 binding sites on MEK1/2 [28]. Noteworthy is that movement of the activation loop to the “DFG-out” conformation exposes an additional hydrophobic site (also referred to as the allosteric site) directly adjacent to the ATP binding pocket, which is separated by the side chains of the conserved Lys97 on the β3-strand and Met143 in the hinge region [28]. Because the amino acids surrounding this region are less conserved than those in the ATP binding pocket, this region provides an advantage for the design of selective inhibitors that can lock kinases in an inactive state [28].

Types of kinase inhibitors

Protein kinases share conserved subdomains that fold into a typical bi-lobed structure that contains the catalytic site in the deep cleft created between these two lobes [45–47]. Many kinase inhibitors take effect by targeting the catalytic site or a surrounding region that is important for enzyme activation. Most kinase inhibitors that form non-covalent reversible bonds are mainly classified as follows.

Type 1 inhibitors typically consist of a heterocyclic ring system that mimic the adenine ring structure of ATP, constituting the majority of ATP-competitive inhibitors. Type 1 inhibitors function by interrupting the molecular interactions in the specific compartments of the catalytic site, i.e., the adenine region, the ribose region and the phosphate-binding region, and the adjacent hydrophobic regions I and II [46]. In these regions, type 1 inhibitors act as a scaffold for the side chains of amino acids that occupy the hydrophobic regions I and II. The resulting hydrogen bonds mimic those normally formed by the exocyclic amino group of adenine. Therefore, type 1 kinase inhibitors compete with ATP for the catalytic site of kinase and are most potent on active kinase conformation [43, 46]. While all type 1 inhibitors invariably occupy the adenine region, these inhibitors can specifically affect those other compartments of the catalytic site, which provides the basis for their selectivity among different kinases [48]. Type 1 inhibitors are generally used to inhibit many kinases, whereas ATP-noncompetitive inhibitors such as type 2 or allosteric inhibitors have been successful for MEK1/2 inhibition.

Type 2 inhibitors occupy the hydrophobic site created directly adjacent to the ATP binding pocket when the activation loop of a kinase undergoes "DFG-out" conformational changes. This is contrasted with the binding mechanism of type 1 inhibitors, which does not require the "DFG-out" conformation of the activation loop. Type 2 kinase inhibitors typically form hydrogen bonds with the amino acids in the region, i.e., one with the conserved glutamate in the αC-helix (Glu114/118 of MEK1/2) and another with the aspartate in the DFG motif [43]. In addition to these hydrogen bonds, type 2 inhibitors also form van der Waals interactions with other residues in this region. This results in locking the kinase in an inactive state. Although occupancy of this site characterizes all the type 2 inhibitors, many of these inhibitors also extend into the adenine region in the ATP binding pocket and exert similar effects as type 1 inhibitors [43]. Because type 2 inhibitors recognize and bind to inactive enzymes, they usually exhibit more potent cellular activity although certain type 1 kinase inhibitors are among the most selective inhibitors [49, 50].

Allosteric inhibitors bind at an allosteric site outside the ATP-binding pocket and modulate kinase activity in an allosteric manner. Allosteric inhibitors exhibit the highest degree of kinase selectivity because they inhibit the regulatory mechanism specific to a kinase [43]. Allosteric MEK1/2 inhibitors usually constrain the movement of the activation loop and thus decrease the rate of Raf-mediated MEK phosphorylation, and/or lock the kinase in a catalytically inactive state.

MEK1/2 inhibitors developed to date

A number of highly selective MEK1/2 inhibitors have been developed, and many of them have been tested in a clinical setting. Notable examples of these inhibitors (Fig. 4, Table 1) are described below for known inhibitory mechanisms. Clinical efficacy of these inhibitors has been extensively reviewed elsewhere [51–53]. Table 1 lists only recent and ongoing clinical trials.

PD98059 and **U0126** are the first generation small molecule inhibitors of MEK1/2. These inhibitors were discovered from the small molecule compound library screening to identify selective inhibitors of the constitutively active MEK1 mutant or of PMA (phorbol 12-myristate 13-acetate)-induced AP-1 transcription [54, 55]. PD98059 and U0126 are potent ATP- and ERK1/2-noncompetitive inhibitors [54–56], and occupy similar regions in MEK1/2 while U0126 exhibited ~100-fold higher affinity to the constitutively active MEK1 mutant than PD98059 [55]. Although potently inhibiting ERK1/2 phosphorylation, both compounds did not affect serum-induced MEK1/2 phosphorylation in NIH3T3 and WM35 cells, which suggests that theses inhibitors do not interfere with Raf access to MEK1/2 [57]. PD98059 and U0126 effectively suppressed *in vitro* Ras transformation [54, 55]. When examined against a panel of protein kinases, U0126 and PD98059 showed very high selectivity to MEK1/2 with very low off-target effects [58]. Given their high specificity, these two inhibitors have been invaluable reagents for academic research. However, both PD98059 and U0126 can also inhibit MEK5, albeit at a higher IC_{50} [59]. A recent study identified additional off-target effects of these inhibitors in cells [60]. Therefore, data obtained using these inhibitors should be carefully interpreted.

Ro 09-2210 is another earlier MEK1/2 inhibitor, which was identified from the screening of microbial extracts for the activity to inhibit anti-CD3-induced T cell activation [61]. Ro 09-2210 is a highly selective MEK1 inhibitor, exhibiting an IC_{50} below 60 nM *in vitro*. The mode of its inhibitory mechanism is not clear.

CI-1040 (PD184352) was identified in 1999 as a highly selective, potent allosteric inhibitor that is ATP- and ERK1/2-noncompetitive [62]. A genetic screen identified five key amino acid residues that bind to CI-1040. These residues were mapped to the Hank's conserved kinase subdomains III and IV, which form a potential hydrophobic binding pocket adjacent to the ATP binding site [63]. The remarkable selectivity of CI-1040 and its analogs is attributed to the low sequence homology of their binding sites in MEK1/2 relative to other kinases, excluding MEK5 [28]. The occupancy of this unique pocket by an inhibitor locks MEK1/2 in an inactive conformation and disrupts the molecular interactions required for catalysis and proper access of the active site of MEK1/2 to the activation loop of ERK1/2, although binding of ATP and ERK1/2 still occurs. Crystal structures of human MEK1 and MEK2 bound to the CI-1040 analogs, PD318088 and PD334581, revealed that concurrent binding of these inhibitors and ATP to MEK1/2 causes several conformational changes, which account for their unique non-competitive mechanisms [28] (Fig. 3B). CI-1040 is the first MEK1/2 inhibitor that was tested in a clinical trial [64].

PD0325901 is a structural analog of CI-1040 with an optimized diphenylamine core and a modified hydroxamate side chain (Fig. 4). PD0325901 is a highly selective allosteric inhibitor that does not compete with ATP or ERK1/2, and its interaction with MEK1/2 does not perturb the ATP binding site [65]. PD325901 presents a synergistic stabilizing effect in the formation of the ternary complex of the inhibitor-MEK1-nucleotide [66]. PD0325901 exhibits significantly increased potency, i.e., IC_{50} of 1 nM for activated MEK1/2 [65], and considerably improved oral bioavailability and metabolic stability compared with CI-1040 [67]. Consistent with this, PD0325901 inhibited proliferation of tumor cells at ~100 fold increased efficacy compared with CI-1040 [65, 68]. PD0325901 potently suppressed B-Raf^{V600E} tumor xenografts in mice, but it showed only partial effects on *RAS*-mutant tumor xenografts [68]. Indeed, PD0325901 increased the interaction between MEK1/2 and C-Raf in *KRAS* mutant tumor cells, although not in *BRAF* mutant tumor cells [69]. Because MEK1/2 inhibition relieved the ERK1/2-mediated feedback-inhibition of C-Raf, which resulted in increased C-Raf activity in *RAS* mutant tumor cells, the increased Raf-MEK interaction led to rebound of MEK/ERK activity in those tumor cells [69] (Fig. 1B). These unexpected effects partly account for the relatively low efficacy of PD0325901 in *RAS* mutant tumors.

Selumetinib (AZD6244/ARRY-142886) is an ATP-noncompetitive allosteric inhibitor. Selumetinib inhibits enzymatic activity of MEK1/2 but not MEK1/2 phosphorylation by Raf [70]. Molecular modeling suggests that selumetinib binds to a unique allosteric site in MEK1/2, which confers a high selectivity. Indeed, selumetinib, even used at 10 μM, did not show any significant off-target effects in a test using more than 40 protein kinases including MEK5. Selumetinib robustly inhibited ERK1/2 phosphorylation (IC_{50} < 40 nM) in *RAS* or *BRAF* mutant tumor cells, and its dose-dependent antitumor activity was observed in a panel of colorectal, pancreatic, liver, skin, and lung cancer xenografts [70–73]. In a single-arm phase II study, selumetinib has demonstrated significant efficacy for recurrent low-grade serous

carcinoma of the ovary or peritoneum and biliary cancers [74, 75], which are the tumor types that present a high basal MEK/ERK activity. Similarly as PD0325901, selumetinib also increased MEK1/2 interaction with C-Raf and subsequently MEK1/2 activation in *KRAS* mutant tumor cells, although not in *BRAF* mutant tumor cells [69]. This partly accounts for its lower potency in *KRAS* mutant tumor cells than in B-Raf^{V600E} tumor cells [68, 76].

Cobimetinib (GDC-0973/XL518/RG7421) is an ATP-noncompetitive allosteric inhibitor. It is a structural analog of CI-1040 optimized for metabolic stability by replacing the hydroxamate ester in CI-1040 with a carboxamide [77] (Fig. 4). The cyclic aminoethanol terminus of cobimetinib is predicted to form a highly complex network of interactions with both the catalytic loop and the γ-phosphate of ATP [78]. Although similarly effective in *BRAF* mutant tumor cells, cobimetinib exhibited much less potency in a panel of *KRAS* mutant tumor cells than two newer allosteric inhibitors, GDC-0623 and G-573[79]. Comparison of their binding modes by structural analysis led to the prediction that the affinity of the inhibitor to Ser212 (MEK1) in the activation loop determines the degree of inhibition of MEK1/2 activation by Raf [79]. In addition to its effect on MEK1/2, cobimetinib induced translocation of C-Raf to plasma membrane in *KRAS* mutant tumor cells [79]. This partly accounts for a mechanism by which C-Raf is activated and subsequently MEK/ERK activity is rebound in cobimetinib-treated *KRAS* mutant tumor cells.

Refametinib (RDEA119/BAY869766) is an ATP-noncompetitive allosteric inhibitor [80]. Structural analysis has revealed that refametinib binds to an allosteric site adjacent to the ATP binding pocket and interacts with ATP, the activation loop, and surrounding amino acids in the region through hydrogen bonding and hydrophobic interactions in a similar manner to PD318088 [28, 80] (Fig. 3B). In addition, the unique cyclopropyl group of refametinib makes direct hydrophobic contacts with the side chain of Met219 in the activation loop, further stabilizing the inhibitor in the closed, DFG-in “active” conformation of MEK1/2 [80]. This imparts strong potency in inhibiting ERK1/2 phosphorylation and suppressing cell proliferation in a panel of human cancer cell lines *in vitro* and in mouse xenografts [80].

Trametinib (GSK1120212/JTP-74057) is an ATP-noncompetitive inhibitor. Trametinib binds to MEK1/2 in a manner similar to PD0325901, inducing allosteric inhibition of MEK1/2 catalysis [81]. Importantly, unlike PD0325901, trametinib does not increase the interaction between MEK1/2 and C-Raf in *KRAS* mutant tumor cells [69]. On the contrary, trametinib promotes dissociation of MEK1/2 from C-Raf, which reduces the rebound of ERK1/2 phosphorylation observed in *KRAS* mutant tumor cells [69]. Therefore, trametinib inhibits not only MEK1/2 activity but also MEK1/2 activation by Raf. Indeed, trametinib demonstrated substantial antitumor effects against not only *BRAF* but also *KRAS* mutant tumor cells in mouse xenografts [81].

Trametinib is the only MEK1/2 inhibitor currently approved by the US Food and Drug Administration for clinical applications as a monotherapy and as a combination therapy with the B-Raf inhibitor dabrafenib. Trametinib exhibited the highest efficacy in patients with *BRAF* mutant melanoma [82], although it also showed efficacy in patients with *NRAS* mutant melanoma, uveal melanoma, pancreatic cancer, and non-small cell lung carcinoma [82, 83]. Of

note, when used in combination with dabrafenib, trametinib significantly improved survival of the patients with B-Raf^{V600E,K} melanomas compared with the dabrafenib monotherapy, indicating that simultaneous inhibition of B-Raf^{V600E,K} and MEK1/2 has a therapeutic advantage [84]. This improvement is partly attributed to the ability of trametinib to suppress the rebound of MEK/ERK activity in *BRAF* mutant tumors, which is a major mechanism of acquired resistance to B-Raf inhibitors triggered via alternate upstream activators such as N-Ras, C-Raf, and Cot/Tpl-2 [85]. Trametinib has also been evaluated in combination with other anticancer agents, including gemcitabine [86], everolimus [87], and the AKT inhibitor afuresertib [88]. These combination therapies are reviewed elsewhere [53].

RO4987655 (CH4987655) is an ATP-noncompetitive inhibitor. This compound potently and selectively inhibits MEK1 at a very low IC_{50} (5 nM), while not inhibiting 400 other kinases even at 10 μM [89]. Co-crystal structure revealed that RO4987655 binds to an allosteric inhibitor binding site in ATP analog-bound MEK1 via interactions with Lys97, Val127, Val211, Ser212, and the nucleotide [89]. RO4987655 exhibited anti-proliferative activity in a panel of *KRAS* and *BRAF* mutant tumor cell lines *in vitro* and *in vivo* [89].

RO5126766 (CH5126766) is a novel allosteric inhibitor. Co-crystal structure revealed that RO512677 binds to an allosteric inhibitor binding site in ATP analog-bound MEK1 [69], which overlaps with the PD0325901 binding site [90]. Specifically, RO512677 interacts with Ser212, Ser222 and Asn221, and induces a conformational change in the activation loop, which prevents MEK1 phosphorylation by C-Raf [69]. RO512677 increased the affinity between inactive MEK and Raf, and the resulting Raf-MEK complex was so stable that it became a dominant-negative inhibitor of Raf [69]. Of note, RO512677 inhibited not only MEK1 but also C-Raf, B-Raf, and B-Raf^{V600E} in an *in vitro* kinase assay, although it did not inhibit 254 other kinases [90]. Therefore, RO5126766 is a first-in-class dual Raf and MEK inhibitor. Because of these properties, RO5126766 prevented C-Raf-mediated MEK/ERK reactivation [69], and exhibited improved tumor suppressive effects in *KRAS* mutant tumor cells, including PD0325901-resistant *KRAS* mutant tumor cells [90].

Binimetinib (MEK162/ARRY-438162) is an ATP-noncompetitive inhibitor [91]. Binimetinib suppressed *NRAS* mutant melanoma cells [92]. Consistent with this, 10% of patients with *NRAS* mutant melanoma and 5% of patients with *BRAF* mutant melanoma responded to binimetinib [93]. Binimetinib also suppressed pancreatic cancer cells expressing K-Ras$^{D12,\ R12,\ or\ C12}$, although it was less effective in pancreatic cancer cells expressing K-Ras^{V12} or increased copy numbers of wild type *KRAS* [91].

Pimasertib (AS703026/MSC1936369B) is an ATP-noncompetitive allosteric inhibitor which did not inhibit 217 other kinases [94]. Pimasertib significantly suppressed human multiple myeloma and other solid tumor xenografts in mice [94]. Pimasertib also suppressed *KRAS*-mutated DLD-1 colorectal cancer cells and their cetuximab-resistant variants in mouse xenografts [95].

TAK733 is an ATP-noncompetitive allosteric inhibitor [96]. Co-crystal structure revealed that TAK733 binds to an allosteric inhibitor binding site in ATP-bound MEK1, wherein it interacts with Lys97 and Ser212 [96]. TAK733 exhibited very low IC_{50} (3.2 nM to MEK1),

while not inhibiting other kinases, receptors, or ion channels [96]. In a preclinical study, TAK733 showed antitumor activity for human breast carcinoma, colorectal cancer, melanoma, non-small cell lung cancer, and pancreatic cancer [96]. Of note, despite its efficacy in *BRAF* mutant melanoma cells, TAK733 upregulated MEK1/2 phosphorylation in melanoma cells harboring *NRAS*, *GNAQ*, or *GNA11* mutations [97], indicating its ability to trigger the rebound of MEK/ERK activity.

GDC-0623 and **G-573** are two recently developed ATP-noncompetitive allosteric inhibitors [79, 98]. Molecular modeling suggests that GDC-0623 and G-573 bind to MEK1 in a similar manner as cobimetinib [79]. Noteworthy is that GDC-0623 and G-573 form stronger interaction with Ser212 in the activation loop than cobimetinib, which account for their superiority in blocking MEK1/2 phosphorylation by Raf [79]. Unlike cobimetinib, these inhibitors did not increase B-Raf and C-Raf hetero-dimerization [79]. Moreover, G-573 blocked Raf translocation to the plasma membrane in *KRAS* mutant tumor cells, suggesting that these inhibitors have an additional role other than MEK inhibition [79]. Consistent with this, GDC-0623 and G-573 did not induce the rebound of MEK/ERK activity in *KRAS* mutant tumor cells while exhibiting much higher potency than cobimetinib [79].

E6201 is unique in that it is an ATP-competitive inhibitor. Molecular docking indicates its positioning in the ATP-binding pocket [99]. E6201 exhibited very low IC_{50} (5.2 nM) to MEK1 and effectively suppressed B-Raf^{V600E} melanoma cells [99–101]. Of note, E6201 effectively blocked MEK/ERK activity and cell growth in melanoma cells expressing MEK1-C121S [99]. Cys121 to Ser switch in MEK1 is a mechanism that imparts vemurafenib resistance to *BRAF* mutant melanomas [102]. MEK1-C121S is also resistant to selumetinib, suggesting that the binding site for selumetinib is altered in this MEK1 mutant [102]. This effect of E6201 indicates a potential benefit of targeting ATP-binding pocket using a type 1 inhibitor when a drug-resistant mutation occurs in the binding sites of ATP-noncompetitive inhibitors.

Other recently developed MEK inhibitors include AZD8330/ARRY-424704 [103] and WX-554 [104]. These inhibitors are currently evaluated in phase I clinical trials (Table 1). The inhibitory mechanisms for these inhibitors are not yet clear.

Current challenges and future perspectives

A plethora of MEK1/2 inhibitors are now available and some of them are expected to improve the treatment of Raf/MEK/ERK-driven cancers. Nevertheless, there are obstacles that limit the use of these specific inhibitors. First, various genetic alterations have been detected in tumor cells that have acquired resistance to MEK1/2 inhibitors. Most of these alterations converge into reactivation of the Raf/MEK/ERK pathway, e.g., amplification of *BRAF* [105] and *MEK1* mutations [106, 107], although mutations in other pathways are also detected, e.g., STAT3 upregulation [108]. Different *MEK1* mutations detected in the tumor specimen from selumetinib-treated patients [107] and in the tumor cells resistant to PD0325901 and G-573 [109] indicate the ability of tumor cells to bypass the effect of advanced MEK1/2 inhibitors by genetically altering the allosteric inhibitor binding sites in MEK1/2 or enhancing intrinsic kinase activity of MEK1/2. Noteworthy is that most of these

tumor cells remain addicted to MEK/ERK activity. Therefore, the MEK/ERK pathway continuously provides the target to treat these tumors. Indeed, it has been demonstrated that use of ERK1/2 inhibitors overcomes acquired resistance to MEK1/2 inhibitors [109], although it is predicted that different ERK1/2 mutations that confer drug resistance would eventually arise in response to ERK1/2 inhibitors. In support of this notion, it has been demonstrated that different ERK2 mutations bypass trametinib effects in *BRAF* mutant melanoma cells [110]. Alternatively, type 1 inhibitors may be used to treat the tumors that developed resistance to the ATP-noncompetitive inhibitors, as exemplified by E6201 in melanoma cells expressing MEK1-C121S [99]. ATP-competitive inhibitors have lower chances to develop resistance mutations because mutations in the ATP-binding pocket are likely to disable the catalytic activity of a kinase, thereby not providing selection advantage.

Second, intrinsic resistance of tumor cells limits the therapeutic window of advanced MEK1/2 inhibitors. Similarly to the cases of B-Raf inhibitors, the intrinsic resistance to MEK1/2 inhibitors is mainly attributed to rapid rebound of MEK/ERK activity after inhibitor treatment, for which an ERK1/2-mediated feedback loop is critical. ERK1/2 feedback inhibits C-Raf via phosphorylation in *RAS* mutant tumor cells [111] (Fig. 1B). Inhibition of MEK1/2 relieves this feedback loop by inactivating ERK1/2, subsequently leading to C-Raf activation when Ras activity is available [111]. Certain ME1/2 inhibitors promote this phenomenon by facilitating C-Raf-MEK interactions. Development of newer inhibitors such as trametinib and RO5126766 has shown promise to prevent this reactivation mechanism [69, 112].

MEK1/2 inhibitors have potential to confer significant therapeutic benefits when combined with other treatments, e.g., combination with dabrafenib [84]. Similarly a number of combination therapies using MEK1/2 inhibitors are currently evaluated and are likely to expand their clinical applications. Of note, combination of MEK1/2 inhibitors with certain drugs induces synthetic lethality in cancer, e.g., combination with Bcl-X_L inhibitors in *KRAS* mutant tumor cells [113]. Further identification of such strategies will require better understanding of the functional relationships between the Raf/MEK/ERK pathway and other key pathways for signal transduction and metabolic regulation in cancer.

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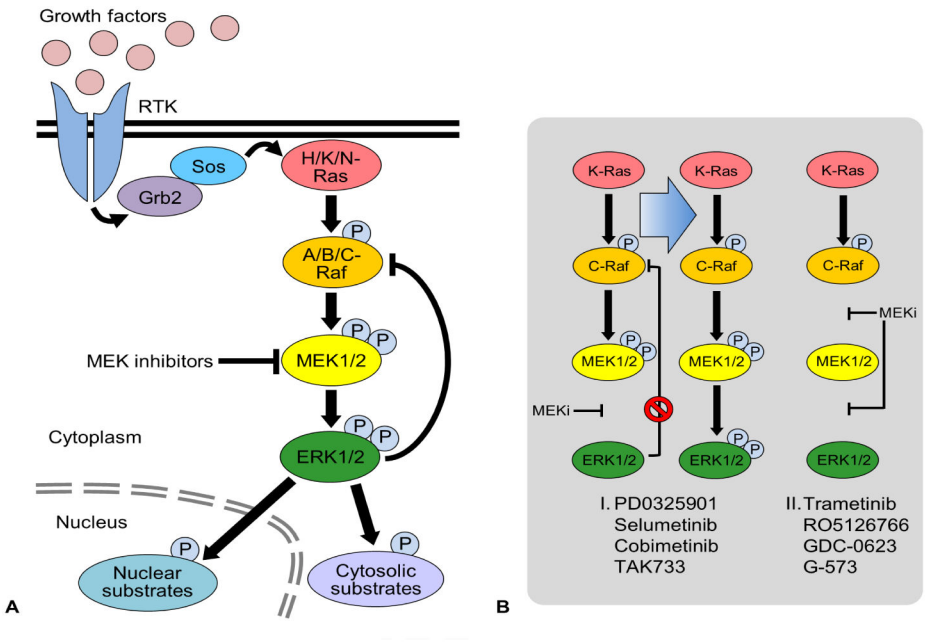

Figure 1.

The Raf/MEK/ERK pathway and MEK1/2 inhibition. (**A**) Extracellular stimuli such as growth factors regulate diverse physiological processes by activating the cell surface receptors, e.g., receptor tyrosine kinases (RTK), which relay the signals to the three-layered kinase cascade, Raf/MEK/ERK, typically via the adapter protein, Growth factor receptor-bound protein 2 (Grb2), the guanine nucleotide exchange factor, Son of sevenless (Sos), and the small GTPase, Ras. Upon activation, ERK1/2 not only activate/inactivate various cytosolic and nuclear substrates but also feedback-inhibit Raf activity to modulate the pathway activity in cells. (**B**) MEK1/2 inhibition relieves ERK1/2-mediated feedback inhibition of C-Raf by inactivating ERK1/2. Certain MEK1/2 inhibitors (I) increase the interaction between MEK1/2 and C-Raf, and, this can promote MEK1/2 phosphorylation by C-Raf, resulting in the rebound of MEK/ERK activity in *RAS* mutant tumors. However, newer MEK1/2 inhibitors (II) that avoid this feedback rebound of MEK/ERK activity in *RAS* mutant tumors are becoming available (see text for details).

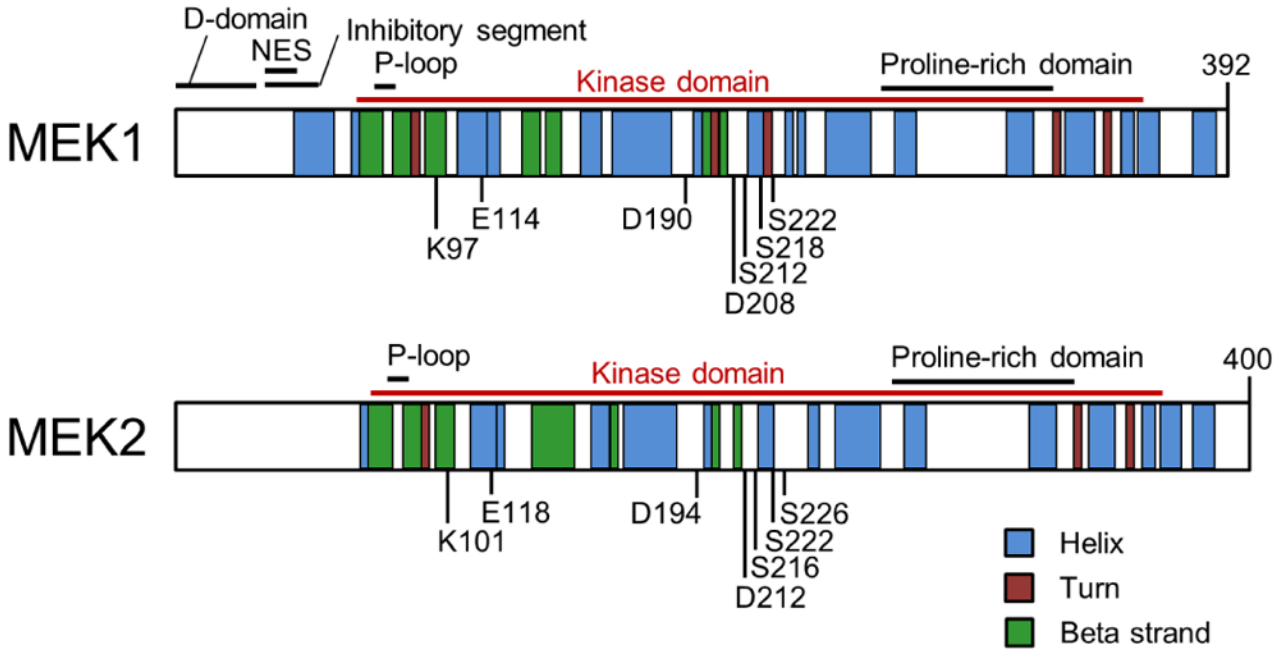

Figure 2.

Secondary structures of MEK1/2. Illustrated are key domains and motifs, including D-domain, the nuclear export sequence (NES), the glycine-rich loop (P-loop), kinase domains, the proline–rich domains, and several key residues for MEK1/2 activation and catalysis (see text for details). Structural information obtained from the UniProtKB database (accession number: MEK1:Q02750; MEK2:P36507).

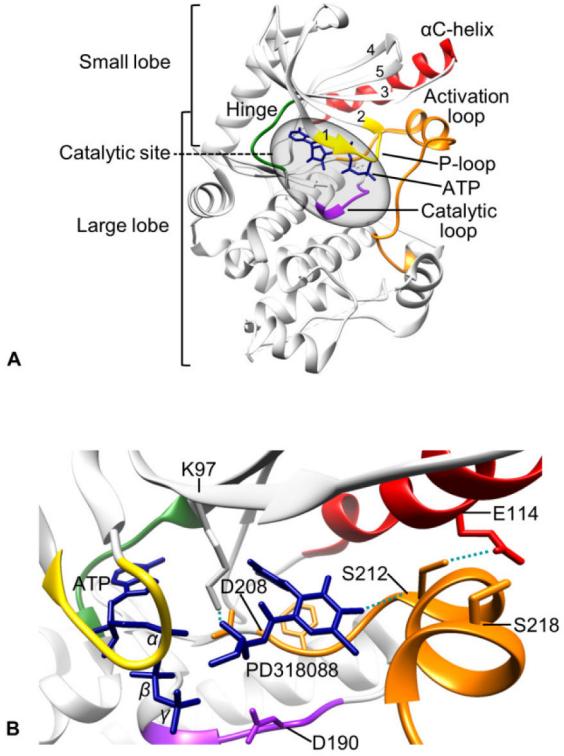

Figure 3.

Tertiary structure of MEK1. (**A**) Crystal structure of ATP-bound unphosphorylated MEK1 (PDB:3EQD). (**B**) Unphosphorylated MEK1 bound by PD318088 (PDB:1S9J). αC-helix, red; hinge region, green; P-loop, yellow; activation loop, orange; catalytic loop, purple; ATP and PD318088, blue. Images produced by the UCSF Chimera software.

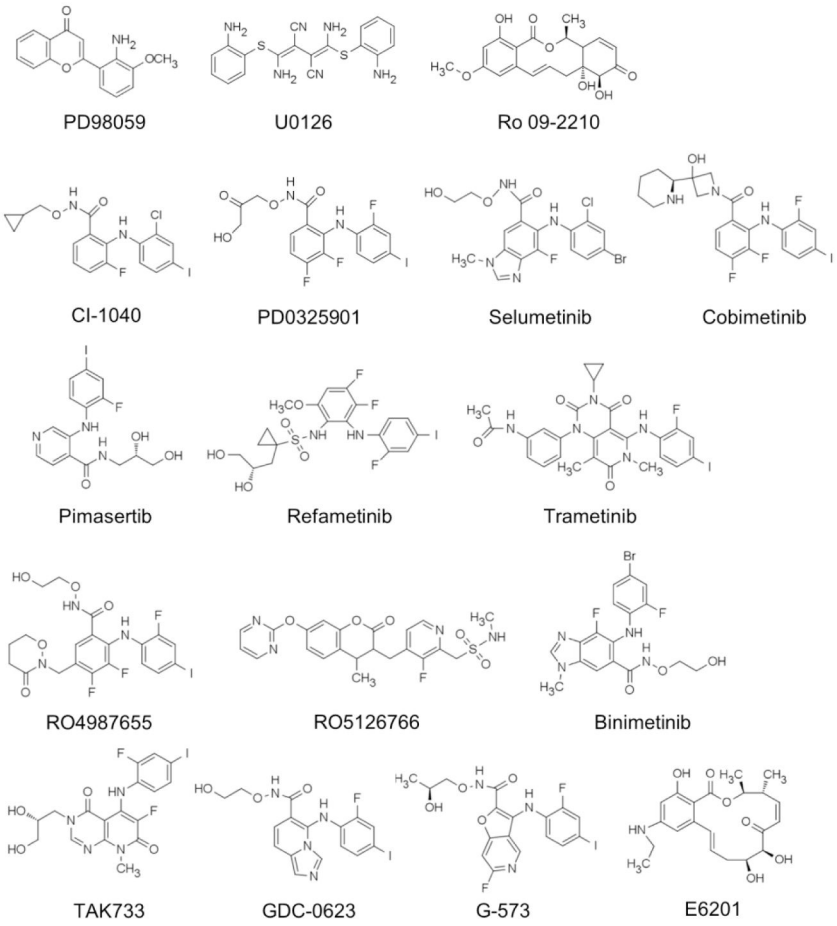

Figure 4.
Chemical structures of MEK1/2 inhibitors. WX-554 not shown.

Table 1

Small molecule inhibitors of MEK1/2

Name	Other name	Company [a]	Year [b]	Mechanism	Binding site	IC^{50} [c]	Clinical trials [d]
PD98059		Warner-Lambert	1995	ATP-noncompetitive	Not overlap with ATP or ERK binding sites	2 μM for MEK1 (Ref. 56)	None
U0126		DuPont	1998	ATP-noncompetitive and allosteric	An allosteric site that overlaps with PD98059 binding site	72 nM for MEK1 58 nM for MEK2 (Ref. 55)	None
Ro 09-2210		Roche	1998	N.D. [e]	N.D.	59 nM for MEK1 (Ref. 61)	None
CI-1040	PD184352	Warner-Lambert/Pfizer	1999	ATP-noncompetitive and allosteric	An allosteric site near the kinase subdomains III and IV, and adjacent to the ATP binding site. Interacts with Val127 in the hinge region and Ser212 in the activation loop. (PDB:1S9J and 1S9I)	17 nM for MEK1 (Ref. 62)	NCT00033384 (Phase II, completed) NCT00034827 (Phase II, completed)
PD0325901		Pfizer	2004	ATP-noncompetitive and allosteric	An allosteric site. Interacts with Ser212 in the activation loop.	1 nM for MEK1/2 (Ref. 65)	NCT00174369 (Phase II, terminated) NCT00147550 (Phase I/II, terminated)
Selumetinib	AZD6244 ARRY-142886	Array Biopharma/AstraZeneca	2005	ATP-noncompetitive and allosteric	An allosteric site	14 nM for MEK1 (Ref. 70)	NCT01843062 (Phase III, recruiting) NCT01933932 (Phase III, recruiting) NCT01974752 (Phase III, active)
Cobimetinib	GDC-0973 XL518	Exelixis/Genentech	2007	ATP-noncompetitive and allosteric	An allosteric site. Interacts with γ-phosphate of ATP, Ser212 in the activation loop and Asp190 in the catalytic loop. (PDB:4LMN)	1 nM for MEK1 (Ref. 78)	NCT01689519 (Phase III, active)
AZD8330	ARRY-424704	Array BioPharma/AstraZeneca	2009	ATP-noncompetitive	N.D.	7 nM for MEK1/2 (Ref. 103)	NCT00454090 (Phase I, completed)
E6201		Eisai	2009	ATP-competitive	ATP binding site	5.2 nM for MEK1 (Ref. 100)	NCT00794781 (Phase I, active)

Name	Other name	Company [a]	Year [b]	Mechanism	Binding site	IC^{50} [c]	Clinical trials [d]
Pimasertib	AS703026 MSC1936369B	EMD Serono	2009	ATP-noncompetitive and allosteric	An allosteric site	N.D.	NCT00957580 (Phase II, terminated) NCT01085331 (Phase II, completed) NCT01693068 (Phase II, active) NCT01936363 (Phase II, active)
Refametinib	RDEA119 BAY 869766	Ardea Biosciences/Bayer	2009	ATP-noncompetitive and allosteric	An allosteric site. Interacts with α-and γ-phosphates of ATP, Lys97 in the β3-strand, Val127 in the hinge region, DFG motif, Ser212 and Met219 in the activation loop. (PDB:3E8N)	19 nM for MEK1 47 nM for MEK2 (Ref. 80)	NCT01915589 (Phase II, not yet recruiting) NCT01915602 (Phase II, recruiting)
Trametinib	JTP-74057 GSK1120212	GlaxoSmithKline	2009	ATP-noncompetitive and allosteric	An allosteric site	14.9 nM for MEK1 (Ref. 81)	FDA approved in 2013
RO4987655	CH4987655	Hoffmann La Roche	2009	ATP-noncompetitive and allosteric	An allosteric site. Interacts with ATP, Lys97 in the β3-strand, Val127 in the hinge region, Val211, Ser212 and Asn221 in the activation loop. (PDB:3ORN)	5.2 nM for MEK1 (Ref. 89)	NCT00817518 (Phase I, completed)
WX-554		Wilex	2009	ATP-noncompetitive	N.D.	N.D.	NCT01581060 (Phase I/II, terminated) NCT01859351 (Phase I, terminated)
G-573		Genentech	2010	ATP-noncompetitive and allosteric	An allosteric site. Interacts with Ser212 in the activation loop.	N.D.	None
Binimetinib	ARRY-438162 MEK162	Array BioPharma/Novartis	2011	ATP-noncompetitive	N.D.	N.D.	NCT01763164 (Phase III, recruiting) NCT01849874 (Phase III, recruiting) NCT01909453 (Phase III, recruiting)
TAK733		Millenium Pharmaceuticals	2011	ATP-noncompetitive and allosteric	An allosteric site. Interacts with Lys97 in the β3-strand and Ser212 in the activation loop. (PDB:3PP1)	3.2 nM for MEK1 (Ref. 96)	NCT00948467 (Phase I, completed) NCT01613261 (Phase Ib, withdrawn)
GDC-0623		Genentech	2013	ATP-noncompetitive and allosteric	An allosteric site. Interacts with Ser212 in the activation loop.	N.D.	NCT01106599 (Phase I, completed)
RO5126766	CH5126766	Hoffmann La Roche	2013	Allosteric	An allosteric site. Interacts with Ser212, Ser222, and Asn221 in the activation loop. (PDB:3WIG)	160 nM for MEK1 (Ref. 90)	NCT00773526 (Phase I, completed)

[a] Invented or licensed by the company listed.

[b] Year in which MEK1/2 inhibitory activity of the listed compound was discovered.

[c] IC_{50} obtained from *in vitro* kinase assays.

[d] Information obtained from ClinicalTrials.gov (as of March 11, 2015)

[e] Not determined.