Emerging MEK inhibitors

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Importance of the field: The Ras/Raf/MEK/ERK pathway is often activated by genetic alterations in upstream signaling molecules. Integral components of this pathway such as Ras and B-Raf are also activated by mutation. The Ras/Raf/MEK/ERK pathway has profound effects on proliferative, apoptotic and differentiation pathways. This pathway can often be effectively silenced by MEK inhibitors.

Areas covered by this review: This review will discuss targeting of MEK which could lead to novel methods to control abnormal proliferation which arises in cancer and other proliferative diseases. This review will cover the scientific literature from 1980-present.

What the reader will gain: By reading this review the reader will understand the important roles that genetics play in the response of patients to MEK inhibitors, the potential of combining MEK inhibitors with other types of therapy, the prevention of cellular aging and the development of cancer stem cells.

Take home message: Targeting MEK has been shown to be effective in suppressing many important pathways involved in cell growth and the prevention of apoptosis. MEK inhibitors have many potential therapeutic uses in the suppression of cancer, proliferative diseases and aging.

Keywords: apoptosis, cancer, ERK, kinases, MEK, MEK inhibitors, proliferative disorders, protein phosphorylation, signal transduction


1. Background

This review is a follow on to a review which focused on Emerging Raf Inhibitors published in this same review series [1]. The Ras/Raf/MEK/ERK signaling pathway transmits signals from extracellular mitogens, growth factors and cytokines. Cytokines trigger and activate cell membrane receptors and the Ras/Raf/MEK/ERK pathway relays these messages from the cytoplasm to the nucleus to control gene expression [2-8]. Furthermore, this pathway also regulates the activity of, and subcellular localization of, many apoptotic molecules which are normally localized and function in the mitochondrion. An introductory overview of this pathway is presented in Figure 1. Also indicated in this figure is the site of intervention with MAPK/ERK kinase (MEK) inhibitors. Many of these inhibitors have been evaluated in various clinical trials and some are even currently being used to treat patients with specific cancers (see below).

The Raf/MEK/ERK pathway regulates the activity of many proteins involved in apoptosis. Many of the effects of the Ras/Raf/MEK/ERK pathway on apoptosis are mediated by extracellular-signal-regulated kinases 1,2 (ERK) phosphorylation of key apoptotic effector molecules (e.g., Bcl-2, Mcl-1, Bad, Bir, Caspase-9 and many others) [4]. Targeting MEK could result in, or contribute to, the induction of apoptosis. An overview of the effects of the Ras/Raf/MEK/ERK pathway on the phosphorylation of critical proteins involved in regulation of apoptosis is presented in Figure 2.
1.1 Introduction

Raf is responsible for serine/threonine (Ser/Thr, S/T) phosphorylation of MEK1 [4-9]. MEK1 phosphorylates ERK1 and 2 at specific Thr and Tyr (Y) residues [4]. Activated ERK1 and ERK2 S/T kinases phosphorylate and activate a variety of substrates, including p90Rsk1 [4]. ERK1,2 have many downstream and even upstream substrates. The number of ERK1,2 targets is easily in the hundreds. Thus, suppression of MEK and ERK will have profound effects on cell growth.
1.2 Biochemical details of the Raf/MEK/ERK pathway

The mammalian Raf gene family consists of A-Raf, B-Raf and Raf-1 (C-Raf). Raf is a S/T kinase and is normally activated by a complex series of events including: i) recruitment to the plasma membrane mediated by an interaction with Ras; ii) dimerization of Raf proteins; iii) phosphorylation/dephosphorylation on different domains; iv) disassociation with the Raf kinase inhibitory protein (RKIP); and v) association with scaffolding complexes (e.g., kinase suppressor of Ras) [4]. Raf activity is further modulated by proteins such as Bag1, 14-3-3 and heat shock protein 90 [1-5].

There are at least 13 regulatory phosphorylation sites on Raf-1 [4]. Some of these sites (e.g., S43, S259 and S621) are phosphorylated when Raf-1 is inactive. This allows 14-3-3 to bind Raf-1 and confer a configuration which is inactive. Upon cell stimulation, S621 becomes transiently dephosphorylated by an unidentified phosphatase. Phosphatases such as protein phosphatase 2A dephosphorylate S259 [1-9]. 14-3-3 then disassociates from Raf-1. This allows Raf-1 to be phosphorylated at S338, Y340 and Y341, rendering Raf-1 active. A Src family kinase is probably responsible for phosphorylation at Y340 and Y341 [1-9].

Y340 and Y341, the phosphorylation targets of Src family kinases, are conserved in A-Raf (Y299 and Y300), but are replaced with aspartic acid (D) at the corresponding positions in B-Raf (D492 and D493) [1-9]. The negatively charged aspartic acid residues mimic activated residues, which makes B-Raf highly active. Maximal activation of Raf-1 and A-Raf requires both Ras and Src activity while B-Raf activation is Src-independent [1-9]. Interestingly, as is discussed later, a greater number of mutations are detected at B-Raf than either Raf-1 or A-Raf in human cancer.

The S338 residue present in Raf-1 is conserved among the three Raf isoforms; however, in B-Raf (S445), this corresponding site is constitutively phosphorylated [4]. S338 phosphorylation on Raf-1 is stimulated by Ras and is dependent on p21-activated protein kinase [4]. Other phosphorylation sites in Raf-1 that may modulate its activity include: S43, S339,
T491, S494, S497, S499, S619 and S621. PKC has been shown to activate Raf and induce cross-talk between PKC and Raf/MEK/ERK signaling pathways [4]. S497 and S499 were identified as the target residues on Raf-1 for PKC phosphorylation. However, other studies suggest that these sites are not necessary for Raf-1 activation [4].

Raf activity is negatively regulated by phosphorylation on the conserved region (CR)2 regulatory domain. Akt and protein kinase A phosphorylate S259 on Raf-1 and inhibit its activity [1-9]. Furthermore, Akt or the related serum/glucocorticoid regulated kinase phosphorylate B-Raf on S364 and S428 and inactivate its kinase activity [1-9]. These S-phosphorylated Rafs associate with 14-3-3 and become inactive.

The scaffolding protein, RKIP, has been shown to inhibit Raf-1 activity [4]. RKIP is a member of the phosphatidylethanolamine-binding protein family. This multi-gene family is evolutionarily conserved and has related members in bacteria, plants and animals [4]. Interestingly, RKIP can bind either to Raf or to MEK/ERK but not to Raf, MEK and ERK all together. Various isoforms of PKC have been shown to phosphorylate RKIP on S153 which results in the disassociation of Raf and RKIP.

The importance of Raf-1 in the Raf/MEK/ERK signal transduction pathway has come into question due to the discovery that B-Raf was a much more potent activator of MEK compared to Raf-1 and A-Raf. Many of the ‘functions’ of Raf-1 persist in Raf-1 knockout mice, and are probably maintained by endogenous B-Raf [4]. Interestingly and controversially, it was recently proposed that B-Raf is not only the major activator of MEK1, but B-Raf is also involved in Raf-1 activation. B-Raf may be temporally activated before Raf-1. However, there may be different subcellular localizations of B-Raf and Raf-1 within the cell that exert different roles in signaling and apoptotic pathways [4]. In some cases, B-Raf may transduce its signal through Raf-1 and B-Raf can form heterodimers with Raf-1. Dimerization is one important component involved in Raf activation [4]. The reasons for these added complexities in the Raf/MEK/ERK kinase cascade are not obvious but may represent another layer of fine-tuning. Alternatively, B-Raf:Raf-Raf heterodimers may have different substrate specificities or affinities than B-Raf:B-Raf and Raf-1:Raf-1 homodimers. Furthermore, as is discussed later, some B-Raf mutants which are kinase deficient may transmit their signals via forming heterodimer with Raf-1 [1-4].

MEK1 is a tyrosine (Y-) and S/T-dual specificity protein kinase [1-9]. Its activity is positively regulated by Raf phosphorylation on S residues in the catalytic domain. All three Raf family members are able to phosphorylate and activate MEK but different biochemical potencies have been observed (B-Raf > Raf-1 >> A-Raf) [1-9]. Another interesting aspect regarding MEK1 is that its predominant downstream target is ERK. In contrast, both upstream Raf and downstream ERK have multiple targets. Thus, therapeutic targeting of MEK1 is relatively specific.

ERK1,2 are S/T kinases and their activities are positively regulated by phosphorylation mediated by MEK1 and MEK2. ERKs can directly phosphorylate many transcription factors including Ets-l, c-Jun and c-Myc. ERKs can also phosphorylate and activate the 90 kDa ribosomal S6 kinase (p90Rsk). p90Rsk can then phosphorylate and activate the cAMP response element binding protein (CREB) transcription factor (Figure 1) [1]. Moreover, through an indirect mechanism, ERKs can lead to activation of the NF-κB transcription factor (nuclear factor immunoglobulin K chain enhancer-B cell) by phosphorylating and activating inhibitor κB kinase (IκB) and ERK1 and ERK2 are differentially regulated. These are only a few examples of the downstream targets of ERKs 1,2. ERK can enter the nucleus to phosphorylate many transcription factors [1].

2. Medical need

In the Western world, cancer has surpassed heart disease and is now the number one killer of adults. As we discuss below, there is often an aberrant activation of the Raf/MEK/ERK pathway in human cancer due to various genetic and epigenetic mechanisms. Furthermore, as we discuss in section 5.4.1, targeting of MEK may be effective in treating other proliferative diseases including aging. MEK lies in a critical position in this pathway as it has few direct upstream activators (e.g., Raf) and few downstream targets (e.g., ERK). Thus, the development of effective MEK inhibitors could prove very useful in cancer therapy. MEK inhibitors have been some of the first small molecule, cell membrane permeable inhibitors developed which have been determined to have a high degree of specificity in extensive preclinical studies with various types of cancer as well as other diseases and syndromes [2,3]. As we discuss below, MEK inhibitors have also been evaluated in combination with other signal transduction pathway inhibitors as well as chemo- and radiotherapeutic approaches. The vast majority of these studies have indicated that the combination of MEK inhibitors and various other types of therapy have resulted in improved outcomes. The development and proper usage of effective MEK inhibitors has the real potential to advance medical treatment.

2.1 Abnormal activation of MEK in human cancer

Perhaps one of the biggest advances in medical science in the 1980s was the confirmation of the proto-oncogene hypothesis, which predicted that the human genome contains genes related to viral oncoproteins which when mutated could cause human cancer. Key genetic members of the Ras/Raf/MEK/ERK pathway (e.g., RAS, RAF) and upstream receptors (e.g., EGFR, ERBB1, ERBB2) were shown to fulfill this hypothesis as they were sometimes mutated/amplified in specific human cancers [9]. Genetic mutations at these cellular oncogenes often confer sensitivity to MEK inhibitors. An illustration of some of the receptors, kinases and phosphatases that are mutated/amplified in human cancer and how they may impact on MEK activity is presented in Figure 3.
Mutations that lead to the expression of constitutively-active Ras proteins have been observed in ~ 20 – 30% of human cancers [5,9,10]. In cholangiocarcinoma, KRAS gene mutations have been identified in 45% of examined tumors [9]. The KRAS gene is also mutated at a very high incidence in pancreatic cancer, as high as 80%. The mutations in KRAS and other genes in pancreatic cancer have recently been reviewed [10]. These are often point mutations that alter Ras activity. Genome RAS amplification or overexpression of Ras, perhaps due to altered methylation of its promoter region, are also detected in some tumors [5,9]. Mutations that result in increased Ras activity may also perturb MEK activity and may confer sensitivity to MEK inhibitors. A key event in the activation of the Ras protein is farnesylation. Inhibitors which target the enzyme farnesyl transferase (FT) have been developed with the goal of targeting Ras. Clinical testing of FT inhibitors (FTI), unfortunately, has yielded disappointing results [11]. The lack of usefulness of FTIs may be due to multiple reasons. First, there are many proteins which are regulated by FT. Second, although H-Ras is exclusively modified by FT and K-Ras to a lesser extent, N-Ras can also be modified by geranylgeranyltransferase. This modified N-Ras is still able to support the biological requirement of Ras in the cancer cell. Geranylgeranylation of K-Ras and N-Ras becomes critical only when farnesylation is inhibited [5]. The majority of RAS mutations in humans occur in KRAS, which is followed by NRAS [9]. The mutation rate at HRAS brings up a distant third. Hence, it is very possible that the effects which FTIs had in initial clinical trials were not due to inhibition of mutant RAS genes present in the cell, but in fact resulted from nonspecific effects which were related to the first point mentioned. Importantly, some of these tumors with RAS mutations may be sensitive to MEK inhibitors.

BRAF mutation occurs in ~ 7% of all cancers [8,12,13]; however, this frequency may change as more and diverse tumors are examined for BRAF mutation (see below). One study has observed that mutated alleles of CRAF are present.

**Figure 3. Dysregulated expression of upstream receptors and kinases can result in activation of MEK.** Sometimes dysregulated expression of growth factor receptors occurs by either increased expression or genomic amplifications (e.g., VEGFR, EGFR, HER2, IGF-1R). Mutations have been detected in EGFR, Flt-3, Kit, PDGF receptor, PI3K, PTEN, Ras and B-Raf. Amplification of HER2 and EGFR is detected in certain cancer types. The BCR-ABL chromosomal translocation is present in virtually all CMLs and some ALLs. Many of these mutations and chromosomal translocations result in the activation of the Raf/MEK/ERK cascade. The PI3K/PTEN/Akt pathway is also activated in certain cancer and can regulate the Raf/MEK/ERK cascade. These pathways can also be activated by autocrine growth stimulation, the genetic basis of which is frequently unknown.

ALL: Acute lymphocytic leukemia; AML: Acute myeloid leukemia; CML: Chronic myeloid leukemia; GIST: Gastrointestinal stromal tumor; PTEN: Phosphatase and tensin homolog.
patients (1 – 3%) [13-15]. Patients with ovarian cancer (30%) and a small minority of lung cancer colorectal cancer (5 – 22%), cholangiocarcinoma (22%), melanoma (27 – 70%), papillary thyroid cancer (36 – 53%), ovarian cancer (30%) and a small minority of lung cancer patients (1 – 3%) [13-15]. Patients with BRAF and CRAF mutations are predicted to be sensitive to MEK inhibitors.

In many cancers with BRAF mutations, the mutations are believed to be initiating events, but are not sufficient for full-blown neoplastic transformation [8,12,16,17]. Mutations at other genes (e.g., in components of the PI3K/PTEN/Ark/mTOR pathway, especially PTEN) have been hypothesized to also be necessary for malignant transformation in some cancers [8].

Most cancers contain multiple mutations [8-23]. Some of these mutations in cancer can be suppressed by inhibitors which target kinases such as MEK and additional kinases which may also be activated in that cancer. For example, melanoma has mutations in genes encoding components of the Ras/Raf/MEK/ERK pathway. Mutations at Ras and BRAF are detected in melanoma and some of these mutant kinases will be sensitive to B-Raf and MEK inhibitors. However, if the melanoma has a mutation at KRAS, it may not be sensitive to the B-Raf and MEK inhibitors as instead the PI3K/PTEN/Akt/mTOR pathway may be activated by the mutant KRAS. In addition, as stated earlier, there are multiple mutations in certain cancers and melanoma is a good example. BRAF mutations may only be part of the mutational events necessary for establishment of a melanoma; additional mutations at genes such as PTEN may also be required for melanoma. Recently, it has been shown that it is more effective to treat melanomas with inhibitors which target both the Raf/MK/ERK and PI3K/PTEN/Akt/mTOR pathways to enhance melanoma treatment [8]. In addition, it has been shown that certain basal-type breast cancers are sensitive to MEK inhibitors and a consequence of treatment of certain cells with MEK inhibitor is the activation of Akt, by the MEK inhibitor suppressing a negative feedback loop [23].

3. Scientific rationale

Clearly, there is a scientific rationale for the development of MEK inhibitors as the Raf/MEK/ERK pathway is frequently aberrantly expressed in human cancer and the targeting of this pathway might improve cancer therapy.

3.1 Altered expression of MEK and sensitivity to therapy

It has been reported that a high frequency of acute myeloid leukemias (AMLs) and acute lymphocytic leukemias (ALLs; > 50%) display constitutive activation of MEK in the absence of any obvious genetic mutation [20,21]. While there may be some unidentified mutations at one component of the pathway or a phosphatase that regulates the activity of the pathway, the genetic mechanism responsible for the constitutive activation of MEK is unknown. Elevated expression of downstream ERK in AMLs and ALLs is associated with a poor prognosis [21]. Often elevated expression of signaling pathways is correlated with increased sensitivity to targeted therapy (e.g., MEK inhibitors) [5,22]. MEK inhibitors may prove useful in the treatment of a large percentage of AMLs and ALLs. The observation that there is often constitutive activation of MEK in these leukemias as well as many other cancers and proliferative disorders (e.g., certain allergies and inflammatory diseases) has propelled the pharmaceutical industry to develop inhibitors which target MEK.

There can be a genetic basis for the sensitivity of some NSCLCs to EGFR inhibitors, such as erlotinib and gefitinib [24-28]. Interestingly, NSCLC with mutations at KRAS are resistant to EGFR inhibitors; these particular NSCLCs may be sensitive to MEK inhibitors [28]. In addition, some melanoma cells carrying BRAF mutations are sensitive to MEK inhibitors, while cells lacking these BRAF mutations are resistant [29]. The introduction of activated EGFR mutants into hematopoietic cells renders them sensitive to MEK inhibitors [30,31]. Furthermore, introduction of activated RAS, RAF and MEK genes into hematopoietic cells makes them sensitive to MEK inhibitors [32-37].

Different BRAF mutations have been mapped to various regions of the B-Raf protein. Mutations at BRAF which result in low kinase activity may signal through Raf-1 [12]. Heterodimerization between B-Raf and Raf-1 proteins may allow the impaired B-Raf to activate Raf-1 [19]. These particular BRAF mutations may be sensitive to MEK inhibitors.
proliferation is dependent upon that particular oncogene product. Cells with the BCR-ABL chromosomal translocation are also sensitive to MEK inhibitors; however, in general the BCR-ABL inhibitors are more effective because they shut off additional signaling pathways.

4. Existing therapy and competitive environment

Existing therapy for many of the cancers which display aberrant Raf/MEK/ERK pathway expression is not optimal. For example, the successful therapy of melanoma, pancreatic, hepatocellular carcinoma (HCC), and advanced breast cancer are poor, as is the successful therapy of adult patients with AML. Development of novel methods to treat these and other cancer patients is essential.

4.1 Advantages and strategies of targeting MEK to enhance cancer therapy

Small-molecule inhibitors, such as imatinib (Novartis), have proven effective in the treatment of CML and certain other leukemias that grow in response to BCR-ABL (e.g., some ALLs) and other cancers which proliferate in response to mutant platelet-derived growth factor receptor (PDGFR) and KIT genes [38,39], such as gastrointestinal stromal tumors, as imatinib also inhibits these related kinases. As previously stated, some lung carcinomas that have EGFR mutations are sensitive to EGFR inhibitors [4]. These cancers represent special examples of tumors that proliferate in response to a defined mutation. Unfortunately, the mechanisms responsible for aberrant proliferation of most cancers are unknown. Many of the mutations in human cancer result in the aberrant activation of signaling pathways which impinge upon MEK. Hence, MEK inhibitors have been developed [41-57] and some have been evaluated in clinical trials (see below).

Other non-mutational-based mechanisms can result in activation of MEK and contribute to either malignant transformation or drug resistance. A consequence of chemotherapeutic drug treatment of breast, hematopoietic, prostate and other cancers is the induction of ERK [4]. Eliminating this deleterious side effect of these therapies using MEK inhibitors may enhance their ability to kill drug resistant cancer. Use of these inhibitors may augment the effects of chemo-, radio- and hormonal-based therapies (see below).

4.2 Specific MEK inhibitors

Specific inhibitors of MEK have been developed (e.g., PD98059 (Pfizer), U0126 (DuPont), PD184352 (CI-1040) (Pfizer), PD0325901 (Pfizer), ARRY-142886 (also known as AZD6244) (Array Biopharma/AstraZeneca), RDEA119 (Ardea Biosciences/Bayer) and XL-518 (Exelixis/Genetech)) (Table 1) [2-4,41-57]. The successful development of MEK inhibitors may be due to the relatively few phosphorylation sites on MEK involved in activation/inactivation. MEK inhibitors differ from most other kinase inhibitors as they do not compete with ATP binding (non-ATP competitive), which confers a high specificity [41,43]. Most MEK inhibitors are specific and do not inhibit many different protein kinases [58] although as is discussed below, certain MEK inhibitors are more specific than others.

The crystal structures of MEK1 and MEK2 have been solved as ternary complexes with ATP and PD184352, and have revealed that both MEK1 and MEK2 have unique inhibitor binding sites located on a hydrophobic pocket adjacent to, but not overlapping with, the ATP-binding site [44]. Furthermore, effective targeting of MEK1/MEK2 is highly specific, as ERK1/ERK2 are the only well-described downstream targets. A distinct advantage of inhibiting the Raf/MEK/ERK cascade is that it can be targeted without knowledge of the precise genetic mutation that results in its aberrant activation. This is important as the nature of the critical mutation(s) that leads to the malignant growth of most cancers is really unknown. An advantage of targeting MEK is that the Raf/MEK/ERK pathway is a convergence point where a number of upstream signaling pathways can be blocked with the inhibition of MEK. For example, MEK inhibitors, such as ARRY-142886 (AZD-6244), are also being investigated for the treatment of diverse cancers including lung, colo-rectal, pancreatic and HCC [45-48].

RDEA119 is a more recently described MEK inhibitor developed by Ardea Biosciences [55]. It is a highly selective MEK inhibitor which displays >100-fold selectivity in kinase inhibition in a panel of 205 kinases. In contrast, in the same kinase specificity analysis, other recently developed MEK inhibitors (e.g., PD0325901) also inhibited the Src and RON kinases.

4.3 MEK inhibitors in clinical studies

ARRY-142886 is an orally-active MEK1 inhibitor that has undergone Phase II clinical trials [45-49]. ARRY-142886 has demonstrated significant tumor suppressive activity in preclinical models of cancer, including melanoma, pancreatic, colon, lung, liver and breast cancers. The effects of ARRY-142886 are enhanced significantly if the tumor has a mutation that activates MEK.

ARRY-142886 inhibits MEK1 in vitro with an IC_{50} value of 14.1 ± 0.79 nM [45]; it is specific for MEK1 as it did not appear to inhibit any of the ~40 other kinases in the panel tested. ARRY-142886 is not competitive with ATP. Molecular modeling studies indicate that ARRY-142886 binds to an allosteric binding site on MEK1/MEK2. The binding sites on MEK1/MEK2 are relatively unique to these kinases and may explain the high specificity of MEK inhibitors. This binding may lock MEK1/2 in an inactive conformation that enables binding of ATP and substrate, but prevents the molecular interactions required for catalysis and access to the ERK activation loop. In basic research studies, treatment with the MEK inhibitor results in the detection of activated MEK1/2 when the proteins present on a western immunoblot are probed with an antibody which recognizes
active MEK1/2, while downstream ERK1/2 will not appear activated with the activation specific ERK1/2 antibody [51]. ARRY-142886 inhibited downstream ERK1/ERK2 activation in \textit{in vitro} cell line assays with stimulated and unstimulated cells, and also inhibited activation in tumor-transplant models. In contrast, ARRY-142886 did not prevent the activation of the related ERK5 that occurs with some older MEK1 inhibitors, which are not being pursued in clinical trials. Inhibition of ERK1,2 suppresses their ability to phosphorylate and modulate the activity of MEK1 but not MEK2 as MEK2 lacks the ERK1/ERK2 phosphorylation site. In essence, by inhibiting ERK1,2 the negative loop of MEK phosphorylation is suppressed and hence there will be an accumulation of activated MEK. This biochemical feedback loop may provide a rational for combining Raf and MEK inhibitors in certain therapeutic situations. The actual concentrations of ARRY-142886 required to inhibit proliferation in various cancer cell lines may depend on how dependent they are on the Raf/MEK/ERK pathway for proliferation. Cells with mutations in Raf or upstream molecules which signal through this pathway may be highly sensitive to ARRY-142886 and IC50s of 10 – 50 nM are observed. In contrast, other cell lines which do not have mutations in neither B-Raf nor upstream molecules may be resistant to ARRY-142886 and doses approaching 5000 nM may be required to reach the IC50. Furthermore, there may be certain mutations in other pathways (e.g., PI3K/PTEN/Akt/mTOR) which confer resistance to ARRY-142886.

In colon, melanoma, pancreatic, liver and some breast cancers, ARRY-142886 inhibited the growth of tumors in tumor xenograph studies performed in mice. The new MEK inhibitors are also at least 10- to 100-fold more effective than earlier MEK inhibitors and hence can be used at lower concentrations [45-48]. ARRY-142886 also inhibits the growth

Table 1. Competitive environment.

<table>
<thead>
<tr>
<th>MEK inhibitors under evaluation</th>
<th>Ref.</th>
<th>Cancer examined</th>
<th>Clinical trial</th>
<th>Result</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEK inhibitor</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CI-1040, PD-184352</td>
<td>[41-43]</td>
<td>Advanced colorectal, NSCLC, pancreatic, kidney, melanoma, breast</td>
<td>Phase II/II</td>
<td>Reduced pERK levels, discontinued clinical trials due to pharmacological problems</td>
<td>Pfizer</td>
</tr>
<tr>
<td>PD0325901</td>
<td>[29,39,53,56]</td>
<td>Breast, colon, NSCLC, melanoma</td>
<td>Phase II/II</td>
<td>Discontinued</td>
<td>Pfizer</td>
</tr>
<tr>
<td>XL518</td>
<td>Exelixis (Internet)</td>
<td>Phase I</td>
<td>Ongoing</td>
<td>Exelixis</td>
<td></td>
</tr>
<tr>
<td>AZD6244/ARRY-142886</td>
<td>[45-48]</td>
<td>Multiple melanoma, HCC, advanced solid cell tumors</td>
<td>Phase II/II</td>
<td>77% pERK reduction</td>
<td>Astra Zeneca/Array BioPharma</td>
</tr>
<tr>
<td>RDEA119</td>
<td>[55]</td>
<td>Advanced tumors</td>
<td>Phase II/II</td>
<td>Ongoing</td>
<td>Ardea/Bayer</td>
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**Combination MEK inhibitor trials**

<table>
<thead>
<tr>
<th>MEK inhibitor + other agent</th>
<th>Ref.</th>
<th>Cancer examined</th>
<th>Clinical trial</th>
<th>Result</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>CI-1040 + ATO</td>
<td>[92-95]</td>
<td>Leukemia</td>
<td>Preclinical</td>
<td>ERK reduction</td>
<td>Ardea/Bayer</td>
</tr>
<tr>
<td>RDEA119 + sorafenib</td>
<td>Ardea (Internet)</td>
<td>Advanced cancer patients, HCC</td>
<td>Phase I</td>
<td>ERK reduction</td>
<td>Ardea/Bayer</td>
</tr>
<tr>
<td>AZD6244/ARRY-142886 + doxorubicin</td>
<td>[48]</td>
<td>HCC</td>
<td>Phase I</td>
<td>ERK reduction</td>
<td>Astra Zeneca/Array BioPharma</td>
</tr>
<tr>
<td>AZD6244/ARRY-142886 + docetaxel</td>
<td>[84]</td>
<td>Various solid tumors</td>
<td>Preclinical</td>
<td>ERK reduction</td>
<td></td>
</tr>
<tr>
<td>AZD6244/ARRY-142886 + radiation</td>
<td>[98]</td>
<td>Various solid tumors</td>
<td>Preclinical</td>
<td>ERK reduction</td>
<td></td>
</tr>
</tbody>
</table>

ERK: Extracellular-signal-regulated kinase; HCC: Hepatocellular carcinoma.
of human leukemia cells, but does not affect the growth of normal human cells. ARRY-142886 also suppressed the growth of pancreatic BxPC3 cells (see below), which do not have a known mutation in this pathway, suggesting that this drug may also be useful for treating cancers that lack definable mutations. However, it is likely that BxPC3 cells have some type of upstream gene mutation/amplification or autocrine growth factor loop which results in the activation of the Raf/MEK/ERK pathway.

ARRY-142886 induced G1/S cell-cycle arrest in colon and melanoma cancer cell lines and activated caspase-3 and -7 in some cell lines (Malme3M and SKMEL2); however, caspase induction was not observed in other melanoma (SKMEL28) or colon cancer cell lines (HT29), demonstrating that further research needs to be performed with this inhibitor to determine if it normally induces apoptosis and whether the induction of apoptosis can be increased with other inhibitors or chemotherapeutic drugs. One report demonstrated that the treatment of HCC cells with ARRY-142886 plus doxorubicin led to synergistic growth inhibition and apoptosis both in vitro and in vivo [48] (further discussed below).

ARRY-142886 has undergone several Phase I and II clinical trials [45-49]. A Phase I clinical trial to assess the safety, tolerability and pharmacokinetics of ARRY-142886 in patients with various solid malignancies was performed. Phase II clinical trials have compared: i) the efficacy of ARRY-142886 versus temozolomide in patients with unresectable stage 3 or 4 malignant melanomas; ii) the efficacy and safety of ARRY-142886 versus capcitabine in patients with advanced or metastatic pancreatic cancer who have failed to respond to gemcitabine therapy; iii) the efficacy and safety of ARRY-142886 compared with pemetrexed in patients with NSCLC who have previously failed to respond to one or two previous chemotherapy regimens; iv) the efficacy and safety of ARRY-142886 versus capcitabine in patients with colorectal cancer who have failed to respond to one or two previous chemotherapy regimens; and v) advanced or metastatic HCC [45-49]. Initial results from clinical trials have not yielded encouraging results [43,53]. This was probably due to low oral bioavailability and high metabolism, which led to plasma drug levels that were inadequate to suppress tumor growth.

The newer PD-0325901 MEK inhibitor is an orally-active, potent, specific, non-ATP competitive inhibitor of MEK. PD-0325901 demonstrated improved pharmacological and pharmaceutical properties compared with PD-184352, including a greater potency for inhibition of MEK, and higher bioavailability and increased metabolic stability.

PD-0325901 has a $K_i$ value of 1 nM against MEK1 and MEK2 in in vitro kinase assays. PD-0325901 inhibits the growth of cell lines which proliferate in response to elevated signaling of the Raf/MEK/ERK pathways in the low nanomolar levels [29,56]. In general, the concentrations of this MEK inhibitor required to inhibit proliferation of cells in vitro ranges from 50 to 5000 nM, cells which are inhibited at 50 nM are referred to as highly sensitive and cells which require 5000 nM are referred to as resistant. The highly sensitive cells often have mutations at key components of the Raf/MEK/ERK pathway which makes their growth highly dependent on MEK.

As stated previously, the $BRAF^{V600E}$ mutation is present in ~ 6 – 8% of human cancers (overall). Interestingly, ~ 5% of lung cancers have mutations at $BRAF$ which are not at $V600E$ [58]. The effects of PD-0325901 were examined in conditional $BRAF^{V600E}$ tumor models where genetically modified mice express normal B-Raf prior to Cre-mediated recombination, after which they express B-Raf$^{V600E}$ at physiological levels [58]. When B-Raf$^{V600E}$ was induced, the mice developed lung tumors which could be inhibited by PD-0325901 (25 mg/(kg day) for ~ 2 weeks, followed by 12.5 mg/(kg day) for an additional 2 weeks). In contrast, mice treated with vehicle alone developed adenomas. This experimental mouse model suggests the effects of the PD-0325901 in the treatment of patients with tumors that proliferate in response to activation of the Raf/MEK/ERK pathway should be re-evaluated. However, these and other models also indicate that therapy needs to include a cytotoxic drug, as the MEK inhibitors are cytostatic and often as soon as the MEK inhibitors are removed, the tumor will re-emerge and eventually kill the host.

Clinical trials with PD-0325901 have documented some successes and some adverse side effects [52-54]. The results of these clinical trials with PD-0325901 as well as other MEK inhibitors have recently been extensively and critically reviewed [52-54]. The use of the PD-0325901 MEK inhibitor to treat cancer patients is currently in limbo. Pfizer has currently (2009) suspended its evaluation in clinical trials [53]. This may have resulted in part from the design of the clinical trials as MEK inhibitors may not be appropriate to treat all types of cancer. MEK inhibitors may be appropriate to treat only those cancers which proliferate in response to activation of the Raf/MEK/ERK pathway. Furthermore, it may also be important to include a chemotherapeutic drug or radiation treatment to induce death of the cancer cell. The reasons for
suspension of the clinical trials with PD-0325901 are well described in [52-54].

4.4 Advantages of targeting MEK in cancers where the pathway components are not specifically mutated: the hepatocellular cancer model

In the following section, we discuss the advantages of using MEK inhibitors to treat a cancer with a poor prognosis, yet this cancer does not have mutations at MEK. HCC is the fifth most common cancer worldwide and the third most prevalent cause of cancer mortality, accounting for ~6% of all human cancers and > 600,000 deaths annually worldwide [59-62]. Although the clinical diagnosis and management of early-stage HCC has improved significantly, HCC prognosis is still extremely poor [59-62]. Therefore, investigating HCC pathogenesis and finding new diagnostic and treatment strategies is important.

Signaling via MEK plays a critical role in liver carcinogenesis [63-68]. Although mutations of Ras and Raf occur infrequently in HCC, a recent study demonstrated that activation of the Ras/Raf/MEK/ERK pathway occurred in 100% of HCC specimens analyzed when compared with non-neoplastic surrounding tissues and normal livers. This increased Ras expression coincided with the decreased expression of genes which serve to inhibit Ras expression, namely the Ras association domain family 1A (RASSF1A) and the novel Ras effector 1A (NORE1A). These genes may be suppressed due to aberrant methylation of their promoters [68]. Decreased expression of these genes, which normally serve to suppress Ras, may lead to activation of downstream MEK.

Human HCC tumors have higher expression and enhanced activity of MEK1/2 and ERK1/2 compared with adjacent non-neoplastic liver [63]. Overexpression of activated MEK1 in HCC HepG2 cells resulted in enhanced tumor growth in vivo [64]. On the other hand, preclinical studies have demonstrated the potential of MEK inhibition to suppress hepatoma cell proliferation and tumorigenicity [47]. Huynh et al. recently reported that treatment of human HCC xenografts with AZD6244 (ARRY-142886) blocked ERK1/2 activation, reduced in vivo tumor growth and induced apoptosis [47]. Moreover, targeting MEK with PD-0325901 had in vivo chemopreventive effects on HCC development in an animal model using TGF-α-transgenic mice in which liver cancers were induced by diethylaminoethylmethane treatment [69]. Therefore, MEK represents a potential therapeutic target for HCC.

Obesity is another important contributing factor for the development of HCC. The important role of Ras/Raf/MEK/ERK signaling has also been suggested for HCC progression in obese patients. A possible explanation for risk associated between obesity and HCC comes from the study of Saxena et al. who for the first time demonstrated that leptin, a key molecule involved in the regulation of energy balance and body weight control, promotes HCC growth and invasiveness through activation of Raf/MEK/ERK signaling [70].

Other well known risk factors for HCC such as hepatitis viruses B and C also utilize the Ras/Raf/MEK/ERK pathway for the control of hepatocyte survival and viral replication [71-77]. Among the four proteins encoded by HBV genome, HBx is involved in hepatocarcinogenesis. HBx activates Ras/Raf/MEK/ERK signaling cascade [71-77]. Among HCV components, the core protein has been reported to activate MEK pathway and thereby might contribute to HCC carcinogenesis [75-77]. Therefore, these studies suggest that MEK is a novel therapeutic target which could be exploited for the treatment of HCC resulting from HBV and HCV infection.

4.5 Advantages of targeting MEK in pancreatic cancer

In the following section, we discuss the advantages of using MEK inhibitors to treat a cancer with a poor prognosis. This cancer does not have mutations at MEK and yet may have mutations in upstream RAS. As we stated earlier, a high percentage of pancreatic cancer have RAS mutations. Certain MEK inhibitors have shown great promise in the treatment of pancreatic cancers (see below), which often have mutations in RAS that can lead to downstream Raf/MEK/ERK pathway activation. In the US, ~37,000 new cases of pancreatic cancer are diagnosed each year and there are 34,000 deaths. It is currently the fourth leading cause of cancer deaths in the US due to the high and rapid mortality rate. The incidence of pancreatic cancer increases with age and peaks between the years 60 and 70. The highest incidence of pancreatic cancer worldwide appears to be in the US, Israel, Sweden and Canada (http://www.cancer.gov/cancertopics/types/pancreatic). This may be related to the diets in these various countries. Cigarette smoking, certain food additives and diabetes may be linked to pancreatic cancer, as well as exposure to certain industrial chemicals. Diets low in fruit and vegetables and high in red meat have been associated with pancreatic cancer. Due to the asymptomatic nature of pancreatic cancer, it is often detected when the disease is in an advanced stage; this leads to poor prognoses for the patients and a short life expectancy (<1 year).

If MEK inhibitors are ever to be used in the treatment of certain pancreatic cancer patients, it will be important to determine if these patients have a RAS mutation which results in Raf/MEK/ERK or PI3K/PTEN/Akt/mTOR activation or activation of both pathways as certain RAS mutations will result in the activation of both pathways. If the PI3K/PTEN/Akt/mTOR pathway is also activated, then it is important to treat these pancreatic cancer patients with a PI3K, Akt or mTOR inhibitor. If both pathways are activated, then treatment with inhibitors which target both pathways is warranted. Due to the frequent detection of pancreatic cancer at advanced stages, it may be necessary to combine signal transduction inhibitor therapy with conventional chemotherapy after surgical removal of the pancreatic cancer if possible.

ARRY-142886 suppressed the tumor growth of pancreatic cells, such as BxPC3, in immune compromised mice more effectively than conventional chemotherapeutic drugs, such as
gemcitabine, which is commonly used to treat pancreatic cancer; however, once treatment with ARRY-142886 was discontinued, the tumors re-grew [46]. Most likely MEK inhibitors do not induce apoptosis, but rather, they inhibit proliferation. That is, MEK inhibitors are cytostatic. As discussed previously, there is a significant amount of overlap between the Raf/MEK/ERK and PI3K/PTEN/Akt/mTOR pathways in terms of the regulation of apoptosis and cell growth. Inhibition of both pathways may more effectively induce apoptosis. To induce apoptosis and eliminate tumor growth, combination treatments may be required. This could include co-treatment with MEK and PI3K/Akt/mTOR inhibitors or co-treatment with MEK inhibitors and chemotherapeutic drugs.

5. Current research goals

The obvious goal of current inhibitor development is to improve the effectiveness of treatment of cancer patients with small molecule signal transduction inhibitors. This has proven to be difficult for multiple reasons: first, as previously discussed, there tends to be a distinct genetic susceptibility for the success of a signal transduction inhibitor in suppressing cancerous growth; second, many of the small molecule signal transduction inhibitors are cytostatic as opposed to being cytotoxic and, therefore, will need to be combined with a therapeutic modality which induces cell death; and third, more than one signal transduction pathway may be activated in the cancer cells.

5.1 Dual pathway inhibition: targeting MEK and PI3K/PTEN/Akt/mTOR

Previously, we have predominately discussed studies which used a MEK inhibitor, sometimes in combination with a chemotherapeutic drug. In the following section, we discuss the potential of combining inhibitors which target two pathways to more effectively limit cancer growth. We present a brief overview of the biochemistry of the PI3K/PTEN/Akt/mTOR pathway to enable the reader to better understand how targeting both pathways may be more effective than just targeting MEK.

PI3K is a heterodimeric protein with an 85-kDa regulatory subunit and a 110-kDa catalytic subunit (PIK3CA). PI3K serves to phosphorylate a series of membrane phospholipids including PtdIns(4)P and PtdIns(4,5)P2, catalyzing the transfer of ATP-derived phosphate to the D-3 position of the inositol ring of membrane phosphoinositides, thereby, forming the second messenger lipids PtdIns(3,4)P2 and PtdIns (3,4,5)P3. Most often, PI3K is activated via the binding of a ligand to its cognate receptor, whereby p85 associates with phosphorylated tyrosine residues on the receptor via an Src-homology 2 domain. After association with the receptor, the p110 catalytic subunit then transfers phosphate groups to the aforementioned membrane phospholipids. It is these lipids, specifically PtdIns(3,4,5)P3, that attract a series of kinases to the plasma membrane thereby initiating the signaling cascade [1]. An overview of the Ras/PI3K/PTEN/Akt/mTOR pathway is presented in Figure 4.

Downstream of PI3K is the primary effector molecule of the PI3K signaling cascade, Akt/protein kinase B. Akt was originally discovered as the cellular homologue of the transforming retrovirus AKT8 and as a kinase with properties similar to protein kinases A and C [1]. Akt contains an amino-terminal pleckstrin homology domain that serves to target the protein to the membrane for activation [1]. Within its central region, Akt has a large kinase domain and is flanked on the carboxy-terminus by hydrophobic and proline-rich regions [1]. Akt is activated via phosphorylation of two residues: T308 and S473.

The phosphotidylinositol-dependent kinases (PDKs) are responsible for activation of Akt. PDK1 is the kinase responsible for phosphorylation of T308 [1]. Akt is also be phosphorylated by the mTOR complex referred to as mTORC2 (rapamycin-insensitive companion of mTOR/mLST8 complex) (Figure 4). Before its discovery, the activity responsible for this phosphorylation event was called PDK2. Phosphorylation of Akt is complicated as it is phosphorylated by a complex which lies downstream of itself. Moreover, it can be dephosphorylated by events mediated by p70S6K which also lies downstream of Akt [1]. So, as with the Ras/Raf/MEK/ERK pathway, there are negative feedback loops which serve to regulate the Ras/PI3K/PTEN/Akt/mTOR pathway. Once activated, Akt leaves the cell membrane to phosphorylate intracellular substrates.

After activation, Akt is able to translocate to the nucleus [1] where it affects the activity of a number of transcriptional regulators, CREB, E2F, NF-kB (via IкB) and the forkhead transcription factors [1]. These are all either direct or indirect substrates of Akt and each can promote either cellular proliferation or survival. Aside from transcription factors, Akt is able to target a number of other molecules to affect the survival state of the cell including caspase-9, the pro-apoptotic molecule BAD and glyco-gen-synthase kinase-3β [1]. When these targets are phosphorylated by Akt, they may either be activated or inactivated but the end result is to promote survival of the cell.

Negative regulation of the PI3K pathway is primarily accomplished through the action of the phosphatase and tensin homologue deleted on chromosome ten (PTEN) tumor suppressor protein. PTEN encodes a lipid and protein phosphatase whose primary lipid substrate is PtdIns(3,4,5)P3. PTEN has four primary structural domains. On the amino terminus is the lipid and protein phosphatase domain, which is flanked by a domain responsible for lipid binding and membrane localization. Next is the PEST domain which regulates protein stability. Last, PTEN has a PDZ domain, which helps facilitate protein–protein interactions. Mutations within the phosphatase domain have been reported to nullify the endogenous function of PTEN [1]. Thus, PTEN is a key therapeutic target, although it is frequently inactivated in
Both ERK and Akt phosphorylate many proteins involved in apoptosis regulation which often result in prevention of apoptosis.

Figure 4. Conceptual overview of targeting MEK and mTOR to suppress malignant growth. The Raf/MEK/ERK and PI3K/PTEN/Akt/mTOR pathways can interact at many different levels. In this diagram, we have focused on how they interact to regulate mTOR, p70S6K and protein synthesis. Dotted lines indicate negative feedback loops which may also include other signaling molecules. Targeting both of MEK and mTOR may be an effective means to regulate cell growth.
human cancer and its inactivation results in elevated Akt activity and abnormal growth regulation.

Next, we discuss some of the key downstream targets of Akt which can also contribute to abnormal cellular growth and are key therapeutic targets. Akt-mediated regulation of mTOR activity is a complex multi-step phenomenon. Akt inhibits tuberous sclerosis 2 (TSC2 or hamartin) function through direct phosphorylation. TSC2 is a GTPase-activating protein (GAP) that functions in association with the putative tuberous sclerosis 1 (TSC1 or tuberin) to inactivate the small G protein Rheb (Ras homolog enriched in brain) [1]. TSC2 phosphorylation by Akt represses GAP activity of the TSC1/TSC2 complex, allowing Rheb to accumulate in a GTP-bound state. Rheb-GTP then activates, through a mechanism not yet elucidated, the protein kinase activity of mTOR when complexed with the Raptor (Regulatory associated protein of mTOR) adaptor protein and mLST8, a member of the Lethal-with-Sac-Thirteen gene family, first identified in yeast [1]. The mTOR/Raptor/mLST8 complex (mTORC1) is sensitive to rapamycin and, importantly, inhibits Akt via a negative feedback loop which involves, at least in part, p70S6k [1].

The relationship between Akt and mTOR is further complicated by the existence of the mTOR/Rictor (mTORC2), which displays rapamycin-insensitive activity. The mTORC2 complex has been found to directly phosphorylate Akt on S473 in vitro and to facilitate T308 phosphorylation. Thus, mTORC2 complex can function as the elusive PDK-2 which phosphorylates Akt on S473 in response to growth factor stimulation [1]. Akt and mTOR are linked to each other via ill-defined positive and negative regulatory circuits, which restrain their simultaneous hyperactivation through a mechanism involving p70S6k and PI3K. Assuming that equilibrium exists between these two complexes, when the mTORC1 complex is formed, it could antagonize the formation of the mTORC2 complex and reduce Akt activity [1]. Thus, at least in principle, inhibition of the mTORC1 complex could result in Akt hyperactivation. This is one problem associated with therapeutic approaches using rapamycin which block some actions of mTOR but not all.

mTOR is a 289-kDa S/T kinase. It regulates translation in response to nutrients/growth factors by phosphorylating components of the protein synthesis machinery, including p70S6K and eukaryotic initiation factor (eIF)-4E binding protein-1 (4EBP-1), the latter resulting in release of the eIF-4E, allowing eIF-4E to participate in the assembly of a translational initiation complex [1]. p70S6K, which can also be directly activated by PDK-1, phosphorylates the 40S ribosomal protein, S6, leading to active translation of mRNAs [1]. Integration of a variety of signals (mitogens, growth factors, hormones) by mTOR assures cell cycle entry only if nutrients and energy are sufficient for cell duplication [1]. Therefore, mTOR controls multiple steps involved in protein synthesis. Hence targeting the mTOR pathway could have many effects on the regulation of cellular growth.

In addition to the BRAF mutations present in melanomas which we have previously discussed, the PTEN phosphatase tumor suppressor gene is also deleted in ~ 45% of melanomas and the downstream Akt gene is amplified in ~ 45% [78]. Both of these mutations result in increased expression of Akt. Elevated Akt expression is often associated with a poor prognosis in human cancer. Downstream of Akt is mTOR. Increased Akt expression will lead to mTOR activation. Increased mTOR activity can increase the efficiency of protein translation. The targeting of mTOR has been examined in melanoma therapy as well as in the treatment options for many diverse cancers. Treatment of inducible lung cancers containing KRAS and PIK3CA mutations with PI3K/mTOR (NVP-BEZ235, Novartis) and MEK (ARRY-142886) inhibitors led to an enhanced response [79].

The combined effects of inhibiting MEK with PD-0329501 and mTOR with rapamycin or its analogue AP-23573 (ARIAD Pharmaceuticals/Merck) were examined in human NSCLC cell lines, as well as in animal models of human lung cancer [80]. PD-0329501 and rapamycin demonstrated synergistic inhibition of proliferation and protein translation. Suppression of both MEK and mTOR inhibited ribosomal biogenesis and was associated with a block in the initiation phase of translation. These results support suppression of both the MEK and mTOR pathways in lung cancer therapy and indicate that both pathways converge to regulate the initiation of protein translation. ERK phosphorylates MAPK signal integrating kinases (Mnk1/2) and p90 ribosomal S6 kinase p90Rsk, which regulate the activity of the eukaryotic translation initiation factor eIF4E [81]. The phosphorylation of 4EBP1 is altered in cells with BRAF mutations. It should also be pointed out that the 4EBP1 is also regulated by Akt, mTOR and p70S6K [1-9,81]. This may result in the efficient translation of certain mRNAs in BRAF-mutant cells. This could explain how co-inhibition of MEK and mTOR synergize to inhibit protein translation and growth in certain lung cancer cells.

5.2 Back to the future: the need to combine MEK inhibitors with chemotherapy and radiotherapy

Classical chemotherapy often remains the most prescribed anticancer therapy for many different types of cancer treatment. Drugs such as doxorubicin and taxol are effective in the treatment of many cancers, even though in some cases drug resistance develops after prolonged treatment. Doxorubicin and taxol target cellular events, such as DNA replication and cell division, which are often downstream of the targets of MEK. Chemotherapeutic drug treatment can also unfortunately lead to drug resistance. Chemotherapeutic drugs can activate the Raf/MEK/ERK pathway by diverse mechanisms (Figure 5). Drugs such as doxorubicin can activate p53 which can lead to increased expression of the discoidin domain receptor (DDR), which in turn can result in Raf/MEK/ERK pathway activation. Activated ERK can phosphorylate p53 and regulate its activity. Doxorubicin can also activate the calcium...
calmodulin-dependent kinase cascade via reactive oxygen species [4]. Activation of this cascade can also result in MEK activation. Activation of this cascade can result in the transcription of genes such as XRCC1 and ERCC1 which are involved in DNA repair and lead to drug resistance [81,82]. Taxols can also stimulate activation of the Raf/MEK/ERK cascade and lead to their increased association with proteins involved in cell division [82-84]. Thus, chemotherapeutic drugs can activate the Raf/MEK/ERK cascade which can promote drug resistance. Thus, by combining classical chemotherapy with targeted therapy, it may be possible to enhance toxicity, while lowering the effective concentrations of classical chemotherapeutics necessary for effective elimination of the tumor [84]. As we have previously discussed, activation of the Raf/MEK/ERK cascade can alter the activity and subcellular localization of many proteins which play critical roles in apoptotic cascades. Also, the Raf/MEK/ERK cascade can regulate the transcription of many critical genes involved in cell cycle progression, growth and differentiation. Thus, combining chemotherapy with MEK inhibitors may enhance the therapeutic effectiveness of chemotherapy.

Cells containing the BCR-ABL chromosomal translocation signal in part through the Raf/MEK/ERK cascade [1] and are sensitive to MEK inhibitors [2]. MEK inhibitors synergize with the BCR-ABL inhibitor imatinib in inhibiting the growth of BCR-ABL positive cells [85]. Furthermore, MEK inhibitors synergize with histone deacetylase inhibitors to suppress the growth of BCR-ABL-positive cells [86]. MEK inhibitors have potentiated the antitumor activity of selective COX-1 and COX-2 inhibitors in suppressing the growth and inducing apoptosis in human liver cancer cells [87].

As mentioned previously, a side effect of some chemotherapeutic drugs, such as paclitaxel, is the induction of the Raf/MEK/ERK pathway. Activation of this pathway can under
certain circumstances promote proliferation, prevent apoptosis and contribute to drug resistance. Combining paclitaxel treatment with MEK inhibitors synergistically enhances apoptosis and inhibits tumor growth [88,89]. The synergistic effects of paclitaxel and MEK inhibitors are complex and have not been fully elucidated, but may be in part mediated by inhibition of Bad phosphorylation at S112 by ERK [88]. Cisplatin also induces the Raf/MEK/ERK pathway in certain cancer cell types such as squamous cell carcinoma [90]. A common problem after treatment of this cancer type with cisplatin is the development of resistance to cisplatin. Combining cisplatin with MEK inhibitors increased cell death in cells normally refractory to cisplatin [90]. Obviously, many other key phosphorylation events mediated by ERK may be suppressed which play critical roles in cell growth.

The cytotoxic effects of combinations of MEK inhibitors and paclitaxel may be specific for cells of certain origins and may depend on the levels of endogenous activated MEK/ERK present in those cells. A study with NSCLC cells, which constitutively-expressed activated MEK, revealed no increase in paclitaxel-induced apoptosis when treated with a MEK inhibitor [89]. In contrast, addition of a dominant negative (DN) MEK gene to these cells potentiated paclitaxel-induced apoptosis.

In neuroblastoma cells, cisplatin-induced apoptosis was associated with increased levels of both p53 and the downstream Bax protein. Activated ERK1/ERK2 levels also increased earlier in these cells upon cisplatin treatment. Cell cultures incubated with MEK inhibitors blocked apoptotic cell death, which prevented the cisplatin-induced accumulation of p53 and Bax proteins [91]. Activation of ERK1/ERK2 also occurs in HCC cells and in other transformed cell types after exposure to nonselective, NSAIDs or selective COX-1 and COX-2 inhibitors. Inhibition of the Raf/MEK/ERK signaling pathway by a MEK inhibitor potentiates the antitumor activity of COX inhibitors, suggesting that the Raf/MEK/ERK pathway does not mediate cytotoxicity induced by COX inhibitors, but may protect cells from death [87].

MEK inhibitors also synergize with arsenic trioxide (ATO) to induce apoptosis in APL and AML cells [92-95]. The p53-related gene p73 is a molecular target of the combined therapy. ATO modulates the expression of the p73 gene by inducing the proapoptotic and antiapoptotic p73 isoforms. P53 requires p63 and p73 for the induction of apoptosis in response to DNA-damaging drugs. P73 exists as multiple transactivation competent (TA) proapoptotic and antiapoptotic p73 COOH-terminal splicing isoforms (α, β, γ, δ, ε and ζ), of which the two major forms are p73α and p73β. DN p73 variants are expressed by a second promoter. These DNp73 variants lack the amino-terminal transactivation domain, act as trans-repressors of p53- and p73-dependent transcription, and have antiapoptotic and proapoptotic potential. APL cells treated with the MEK inhibitor PD-184352 displayed reduced levels of DNp73 and decreased the ATO-mediated upregulation of DNp73, thus, causing an increase in the TA:DNp73 ratio of dual-treated cells. High doses of ATO induced p53 accumulation in 11/21 patients. Combined treatment resulted in the induction of the proapoptotic p53/p73 target gene p53AIP1 and greatly enhanced the apoptosis of treated cells [93]. Thus, this study documented the effectiveness of combining ATO with MEK inhibitors in the treatment of APL and identified the molecular mechanism responsible for the observed synergism.

MEK inhibitors synergize with UCN-01 (NCI/Keryx Biopharmaceuticals, Inc.) and induce apoptosis in multiple myeloma cells [96]. UCN-01 is a modified staurosporine and inhibits many kinases. UCN-01 has been evaluated in some clinical trials. The synergy may be partially caused by UCN-01 inducing ERK activation, which is suppressed by the MEK inhibitor.

It should be noted that the combination of MEK inhibitors and chemotherapeutic drugs may not always result in a positive interaction and in some cases, combination therapy results in an antagonistic response. For example, combining MEK inhibitors with betulinic acid, a drug lethal for melanoma cells, antagonized the normal enhancing effects of betulinic acid on apoptosis [97]. Furthermore, the precise timing of the addition of two agents is important as they may differentially affect cell-cycle progression; therefore, the order of administration may be important for a synergistic response to be obtained and perhaps to prevent an antagonistic response [97].

5.3 Back to the future again: combining MEK inhibitors with radiotherapy

Radiotherapy is a common therapeutic approach for the treatment of many diverse cancers. A side effect of radiotherapy in some cells is the induction of the Ras/Raf/MEK/ERK cascade [4]. Recently, various signal transduction inhibitors have been evaluated as radiosensitizers. The effects of pretreatment of lung, prostate, and pancreatic cancer cells with the ARRY-142886 MEK inhibitor were evaluated [98]. The MEK inhibitor treatment was observed to radiosensitize the various cancer cell lines in vitro and in vivo. The MEK inhibitor treatment was correlated with decreased Chk1 phosphorylation 1–2 h after radiation. The authors noticed the effects of the MEK inhibitor on the G2 checkpoint activation after irradiation, as the MEK inhibitor suppressed G2 checkpoint activation. Because ERK1/ERK2 activity is necessary for carcinoma cells to arrest at the G2 checkpoint, suppression of phosphorylated Chk1 was speculated to lead to the abrogated G2 checkpoint, increased mitotic catastrophe and impaired activation of cell cycle checkpoints. Mitotic catastrophe was increased in cells receiving both the MEK inhibitor and radiation when compared to the solo-treated cells. It was also postulated in this study that the MEK inhibitor suppressed the autocrine cascade in DU145 prostate cancer cells which normally resulted from EGF secretion and EGFR activation. Suppression of this autocrine cascade by the MEK inhibitor may have served as a radiosensitizer to the radiation.
therapy. The other two cancer cell lines examined in this study (A549 and MiaPaCa2) had KRAS mutations and both were radiosensitized by the MEK inhibitor. Although these studies document the ability of a MEK inhibitor to radiosensitize certain cells, clearly other cancer cell lines without activating mutations in the Ras/Raf/MEK/ERK pathway or autocrine growth stimulation should be examined for radiosensitization by the MEK inhibitor.

5.4 Novel concepts involving MEK inhibitors

In the following two sections, we discuss novel concepts regarding MEK inhibitors. These scientific topics are in their infancy but should be considered as they have important implications regarding the potential uses of these inhibitors.

5.4.1 Targeting MEK and PI3K/PTEN/Akt/mTOR pathways to suppress cellular senescence and aging

A recent series of manuscripts by Blagosklonny and colleagues have demonstrated that treatment of cells induced to undergo senescence with MEK, PI3K and mTOR inhibitors will prevent the induction of cellular senescence and aging [99-109]. These experiments have led to the innovative hypothesis that cellular senescence results from the hyper-activation of proliferative pathways and that drugs (e.g., metformin used to treat diabetes) and signal transduction inhibitors (e.g., MEK, PI3K, mTOR inhibitors) can inhibit cellular proliferation and aging [105-110]. Similar effects on the prevention of cellular senescence were observed with reversitol, the active component contained in the skins of red grapes which was shown to also inhibit mTOR and cellular senescence [107]. Additional studies have shown that the commonly-prescribed diabetes drug metformin will also inhibit mTOR and prevent cellular aging [110]. Because both the Raf/MEK/ERK and PI3K/PTEN/Akt/mTOR pathways interact to regulate the activity of mTOR and downstream components of this pathway which are critical for both mRNA stability and protein translation, it is believed that by inhibiting some of these key pathways, it may be possible to prevent cellular aging.

5.4.2 Targeting Raf/MEK/ERK in cancer initiating cells

An area of intense interest in cancer biology is the cancer stem cell, more appropriately referred to as the cancer initiating cell [38]. It is widely believed that after therapy of cancer patients by various approaches which eliminate the bulk of the cancer that the cancer may eventually raise its ugly head again. This re-emergence of the cancer may be due to the outgrowth of therapy resistant cancer initiating cells. The abilities of the various MEK inhibitors to target and suppress the proliferation of the cancer initiating cells (cancer stem cells) are only beginning to be examined. It is not clear whether MEK inhibitors will specifically target the cancer initiating cells. Cancer stem cells have unique properties as they can be both quiescent and also resistant to chemotherapeutic drugs. However, under certain conditions, they resume proliferation and hence should be potentially susceptible to MEK inhibitors. We have observed that some drug resistant cells which display properties similar to cancer initiating cells exhibit elevated activation of the Raf/MEK/ERK signaling cascade [40]. Hence, these cells could be preferentially sensitive to MEK inhibitors. Targeting MEK could be very important in terms of cancer stem cell elimination.

6. Conclusions

Various pharmaceutical companies have developed inhibitors to the MEK pathway which were shown to be both highly specific and effective. However, these inhibitors may have limited effectiveness in treating human cancers, unless the particular cancer proliferates directly in response to the Raf/MEK/ERK pathway. Moreover, MEK inhibitors are often cytostatic as opposed to cytotoxic; thus, their ability to function as effective anticancer agents in a monotherapeutic setting is limited, and they may be more effective when combined with chemo- or radiotherapy.

Combination therapy with either a traditional drug/physical treatment or another inhibitor which targets a specific molecule in a different signal transduction pathway is also a key approach for improving the effectiveness and usefulness of MEK inhibitors. This avenue of investigation has not been pursued as actively often due to the conflicting interests of pharmaceutical companies as different companies will often have competing interests for the different inhibitors/chemotherapeutic drugs.

7. Expert opinion

Over the past 25 years, there has been significant progress in elucidating the involvement of the Ras/Raf/MEK/ERK cascade in promoting cell growth, regulating apoptosis, as well as the induction of chemotherapeutic drug resistance. Initial seminal studies performed in the late 1970s and early 1980s elucidated that oncogenes were present in the genomes of avian and murine retroviruses. Many of the viral oncogenes: ErbB, Fms, Ras, PI3K, Akt, Src, Abl, Raf, Fos, Ets and NF-kB (Rel) were subsequently identified as cellular genes which in some cases were captured by retroviruses. Now, we know that these cellular genes are frequently abnormally regulated in human cancer and many of them serve to activate the Raf/MEK/ERK and PI3K/Akt pathways which have been discussed as playing critical roles in cellular proliferation in this review. Biochemical studies continue to elucidate the roles that these cellular and viral oncogenes have in cellular transformation. Mutations at many of these genes can result in abnormal MEK activation. Hence, targeting MEK with small-molecule inhibitors may inhibit cell growth of these cells. Specific MEK inhibitors have been developed and represent promising therapies for cancer and other proliferative diseases. The usefulness of these inhibitors may depend on the mechanism of transformation of the particular cancer. If the tumor exhibits a dependency
on the Raf/MEK/ERK pathway, then it may be sensitive to MEK inhibitors. In contrast, tumors which do not display enhanced expression of the Raf/MEK/ERK pathway may not be sensitive to MEK inhibitors. Finally, it is likely that MEK inhibitors will only be effective in inhibiting tumor growth in combination with cytotoxic chemotherapeutic drugs or radiation.

Scientists and clinicians often have an intentionally narrow view of a particular topic. For example, cancer researchers pre-dominantly feel that MEK inhibitors will suppress the growth of malignant cancer cells. Yet, MEK and other inhibitors may also be useful in the treatment of autoimmune and allergic disorder where there is abnormal cellular proliferation. Recent reports have also demonstrated that the suppression of MEK may prevent the induction of cellular senescence and aging. Clearly, these last two clinical topics, immune disorders and aging, greatly enhance the potential clinical uses of these targeted therapeutic drugs.

**Declaration of interest**

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Emerging MEK inhibitors

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• A key manuscript documenting ability of mutant B-Raf to transmit signal through Raf-1 and preserve the mutant signaling ability.


• A key manuscript demonstrating involvement of Raf/MEK/ERK pathway in the phosphorylation of Bcl-2 and regulation of apoptosis and chemoresistance of hematopoietic cells.


• A key manuscript documenting differential sensitivity of breast cancer to MEK inhibitors and demonstration of a negative feedback loop suppressed by MEK inhibitors which results in Akt activation and resistance to MEK inhibitors. The manuscript also documents the ability of MEK inhibitors to synergize with PI3K inhibitors to suppress breast cancer growth.


• A key manuscript documenting sensitivity of NSCLC patients to targeted inhibitor depends on genetic mutations.


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• A key manuscript discussing the potential for rapamycin to prevent aging in stem cells.