HHS Public Access

Author manuscript

*Trends Cancer*. Author manuscript; available in PMC 2016 November 01.

Published in final edited form as:

*Trends Cancer*. 2015 November 1; 1(3): 183–198. doi:10.1016/j.trecan.2015.10.001.

# Targeting *RAS*-mutant cancers: is ERK the key?

**Meagan B. Ryan**[1], **Channing J. Der**[2], **Andrea Wang-Gillam**[3], and **Adrienne D. Cox**[4]

[1]Department of Pharmacology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA; mryan4@email.unc.edu

[2]Department of Pharmacology, and the Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA; cjder@med.unc.edu

[3]Division of Oncology, Department of Internal Medicine, Alvin J. Siteman Cancer Center, Washington University School of Medicine, ST. Louis, MO 63110, USA; awang@dom.wustl.edu

[4] Departments of Pharmacology and Radiation Oncology, and the Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA; adrienne_cox@med.unc.edu

## Abstract

The three *RAS* genes comprise the most frequently mutated oncogene family in cancer. With significant and compelling evidence that continued function of mutant *RAS* is required for tumor maintenance, it is widely accepted that effective anti-RAS therapy will have a significant impact on cancer growth and patient survival. However, despite more than three decades of intense research and pharmaceutical industry efforts, a clinically effective anti-RAS drug has yet to be developed. With the recent renewed interest in targeting RAS, exciting and promising progress has been made. In this review, we discuss the prospects and challenges of drugging oncogenic RAS. In particular we focus on new inhibitors of RAS effector signaling and the ERK mitogen-activated protein kinase cascade.

## RAS: in fashion, again

The discoveries in 1982 that human *RAS* genes are mutationally activated in cancer (Figure 1 and Supplementary Figure 1) initiated intensive efforts to identify pharmacological strategies that could disrupt the aberrant function of the corresponding RAS proteins[1]. Two decades later, when it became disappointingly apparent that farnesyltransferase inhibitors (FTIs) were not the answer, enthusiasm diminished dramatically. This failure coincided with the dawn of the current post-genomic era of cancer research, when sequencing of the cancer genome began to reveal the complexities of the genetic basis of cancer[2,3]. What these studies did not yield, however, were attractive new targets for cancer drug discovery. Instead, exome sequencing of colorectal, lung and pancreatic cancers verified that *RAS*

*Corresponding author:* Der, C.J. (cjder@med.unc.edu).

mutations are the most prevalent gain-of-function genetic alterations in the cancers that comprise three of the top four causes of cancer deaths in the United States[2-4]. With this reality check, it became apparent that further efforts to seek an effective anti-RAS therapy, long an elusive holy grail of cancer research, must become a renewed priority, however difficult the task[5]. In this review, we provide an overview and perspective on the most promising directions for these efforts. We then focus on the direction where the greatest promise lies in the near future: with inhibitors already under clinical evaluation, there is guarded optimism that blocking RAS effector signaling may produce a clinically effective anti-RAS drug. In particular, we focus on the prospects and challenges faced by inhibitors of what is arguably the most significant signaling network driving cancer growth, the RAF-MEK-ERK protein kinase cascade.

## Targeting RAS in cancer

The three *RAS* genes (*HRAS*, *NRAS* and *KRAS*) comprise the most frequently mutated gene family in cancer, with *KRAS* by far the most commonly mutated of these[2,3] (Figure 2a). There is substantial experimental evidence in cell culture and mouse model studies that mutant *RAS* is a critical driver of cancer initiation and maintenance. Thus, an effective anti-RAS therapy is expected to significantly impact cancer growth. Oncogenic RAS mutations are typically found in hotspots critical for the GTP/GDP on-off switch (Figure 2b), so the mutated RAS proteins escape normal regulation and are constitutively GTP-bound and active (Figure 2c). Unlike the successful development of ATP-competitive inhibitors of protein kinases, similar strategies to disrupt persistent GTP binding to mutant RAS have been seen as unsuccessful due to the apparent high picomolar binding affinities of RAS for GTP. Moreover, the smooth topology of RAS proteins originally discouraged efforts to search for small molecules that bound RAS directly, prompting perceptions that RAS is “undruggable”. Yet, recent intriguing success in this area includes identification of cell-active small molecules that bind directly to RAS and disrupt RAS interaction with regulators and/or effectors[6-8]. Particularly significant are the small molecules that target a specific KRAS mutation (G12C)[9,10], although it remains uncertain as to whether these can be advanced to clinically active and selective inhibitors of mutant RAS.

In addition to the challenging attempts to directly inhibit RAS itself, four approaches to inhibit RAS involve indirect targeting of proteins that support mutant RAS function (Figure 3). These approaches include: ***i) Inhibition of RAS-membrane association***- RAS proteins undergo posttranslational modification and covalent addition of prenyl and fatty acid lipids that promote association with the plasma membrane[11]. While FTIs effectively disrupt plasma membrane association of HRAS, they do not interfere with KRAS or NRAS. Therefore, it was not surprising that FTIs were clinically ineffective in pancreatic and colon cancer, where there is nearly exclusive mutation of KRAS. Another recent approach is to inhibit phosphodiesterase delta (PDEδ), a chaperone that is thought to facilitate RAS membrane trafficking[12]. A potential limitation of these approaches is that the proteins targeted also support the function of numerous other proteins; ***ii) Inhibition of synthetic lethality interaction***- Functional genetic screens have identified synthetic lethal interactors of mutant RAS, proteins whose functions are critical only in the context of *RAS*-mutant cancer cells[13]. However, the initial excitement in this area was dampened considerably

when follow-up analyses failed to support the strong association of these proteins specifically with mutant RAS. Despite mixed opinions on the ultimate promise of this direction, ongoing studies still seek to improve the methodologies and biological screens in hopes of overcoming earlier limitations; iii) Inhibition of RAS-regulated metabolic processes- A recent new direction has been prompted by findings that mutant RAS function deregulates cellular processes (e.g., autophagy, glucose and glutamine metabolism) that support the increased metabolic needs of cancer cells[14]. These efforts are still in their infancy, with attractive targets and selective inhibitors for those targets still to be developed. A key limitation of the latter three approaches is that these proteins do not support RAS function exclusively and, hence, their inhibition can have significant non-RAS cellular effects. Currently, the area with the most advanced activity is the ***iv) Inhibition of RAS effector signaling***- Numerous candidate inhibitors are presently under clinical evaluation, including inhibitors of the RAF and PI3K effector pathways[3] (RAF-MEK- ERK inhibitors detailed in Table 1). While conceptually simple, in practice this approach is complicated by the diversity of RAS downstream signaling networks, extensive signaling crosstalk and the highly dynamic nature of these networks. In this review, we ask, "Can inhibitors of the RAF-MEK-ERK mitogen-activated protein kinase (MAPK) cascade fulfill the promise of targeting RAS?".

## The RAF-MEK-ERK cascade: sufficient and necessary for mutant *RAS*-driven tumor development

Active RAS-GTP can bind to and regulate a spectrum of catalytically diverse effectors (Figure 2c). Of these, the three-tiered RAF-MEK-ERK protein kinase cascade is the best characterized and validated driver of normal and mutant RAS function (Figure 4). The RAF-MEK-ERK cascade is under tight spatio-temporal regulation, dictating both quantitative and qualitative differences in ERK signaling output and biological outcomes. Among the numerous ERK substrates are components that comprise negative feedback mechanisms to attenuate the strength of ERK signaling. While ERK activation generally stimulates growth, excessive ERK activation can instead cause growth arrest[15]. Thus, finely tuned dynamic regulation of signaling flux through this cascade is critical in dictating the cellular consequences of ERK activation. Accordingly, there are diverse mechanisms of ERK feedback inhibition (Figure 5). One key mechanism involves ERK phosphorylation of CRAF and BRAF, thereby decreasing RAF dimerization and association with activated RAS[16].

The importance of the RAF-MEK-ERK cascade as a therapeutic target in cancer is supported by several lines of evidence. *BRAF* is frequently mutationally activated (19%; COSMIC). The non-overlapping occurrence of *RAS* and *BRAF* mutations in cancer types where both are found is consistent with equivalent driver roles for each activated oncogene. Supporting a key driver role of *BRAF* in *KRAS*-driven oncogenesis, mutationally activated $Braf^{V600E}$ but not $Pik3ca^{1047R}$ was sufficient to phenocopy activated $Kras^{G12D}$ in a mouse model of pancreatic cancer and to induce pancreatic ductal adenocarcinoma together with mutant $Tp53^{R270H}$[17]. Genetic ablation of components of this pathway further supports the therapeutic value of targeting each level of this cascade. For example, in a *Kras*-driven

mouse model of lung tumorigenesis, loss of either *Mek1* or *Mek2* increased survival by ~20%, while loss of both genes induced a near 100% increase in survival[18]. Also, the loss of *Erk1* or *Erk2* increased survival by 20% and 16%, respectively, and deficit of both genes increased survival by 40%[18]. More importantly, the few tumors that did arise in the *Erk1* null background were "escapers" that continued to express Erk2[18]. However, the complete genetic ablation of both *Erk1* and *Erk2* was deleterious for normal adult tissue homeostasis[18]. Genetic ablation of *Craf* alone (but not *Braf*) impaired mutant *Kras*- driven lung tumor formation and increased survival[18,19]. However, *Craf* deficiency did not impair mutant *Kras*-driven pancreatic cancer development, indicating that there are cancer-type differences in RAF isoform dependencies[20]. These genetic studies support both the sufficiency and necessity of the RAF-MEK-ERK cascade in mutant *RAS*-driven tumor initiation and progression. However, since each MAPK component was ablated concurrently with RAS activation, their requirement in tumor maintenance was not addressed. Additionally, genetic loss of an entire protein may not accurately model the consequences of the pharmacologic inhibition of its catalytic kinase domain and activity. How far along is the development of RAF-MEK-ERK inhibitors and how are these drugs performing in the clinic?

## RAF Inhibitors

The FDA-approved drug sorafenib was developed originally as an ATP-competitive CRAF inhibitor, but its clinical efficacy is attributed to its unspecific multi-kinase inhibitory activity, particularly the inhibition of receptor tyrosine kinases (RTKs) that drive tumor angiogenesis [21]. While sorafenib can inhibit ERK signaling, the degree of ERK inhibition may not be sufficient for effective suppression of ERK-driven cancer growth[21]. Second generation ATP-competitive BRAF-selective inhibitors, vemurafenib and dabrafenib, have been approved by the FDA for use in *BRAF*-mutant malignant melanoma and lead to clinically significant progression-free and overall survival[22-24]. However, while both cause initial rapid tumor regression in 70 to 80% of *BRAF*-mutant melanoma patients, mechanisms of resistance leading to relapse also occur rapidly in the majority of cases. Additionally, many *BRAF*-mutant colorectal, thyroid, and lung cancers exhibit *de novo* resistance to these BRAF-selective inhibitors[25]. Identifying resistance mechanisms will therefore be critical to use more effectively these inhibitors in the clinic.

Much of the information regarding mechanisms that drive *de novo* and/or acquired resistance to inhibitors of RAF-MEK-ERK inhibition (Figure 6) comes from cell culture experiments in which resistance is induced by long-term treatment with inhibitors. These mechanisms include activation of upstream components (e.g., *NRAS* mutation, *NF1* inactivation, increased RTK expression and/or activation)[26,27] or increased RAF activity (via truncation and increased BRAF dimerization or increased BRAF expression) that lead to ERK reactivation. Since more than 80% suppression of ERK is required for a clinical response[28], increased flux through the cascade and increased ERK activation is sufficient to render cancer cells drug-insensitive. Other resistance mechanisms that reactivate the pathway downstream of the inhibitor blockade include activating mutations in MEK1 and MEK2[29] or amplification of TPL2/COT[30], which phosphorylates and activates MEK1/2. Additional mechanisms that do not restore ERK activation, but that instead decrease

dependency on ERK-driven growth, include activation of PI3K-AKT-mTOR signaling and mutational activation of the small GTPase RAC1[31,32]. The clinical significance of some mechanisms remains to be established.

In contrast to their efficacy in *BRAF*-mutant cancers, vemurafenib and dabrafenib not only are ineffective in *RAS*-mutant cancers, but instead stimulate their growth[33-37]. This effect is due to paradoxical activation of ERK, rather than inhibition. In this setting, drug-inactivated BRAF forms an heterodimer with drug-free CRAF that complexes with mutant RAS, which causes allosteric activation of CRAF by the inactive BRAF dimerization partner, thereby increasing ERK signaling (Figure 6).

Third-generation BRAF inhibitors (Table 1) are not limited by this activation and are known as "paradox breakers". Currently, there is one paradox-breaker inhibitor, PLX8394, in clinical Phase I evaluation (NCT02428712). Compared to vemurafenib, PLX8394 has unique binding sites in the BRAF activation site and is also a superior inhibitor of CRAF[38,39]. PLX8394 can also effectively block ERK activation and the growth of *RAS*-mutant vemurafenib-resistant melanoma cells[39]. Pan-RAF inhibitors - MLN2480, HM95573 and LY3009120- have also entered Phase I trials[40-42]. LY3009120 has shown *in vitro* and *in vivo* efficacy in inhibiting the ERK pathway without eliciting the effect of paradoxical activation [42]. An alternative strategy for effective RAF inhibition in *RAS*-mutant cancers may be the use of small molecule inhibitors of RAF dimerization[43]. While these strategies can overcome upstream signaling resistance mechanisms, they will still be, however, susceptible to downstream mechanisms of resistance (e.g., mutational activation of MEK) or to those that reduce ERK dependency (e.g., increased PI3K-AKT-mTOR activity).

## MEK Inhibitors

Currently, there is one FDA-approved MEK1/2 inhibitor for the treatment of *BRAF*-mutant melanoma- trametinib, and at least 11 other agents in clinical trial evaluation (Table 1). Trametinib and the majority of MEK drugs are allosteric non-ATP-competitive inhibitors and, consequently, exhibit greater target selectivity than ATP-competitive protein kinase inhibitors. These drugs work by blocking the ability of activated MEK to phosphorylate and activate ERK.

In preclinical studies, MEK inhibitors that were effective in *BRAF*-mutant cancer cell lines were not effective in a majority of *KRAS*- or *NRAS*-mutant tumor lines[44-46]. Consistent with this, clinical trials showed limited to no response of *RAS*-mutant non-small-cell lung carcinoma (NSCLC) patients to these drugs [47,48]. Phase II trials failed to show an advantage of combining trametinib with gemcitabine in *KRAS*-mutant pancreatic cancer[49]. In contrast, the MEK inhibitor selumetinib plus docetaxel showed increased overall survival (9.4 months) compared with docetaxel alone (5.2 months) in Phase II trials for *KRAS*-mutant lung cancer patients[50,51]. Mutation-selective trends were seen, in that patients with G12V mutation-positive cancers responded better than others[50,51]. Other clinical Phase II studies have shown that trametinib induces similar progression-free survival and response rates as docetaxel in patients with *KRAS*-mutant-positive NSCLC [52]. MEK162 also showed limited activity in *NRAS*-mutant melanomas[53], where a partial response was seen in 20% of *NRAS*-

mutant patients, although the response was transient, with rapid onset of resistance. Collectively, the clinical data suggest that combination therapies will likely be warranted.

Like RAF inhibitors, MEK inhibitors are also limited by mechanisms of drug resistance that typically involve the loss of multiple ERK-driven negative feedback loops that normally modulate flux through the cascade (Figure 5). Further, while initial treatment with MEK inhibitors effectively blocks ERK activation, kinome reprogramming (sometimes described as the rewiring of kinase signaling networks) drives a rebound in ERK activity within 24 h[54]. Acute inhibition of ERK impairs its ability to regulate stability of the MYC oncoprotein[54,55], resulting in loss of RTK suppression by this nuclear transcription factor. Upregulation of RTK expression and signaling then overcomes MEK inhibitor activity (Figure 6).

Two novel MEK inhibitors have distinct mechanisms of action that reduce their vulnerability to the loss of ERK-dependent negative feedback loops, and consequently may be more effective against *RAS*-mutant tumors. The clinical candidate GDC-0623 stabilizes the RAF-MEK complex in cells, preventing the activation of MEK by RAF[44,45]. GDC-0623 showed greater efficacy than conventional MEK inhibitors in *KRAS*-mutant cancer cells. Similarly, the clinical candidate RO5126766 forms a stable RAF-MEK-drug complex in cells, preventing both MEK and ERK phosphorylation[44,56,45,57,58]. However, these inhibitors remain susceptible to resistance mechanisms at the levels of MEK and ERK, as well as non-ERK mechanisms.

## ERK Inhibitors

Until recently, it was assumed that RAF and/or MEK inhibitors would be sufficient to inhibit ERK1/2 activity and that there would be no additional benefit of directly blocking ERK. Thus, development of ERK inhibitors lagged behind RAF and MEK drugs. However, because the majority of resistance mechanisms to RAF and MEK drugs results in reactivation of ERK1/2, blocking ERK1/2 directly may overcome the current limitations of RAF or MEK inhibitors. Furthermore, although reactivation of ERK alone can overcome the loss of MEK function, it is likely that no single ERK substrate will be capable of restoring loss of ERK function. Hence, the mechanisms of resistance to ERK inhibitors will likely be both diverse and distinct from those of resistance to MEK inhibitors.

To date, two potent and selective cell-active preclinical ERK inhibitors have been described in the literature: VTX-11e and SCH772984, an analog of the orally available clinical candidate MK-8353/SCH900353 (Table 1)[59,60]. VTX-11e is a type I ATP-competitive inhibitor, whereas SCH772984 has a dual mechanism of action, causing the allosteric inhibition of MEK1/2 binding and ERK phosphorylation and also the ATP-competitive inhibition of ERK phosphorylation of its substrates. SCH772984 binding adjacent to the ATP binding pocket induces formation of a new allosteric pocket that then optimally accommodates the inhibitor[61]. Although VTX-11e and SCH772984 exhibit different interactions with ERK and distinct mechanisms of ERK inhibition[61], both inhibitors exhibit a slow off-rate[61,62], a property that prolongs their cellular inhibitory activities.

In *in vitro* studies, SCH772984 inhibited cellular proliferation in a subset of 121 *RAS*- (49%) or *BRAF*- (88%) mutant cancer cell lines[60]. Further, the majority (11 of 14) *NRAS*-mutant melanoma cell lines were sensitive to SCH772984 but not to vemurafenib [63]. Four ERK1/2 inhibitors are currently undergoing Phase I or I/II clinical evaluation (Table 1). GDC-0994[64] and BVD-523 (ulixertinib)[65] have shown potency in *RAS*-mutant cancer cells. In a Phase I dose escalation in patients with advanced solid tumors, BVD-523 achieved ERK inhibition and showed manageable tolerability, with adverse events most commonly including diarrhea, nausea, vomiting or constipation[66]. Ongoing trials will demonstrate whether sufficient inhibition can be achieved for therapeutic benefit.

## Vertical inhibition of the RAF-MEK-ERK cascade

Current evidence indicates that inhibition of RAF or MEK alone is not sufficient for prolonged arrest of *RAS*-mutant cancers. Furthermore, the emergence of tumor cell resistance and normal tissue toxicity due to blockade of the critical RAF-MEK-ERK cascade are anticipated to pose additional limitations. Instead, combination approaches will be needed to effectively 1) overcome bypass of inhibitor action that drive ERK reactivation, 2) block ERK-independent mechanisms that overcome cancer cell addiction to ERK, and 3) concurrently block other RAS effector pathways important for cancer growth. Which combined therapies may provide the answer?

The restricted number of substrates of RAF and MEK led to the earlier perception that the RAF-MEK-ERK kinase cascade was a simple linear unidirectional pathway. However, there is now greater appreciation that there are multiple input and output signals at different levels and that ERK activation stimulates feedback inhibitory mechanisms to reduce flux through the pathway. Consequently, concurrent inhibition of the pathway at multiple levels may induce a more effective inhibition of ERK. In fact, the combination of the BRAF inhibitor dabrafenib with the MEK inhibitor trametinib enhanced progression-free survival and reduced toxicity as compared to dabrafenib alone in *BRAF*-mutant melanoma[67-69], leading to FDA approval of this combination for these tumors. Also, in *KRAS*-mutant tumor cells, unbiased shRNA screening showed that genetic ablation of *CRAF* enhanced MEK inhibitor response[44,70,45]. And the combination of a pan-RAF inhibitor (PRi, Amgen Compd A) with trametinib showed a synergistic effect on the growth inhibition of *NRAS*-mutant melanoma cells[71].

Although combining RAF and MEK inhibitors has shown greater clinical efficacy in *BRAF*-mutant melanoma cancers than either drug alone, reactivation of ERK signaling limits the long-term effectiveness of this combination[72,29]. *BRAF*-mutant melanomas acquired resistance to combined dabrafenib and trametinib treatment by several alterations (*BRAF* amplification and *NRAS* or *MEK1/2* mutational activation) that ultimately led to ERK reactivation. These results prompted studies to evaluate if blockade of ERK can overcome resistance to RAF and/or MEK inhibition. Data from multiple studies in different cancers has shown that this is the case. In fact, resistance of a *BRAF*-mutant melanoma cell line to concurrent vemurafenib and trametinib treatment was overcome by the ERK-selective inhibitor SCH772984[60]. Similarly, a *BRAF*-mutant melanoma cell line resistant to a RAF/MEK inhibitor combination due to MEK2 mutation remained sensitive to the

preclinical ERK inhibitor VTX-11e[72,29]. Further, *KRAS*-mutant tumor cell lines resistant to MEK inhibitor (PD0325901) retained sensitivity to VTX-11e[73]. Co-treatment with VTX-11e enhanced the growth inhibitory activity of selumetinib and trametinib by preventing RAF-dependent rebound of flux through the RAF-MEK-ERK cascade, and caused apoptosis in *NRAS*-mutant melanoma cells[74]. Finally, SCH772984 was also effective in both *NRAS*- and *BRAF*-mutant melanoma cell lines, and synergized with vemurafenib in *BRAF*-mutant lines[63]. Thus, ERK inhibition in combination with RAF and/or MEK inhibition may be a superior therapeutic strategy to inhibition of any single step alone.

Despite these promising findings, ERK inhibitors will also likely be limited by both de novo and acquired mechanisms of resistance. A recent study found that experimentally induced mutations in ERK1 and ERK2 conferred resistance to VTX-11e or SCH772984 treatment[15]. However, the fact that these mutations did not confer cross-resistance to RAF or MEK inhibitors supports the value of combining ERK inhibitors with RAF or MEK inhibitors.

## ERK substrates

ERK1/2 kinases undergo nuclear/cytoplasmic shuttling and translocate to the nucleus upon phosphorylation. ERK subcellular localization is further regulated by dimerization and by interaction with scaffold proteins (e.g., kinase suppressor of Ras (KSR)) and this localization in turn regulates ERK selectivity towards its substrates [75]. Unlike the restricted substrate profile for RAF and MEK, >200 nuclear and cytoplasmic ERK substrates have been identified[76,77]. The specific ERK substrates that are critical for ERK-dependent cancer growth remain poorly understood, with opposing conclusions reached regarding whether nuclear or cytoplasmic substrates, or both, are critical for cancer progression. For example, the multi-functional protein PEA-15 binds and sequesters ERK in the cytoplasm, and genetic ablation of PEA-15 increased ERK nuclear localization and promoted cellular proliferation[78]. In a study where whole-body $Kras^{G12D}$ activation was induced, tumorigenesis was driven in a subset of mouse tissues that was associated with nuclear accumulation of activated ERK and activation of nuclear substrates. In contrast, nonresponsive tissue was associated with cytoplasmic ERK[79]. Further, the nuclear import protein importin7 facilitates ERK nuclear translocation by recognition of the phosphorylated nuclear translocation signal (NTS), and an NTS-derived phosphomimetic peptide that blocks nuclear translocation of ERK impairs the growth of *RAS*- or *BRAF*-mutant tumor cell lines[80]. Because many nuclear ERK substrates are associated with cell proliferation, whereas ERK negative feedback targets are cytosolic (Figure 5), the selective inhibition of phosphorylation of ERK nuclear substrates might favor inhibition of tumor growth. Among the multitude of nuclear transcription factors that are ERK substrates, MYC is likely a critical mediator of ERK effects in *RAS*-mutant cancers. Substantial evidence shows that MYC is essential for *RAS*-driven cancer initiation and growth[81-83]. MYC is a critical driver of *Kras*G12D-dependent up-regulation of genes that support the increased glycolytic and metabolic needs of pancreatic tumors[84] and ERK phosphorylation of MYC prevents MYC protein degradation[85]. In contrast, the therapeutic response to vemurafenib correlated with reduction in cytoplasmic rather than nuclear ERK phosphorylation, arguing for a critical role

of cytoplasmic ERK substrates[28]. This finding is consistent with observations that ERK dimerization is essential for the activation of cytoplasmic but not nuclear substrates, and that preventing ERK dimerization impaired the tumorigenic growth of *RAS*-mutant cancer cell lines[86]. Recent efforts have demonstrated that pharmacologically targeting ERK dimerization can lead to a significant reduction in RAS-driven tumor growth by potently inhibiting phosphorylation of ERK cytoplasmic substrates. The ERK dimerization inhibitor DEL-22379 reduced tumor growth in mutant KRAS xenograft models and was able to overcome upstream resistance mechanisms, including NRAS overexpression and MEK mutation[87]. Among ERK1/2 cytoplasmic substrates that drive tumorigenesis are the RSK serine/threonine kinases. RSKs are major effectors of the ERK1/2 kinases and have been identified as drivers of motility and invasiveness in cancer, as regulators of mTOR in *BRAF*-mutant cancers, and as drivers of chemoresistance[88,89]. Clearly, further work is needed to fully understand the importance of the diverse spectrum of ERK substrates in *RAS*-driven cancers.

## Combined inhibition of RAF-MEK-ERK and PI3K-AKT-mTOR signaling

In addition to ERK reactivation downstream of RAF and MEK inhibitors, increased activation of the PI3K-AKT-mTOR pathway has also been observed. This can occur by increased RTK signaling[90] and, therefore, concurrent treatment with RTK inhibitors may enhance inhibition of RAF-MEK-ERK signaling. Combining inhibitors of the PI3K-AKT-mTOR pathway with MEK inhibitors effectively inhibited *NRAS*-mutant melanoma growth both *in vitro* and *in vivo*[91]. In *KRAS*-mutant pancreatic cancer, the dual PI3K-mTOR inhibitor BEZ235 enhanced MEK/ERK signaling, which could be reversed by the addition of a MEK inhibitor, leading to enhanced growth suppression compared to targeting either pathway alone[92]. Similarly, combination of the PI3K inhibitor GDC-0973 with the MEK inhibitor GDC-0941 was able to confer a greater survival advantage in a $Kras^{G12D}$-driven mouse model of pancreatic cancer than either inhibitor alone[93]. Pre-clinical findings with combined PI3K-AKT-mTOR and RAF-MEK-ERK inhibition have been followed by early clinical trials in a small series of *KRAS*-driven cancers including NSCLC, colorectal, pancreatic and ovarian[94,95]. Occasional partial responses were noted, particularly in ovarian cancers, although normal tissue toxicity remains a concern[94,95].

## Concluding Remarks

While direct inhibitors of RAS remain the ideal strategy for clinically active anti-RAS drug discovery, inhibitors of the RAF-MEK-ERK cascade arguably hold the greatest promise for the immediate future. With earlier perceptions that this protein kinase cascade operated as a simple linear unidirectional pathway, initial efforts centered on MEK inhibitors, and subsequently on RAF inhibitors, to block ERK activation. As the development of RAF and MEK inhibitors progressed, it became painfully apparent that cancer cells can dynamically rewire their signaling networks to restore ERK activity and override the actions of inhibitors that act upstream of ERK. These revelations have led the field to consider ERK itself as perhaps the “best” node for effective disruption of ERK signaling.

As ERK inhibitors transit through clinical evaluation, new issues will likely arise that will challenge the usefulness of ERK inhibitors for cancer treatment. While ERK is clearly a key driver of cancer growth, it is also an essential component in normal cell physiology. Therefore, achieving a therapeutic index and minimizing normal tissue toxicity will be one challenge. Another will be acquired mechanisms of cancer cell resistance to ERK inhibition. However, unlike RAF or MEK, ERK action cannot be attributed to a single substrate. Thus, mechanisms of resistance to ERK inhibitors will likely be distinct from those that overcome the actions of RAF or MEK inhibitors, and likely more complex and varied as well. Defining combination approaches with ERK inhibitors that might overcome cancer cell resistance and normal cell toxicity will be key challenges for the development of ERK inhibitors. Innovative chemical library or genetic functional screens will provide helpful unbiased functional strategies to address this need[96,97].

Other strategies beyond protein kinase inhibitors to block growth dependent on RAF-MEK-ERK signaling, for example inhibitors of RAF or ERK dimerization, are also being pursued. Defining the key ERK substrates critical for ERK-dependent cancer growth remains to be fully elucidated and may provide additional targets for effective blockade of ERK activation in cancer.

Finally, even if direct inhibitors of RAS can be developed, given experimental evidence that cancers can overcome their addiction to mutant RAS, defining the mechanisms by which they accomplish this will also be important. Nevertheless, despite the considerable uncertainty ahead (see Outstanding Questions), there is renewed albeit cautious optimism that an effective anti-RAS strategy may finally be at hand.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

We apologize to colleagues whose studies we were unable to cite due to space limitations. CJD and ADC are supported by grants from the NIH (CA42978, CA179193 and CA175747), Pancreatic Cancer Action Network-AACR and the Lustgarten Pancreatic Cancer Foundation. AGW is supported by Washington University Institute of Clinical and Translational Sciences grants UL1TR000448 and KL2TR000450. MBR is supported by an F31 CA192829 NRSA predoctoral fellowship.

## Glossary Box

**ERK** Extracellular signal-regulated kinases, members of the mitogen-activated protein kinases (MAPKs). The two related ERK1 (MAPK2) and ERK2 (MAPK3) paralogs are serine/threonine kinase components of the last step of the three-tiered RAF-MEK-ERK protein kinase cascade. ERKs are activated downstream of cell surface receptors for diverse extracellular ligands that regulate cell growth. ERKs are activated by MEK1 and MEK2 phosphorylation of their Thr-Glu-Tyr (TEY) motifs.

| | |
|---|---|
| **Farnesyltransferase (FTase)** | One of three mammalian protein prenyltransferases, FTase catalyzes the covalent addition of a 15-carbon farnesyl isoprenoid lipid to the cysteine residue of C-terminal CAAX (Cysteine, Aliphatic, Aliphatic, Any amino acid) tetrapeptide motifs. FTase is comprised of a common α subunit, which shared with a second CAAX-directed prenyltransferase, geranylgeranyltransferase-I, and a distinct β subunit. |
| **MEK** | Dual specificity threonine/tyrosine kinases, that function at the second step of the three-tiered RAF-MEK-ERK MAPK cascade. The two highly related paralogs, MEK1 mitogen-activated protein kinase kinase 1/MAP2K1) and MEK2 (mitogen-activated protein kinase kinase 2/MAPK2K2), are MAPK kinases (MAPKKs or MAP2Ks) that phosphorylate threonine and tyrosine residues in the TEY sequences located in ERK MAPKs. |
| **Mitogen-activated protein kinases** | Comprised of four families of MAPKs that include the related ERK, p38 and JNK serine/threonine kinase families. MAPKs comprise the last step in the three-tiered MAPKKK-MAPKK-MAPK cascades, of which RAF-MEK-ERK is a prominent example. |
| **RAF** | Serine/threonine kinases named after the protein encoded by the rat fibrosarcoma retrovirus oncogene. There are three highly related human RAF paralogs, ARAF, BRAF and CRAF. RAF kinases act as MAP kinase kinase kinases (MAPKKKs or MAP3Ks) at the first step of the three-tiered RAF-MEK-ERK MAPK cascade. The mechanism of activation of RAF kinases is initiated by the binding of activated Ras-GTP to N-terminal Ras-binding domains (RBDs) of RAF, and involves an additional complex set of positive and negative regulatory phosphorylation events. |
| **Small GTPases** | Enzymes that bind and hydrolyze GTP, where the GTP-bound state is the activated state by virtue of increased affinity for effectors and the GDP-bound state is the resting state. RAS proteins are the founding members of a large superfamily of small GTPases comprised of more than 150 members. |

## References


1. Cox AD, Der CJ. Ras history: The saga continues. Small GTPases. 2010; 1:2–27. [PubMed: 21686117]
2. Vogelstein B, et al. Cancer genome landscapes. Science. 2013; 339:1546–58. [PubMed: 23539594]
3. Cox AD, et al. Drugging the undruggable RAS: Mission possible? Nat Rev Drug Discov. 2014; 13:828–51. [PubMed: 25323927]
4. Stephen AG, et al. Dragging ras back in the ring. Cancer Cell. 2014; 25:272–81. [PubMed: 24651010]

5. Ledford H. Cancer: The Ras renaissance. Nature. 2015; 520:278–80. [PubMed: 25877186]
6. Maurer T, et al. Small-molecule ligands bind to a distinct pocket in Ras and inhibit SOS-mediated nucleotide exchange activity. Proc Natl Acad Sci U S A. 2012; 109:5299–304. [PubMed: 22431598]
7. Sun Q, et al. Discovery of small molecules that bind to K-Ras and inhibit Sos-mediated activation. Angew Chem Int Ed Engl. 2012; 51:6140–3. [PubMed: 22566140]
8. Shima F, et al. In silico discovery of small-molecule Ras inhibitors that display antitumor activity by blocking the Ras-effector interaction. Proc Natl Acad Sci U S A. 2013; 110:8182–7. [PubMed: 23630290]
9. Ostrem JM, et al. K-Ras(G12C) inhibitors allosterically control GTP affinity and effector interactions. Nature. 2013; 503:548–51. [PubMed: 24256730]
10. Hunter JC, et al. In situ selectivity profiling and crystal structure of SML-8-73-1, an active site inhibitor of oncogenic K-Ras G12C. Proc Natl Acad Sci U S A. 2014; 111:8895–900. [PubMed: 24889603]
11. Cox AD, et al. Targeting RAS Membrane Association: Back to the Future for Anti-RAS Drug Discovery? Clin Cancer Res. 2015; 21:1819–27. [PubMed: 25878363]
12. Zimmermann G, et al. Small molecule inhibition of the KRAS-PDEdelta interaction impairs oncogenic KRAS signalling. Nature. 2013; 497:638–42. [PubMed: 23698361]
13. Downward J. RAS Synthetic Lethal Screens Revisited: Still Seeking the Elusive Prize? Clin Cancer Res. 2015; 21:1802–9. [PubMed: 25878361]
14. Bryant KL, et al. KRAS: feeding pancreatic cancer proliferation. Trends Biochem Sci. 2014; 39:91–100. [PubMed: 24388967]
15. Goetz EM, et al. ERK mutations confer resistance to mitogen-activated protein kinase pathway inhibitors. Cancer Res. 2014; 74:7079–89. [PubMed: 25320010]
16. Morrison DK. MAP kinase pathways. Cold Spring Harb Perspect Biol. 2012; 4
17. Collisson EA, et al. A central role for RAF-->MEK-->ERK signaling in the genesis of pancreatic ductal adenocarcinoma. Cancer Discov. 2012; 2:685–93. [PubMed: 22628411]
18. Blasco RB, et al. c-Raf, but not B-Raf, is essential for development of K-Ras oncogene-driven non-small cell lung carcinoma. Cancer Cell. 2011; 19:652–63. [PubMed: 21514245]
19. Karreth FA, et al. C-Raf is required for the initiation of lung cancer by K-Ras(G12D). Cancer Discov. 2011; 1:128–36. [PubMed: 22043453]
20. Eser S, et al. Selective requirement of PI3K/PDK1 signaling for Kras oncogene-driven pancreatic cell plasticity and cancer. Cancer Cell. 2013; 23:406–20. [PubMed: 23453624]
21. Wilhelm S, et al. Discovery and development of sorafenib: a multikinase inhibitor for treating cancer. Nat Rev Drug Discov. 2006; 5:835–844. [PubMed: 17016424]
22. Flaherty KT, et al. Inhibition of mutated, activated BRAF in metastatic melanoma. N Engl J Med. 2010; 363:809–19. [PubMed: 20818844]
23. Chapman PB, et al. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. N Engl J Med. 2011; 364:2507–16. [PubMed: 21639808]
24. Hauschild A, et al. Dabrafenib in BRAF-mutated metastatic melanoma: a multicentre, open-label, phase 3 randomised controlled trial. Lancet. 2012; 380:358–65. [PubMed: 22735384]
25. Hyman DM, et al. Vemurafenib in Multiple Nonmelanoma Cancers with BRAF V600 Mutations. N Engl J Med. 2015; 373:726–36. [PubMed: 26287849]
26. Nazarian R, et al. Melanomas acquire resistance to B-RAF(V600E) inhibition by RTK or N-RAS upregulation. Nature. 2010; 468:973–7. [PubMed: 21107323]
27. Lidsky M, et al. Mitogen-activated protein kinase (MAPK) hyperactivation and enhanced NRAS expression drive acquired vemurafenib resistance in V600E BRAF melanoma cells. J Biol Chem. 2014; 289:27714–26. [PubMed: 25063807]
28. Bollag G, et al. Clinical efficacy of a RAF inhibitor needs broad target blockade in BRAF-mutant melanoma. Nature. 2010; 467:596–9. [PubMed: 20823850]
29. Wagle N, et al. MAP kinase pathway alterations in BRAF-mutant melanoma patients with acquired resistance to combined RAF/MEK inhibition. Cancer Discov. 2014; 4:61–8. [PubMed: 24265154]

30. Johannessen CM, et al. COT drives resistance to RAF inhibition through MAP kinase pathway reactivation. Nature. 2010; 468:968–72. [PubMed: 21107320]
31. Hodis E, et al. A landscape of driver mutations in melanoma. Cell. 2012; 150:251–63. [PubMed: 22817889]
32. Watson IR, et al. The RAC1 P29S hotspot mutation in melanoma confers resistance to pharmacological inhibition of RAF. Cancer Res. 2014; 74:4845–52. [PubMed: 25056119]
33. Callahan MK, et al. Progression of RAS-mutant leukemia during RAF inhibitor treatment. N Engl J Med. 2012; 367:2316–21. [PubMed: 23134356]
34. Oberholzer PA, et al. RAS mutations are associated with the development of cutaneous squamous cell tumors in patients treated with RAF inhibitors. J Clin Oncol. 2012; 30:316–21. [PubMed: 22067401]
35. Su F, et al. RAS mutations in cutaneous squamous-cell carcinomas in patients treated with BRAF inhibitors. N Engl J Med. 2012; 366:207–15. [PubMed: 22256804]
36. Abdel-Wahab O, et al. Efficacy of intermittent combined RAF and MEK inhibition in a patient with concurrent BRAF- and NRAS-mutant malignancies. Cancer Discov. 2014; 4:538–45. [PubMed: 24589925]
37. Carlino MS, et al. New RAS-mutant pancreatic adenocarcinoma with combined BRAF and MEK inhibition for metastatic melanoma. J Clin Oncol. 2015; 33:e52–6. [PubMed: 24821886]
38. Basile KJ, et al. Inhibition of mutant BRAF splice variant signaling by next-generation, selective RAF inhibitors. Pigment Cell Melanoma Res. 2014; 27:479–84. [PubMed: 24422853]
39. Choi J, et al. Identification of PLX4032-resistance mechanisms and implications for novel RAF inhibitors. Pigment Cell Melanoma Res. 2014; 27:253–62. [PubMed: 24283590]
40. Rasco DW, et al. MLN2480, an investigational oral pan-RAF kinase inhibitor, in patients (pts) with relapsed or refractory solid tumors: Phase I study. J Clin Oncol. 2013 2013 ASCO Annual meeting,suppl; abstr 2547.
41. Dickson MA, et al. Phase I study of XL281 (BMS-908662), a potent oral RAF kinase inhibitor, in patients with advanced solid tumors. Invest New Drugs. 2015; 33:349–56. [PubMed: 25476894]
42. Henry JR, et al. Discovery of 1-(3,3-Dimethylbutyl)-3-(2-fluoro-4-methyl-5-(7-methyl-2-(methylamino)pyrido[2,3-d]pyrimidin-6-yl)phenyl)urea (LY3009120) as a Pan-RAF Inhibitor with Minimal Paradoxical Activation and Activity against BRAF or RAS Mutant Tumor Cells. J Med Chem. 2015
43. Freeman AK, et al. Effects of Raf dimerization and its inhibition on normal and disease-associated Raf signaling. Mol Cell. 2013; 49:751–8. [PubMed: 23352452]
44. Hatzivassiliou G, et al. Mechanism of MEK inhibition determines efficacy in mutant KRAS- versus BRAF-driven cancers. Nature. 2013; 501:232–6. [PubMed: 23934108]
45. Lito P, et al. Disruption of CRAF-mediated MEK activation is required for effective MEK inhibition in KRAS mutant tumors. Cancer Cell. 2014; 25:697–710. [PubMed: 24746704]
46. Neuzillet C, et al. MEK in cancer and cancer therapy. Pharmacol Ther. 2014; 141:160–71. [PubMed: 24121058]
47. Hainsworth JD, et al. A phase II, open-label, randomized study to assess the efficacy and safety of AZD6244 (ARRY-142886) versus pemetrexed in patients with non-small cell lung cancer who have failed one or two prior chemotherapeutic regimens. J Thorac Oncol. 2010; 5:1630–6. [PubMed: 20802351]
48. Haura EB, et al. A phase II study of PD-0325901, an oral MEK inhibitor, in previously treated patients with advanced non-small cell lung cancer. Clin Cancer Res. 2010; 16:2450–7. [PubMed: 20332327]
49. Infante JR, et al. A randomised, double-blind, placebo-controlled trial of trametinib, an oral MEK inhibitor, in combination with gemcitabine for patients with untreated metastatic adenocarcinoma of the pancreas. Eur J Cancer. 2014; 50:2072–81. [PubMed: 24915778]
50. Janne PA, et al. Selumetinib plus docetaxel for KRAS-mutant advanced non-small-cell lung cancer: a randomised, multicentre, placebo-controlled, phase 2 study. Lancet Oncol. 2013; 14:38–47. [PubMed: 23200175]

51. Blumenschein GR Jr. et al. A randomized phase II study of the MEK1/MEK2 inhibitor trametinib (GSK1120212) compared with docetaxel in KRAS-mutant advanced non-small-cell lung cancer (NSCLC)dagger. Ann Oncol. 2015; 26:894–901. [PubMed: 25722381]
52. Janne PA, et al. Impact of KRAS codon subtypes from a randomised phase II trial of selumetinib plus docetaxel in KRAS mutant advanced non-small-cell lung cancer. Br J Cancer. 2015
53. Ascierto PA, et al. MEK162 for patients with advanced melanoma harbouring NRAS or Val600 BRAF mutations: a non-randomised, open-label phase 2 study. Lancet Oncol. 2013; 14:249–56. [PubMed: 23414587]
54. Duncan JS, et al. Dynamic reprogramming of the kinome in response to targeted MEK inhibition in triple-negative breast cancer. Cell. 2012; 149:307–21. [PubMed: 22500798]
55. Johnson GL, et al. Molecular pathways: adaptive kinome reprogramming in response to targeted inhibition of the BRAF-MEK-ERK pathway in cancer. Clin Cancer Res. 2014; 20:2516–22. [PubMed: 24664307]
56. Ishii N, et al. Enhanced inhibition of ERK signaling by a novel allosteric MEK inhibitor, CH5126766, that suppresses feedback reactivation of RAF activity. Cancer Res. 2013; 73:4050–60. [PubMed: 23667175]
57. Wada M, et al. The dual RAF/MEK inhibitor CH5126766/RO5126766 may be a potential therapy for RAS-mutated tumor cells. PLoS One. 2014; 9:e113217. [PubMed: 25422890]
58. Zimmer L, et al. Phase I expansion and pharmacodynamic study of the oral MEK inhibitor RO4987655 (CH4987655) in selected patients with advanced cancer with RAS-RAF mutations. Clin Cancer Res. 2014; 20:4251–61. [PubMed: 24947927]
59. Aronov AM, et al. Structure-guided design of potent and selective pyrimidylpyrrole inhibitors of extracellular signal-regulated kinase (ERK) using conformational control. J Med Chem. 2009; 52:6362–8. [PubMed: 19827834]
60. Morris EJ, et al. Discovery of a novel ERK inhibitor with activity in models of acquired resistance to BRAF and MEK inhibitors. Cancer Discov. 2013; 3:742–50. [PubMed: 23614898]
61. Chaikuad A, et al. A unique inhibitor binding site in ERK1/2 is associated with slow binding kinetics. 2014; 10:853–60.
62. Rudolph J, et al. Slow inhibition and conformation selective properties of extracellular signal-regulated kinase 1 and 2 inhibitors. Biochemistry. 2015; 54:22–31. [PubMed: 25350931]
63. Wong DJ, et al. Antitumor activity of the ERK inhibitor SCH722984 against BRAF mutant, NRAS mutant and wild-type melanoma. Mol Cancer. 2014; 13:194. [PubMed: 25142146]
64. Robarge K, et al. Abstract DDT02-03: Discovery of GDC-0994, a potent and selective ERK1/2 inhibitor in early clinical development. Cancer Research. 2014; 74:DDT02–03.
65. Germann, UA., et al. ABSTRACT The selective ERK inhibitor BVD-523 is active in models of MAPK pathway-dependent cancers, including those with intrinsic and acquired drug resistance; AACR 2015 annual meeting; 2015.
66. Infante J, et al. Dose escalation stage of a first-in-class phase I study of the novel oral ERK 1/2 kinase inhibitor BVD-523 (ulixertinib) in patients with advanced solid tumors (2015 ASCO Annual Meeting suppl; abstr 2506). J Clin Oncol. 2015; 33
67. Flaherty KT, et al. Combined BRAF and MEK inhibition in melanoma with BRAF V600 mutations. N Engl J Med. 2012; 367:1694–703. [PubMed: 23020132]
68. Long GV, et al. Combined BRAF and MEK inhibition versus BRAF inhibition alone in melanoma. N Engl J Med. 2014; 371:1877–88. [PubMed: 25265492]
69. Robert C, et al. Improved overall survival in melanoma with combined dabrafenib and trametinib. N Engl J Med. 2015; 372:30–9. [PubMed: 25399551]
70. Lamba S, et al. RAF suppression synergizes with MEK inhibition in KRAS mutant cancer cells. Cell Rep. 2014; 8:1475–83. [PubMed: 25199829]
71. Atefi M, et al. Combination of pan-RAF and MEK inhibitors in NRAS mutant melanoma. Mol Cancer. 2015; 14:27. [PubMed: 25645078]
72. Long GV, et al. Increased MAPK reactivation in early resistance to dabrafenib/trametinib combination therapy of BRAF-mutant metastatic melanoma. Nat Commun. 2014; 5:5694. [PubMed: 25452114]

73. Hatzivassiliou G, et al. ERK inhibition overcomes acquired resistance to MEK inhibitors. Mol Cancer Ther. 2012; 11:1143–54. [PubMed: 22402123]
74. Rebecca VW, et al. Vertical inhibition of the MAPK pathway enhances therapeutic responses in NRAS-mutant melanoma. Pigment Cell Melanoma Res. 2014; 27:1154–8. [PubMed: 25130256]
75. Wortzel I, Seger R. The ERK Cascade: Distinct Functions within Various Subcellular Organelles. Genes Cancer. 2011; 2:195–209. [PubMed: 21779493]
76. Yoon S, Seger R. The extracellular signal-regulated kinase: multiple substrates regulate diverse cellular functions. Growth Factors. 2006; 24:21–44. [PubMed: 16393692]
77. von Kriegsheim A, et al. Cell fate decisions are specified by the dynamic ERK interactome. Nat Cell Biol. 2009; 11:1458–64. [PubMed: 19935650]
78. Formstecher E, et al. PEA-15 mediates cytoplasmic sequestration of ERK MAP kinase. Dev Cell. 2001; 1:239–50. [PubMed: 11702783]
79. Parikh N, et al. Mouse tissues that undergo neoplastic progression after K-Ras activation are distinguished by nuclear translocation of phospho-Erk1/2 and robust tumor suppressor responses. Mol Cancer Res. 2012; 10:845–55. [PubMed: 22532587]
80. Plotnikov A, et al. The nuclear translocation of ERK1/2 as an anticancer target. Nat Commun. 2015; 6:6685. [PubMed: 25819065]
81. Soucek L, et al. Modelling Myc inhibition as a cancer therapy. Nature. 2008; 455:679–83. [PubMed: 18716624]
82. Soucek L, et al. Inhibition of Myc family proteins eradicates KRas-driven lung cancer in mice. Genes Dev. 2013; 27:504–13. [PubMed: 23475959]
83. Annibali D, et al. Myc inhibition is effective against glioma and reveals a role for Myc in proficient mitosis. Nat Commun. 2014; 5
84. Ying H, et al. Oncogenic Kras maintains pancreatic tumors through regulation of anabolic glucose metabolism. Cell. 2012; 149:656–70. [PubMed: 22541435]
85. Farrell AS, Sears RC. MYC degradation. Cold Spring Harb Perspect Med. 2014; 4
86. Casar B, et al. Essential role of ERK dimers in the activation of cytoplasmic but not nuclear substrates by ERK-scaffold complexes. Mol Cell. 2008; 31:708–21. [PubMed: 18775330]
87. Herrero A, et al. Small Molecule Inhibition of ERK Dimerization Prevents Tumorigenesis by RAS-ERK Pathway Oncogenes. Cancer Cell. 2015; 28:170–82. [PubMed: 26267534]
88. Romeo Y, et al. RSK regulates activated BRAF signalling to mTORC1 and promotes melanoma growth. Oncogene. 2013; 32:2917–26. [PubMed: 22797077]
89. Sulzmaier FJ, Ramos JW. RSK isoforms in cancer cell invasion and metastasis. Cancer Res. 2013; 73:6099–105. [PubMed: 24097826]
90. Wilson TR, et al. Widespread potential for growth-factor-driven resistance to anticancer kinase inhibitors. Nature. 2012; 487:505–9. [PubMed: 22763448]
91. Posch C, et al. Combined targeting of MEK and PI3K/mTOR effector pathways is necessary to effectively inhibit NRAS mutant melanoma in vitro and in vivo. Proc Natl Acad Sci U S A. 2013; 110:4015–20. [PubMed: 23431193]
92. Soares HP, et al. Dual PI3K/mTOR Inhibitors Induce Rapid Overactivation of the MEK/ERK Pathway in Human Pancreatic Cancer Cells through Suppression of mTORC2. Mol Cancer Ther. 2015; 14:1014–23. [PubMed: 25673820]
93. Junttila MR, et al. Modeling targeted inhibition of MEK and PI3 kinase in human pancreatic cancer. Mol Cancer Ther. 2015; 14:40–7. [PubMed: 25376606]
94. Bedard PL, et al. A phase Ib dose-escalation study of the oral pan-PI3K inhibitor buparlisib (BKM120) in combination with the oral MEK1/2 inhibitor trametinib (GSK1120212) in patients with selected advanced solid tumors. Clin Cancer Res. 2015; 21:730–8. [PubMed: 25500057]
95. Tolcher AW, et al. Antitumor activity in RAS-driven tumors by blocking AKT and MEK. Clin Cancer Res. 2015; 21:739–48. [PubMed: 25516890]
96. Pemovska T, et al. Individualized systems medicine strategy to tailor treatments for patients with chemorefractory acute myeloid leukemia. Cancer Discov. 2013; 3:1416–29. [PubMed: 24056683]
97. artz CA, et al. Systematic identification of signaling pathways with potential to confer anticancer drug resistance. Sci Signal. 2014; 7:ra121. [PubMed: 25538079]

98. Haarberg HE, Smalley KS. Resistance to Raf inhibition in cancer. Drug Discov Today Technol. 2014; 11:27–32. [PubMed: 24847650]

99. Matallanas D, et al. Raf family kinases: old dogs have learned new tricks. Genes Cancer. 2011; 2:232–60. [PubMed: 21779496]

100. Zhang Z, et al. Dual specificity phosphatase 6 (DUSP6) is an ETS-regulated negative feedback mediator of oncogenic ERK signaling in lung cancer cells. Carcinogenesis. 2010; 31:577–86. [PubMed: 20097731]

101. Red Brewer M, et al. The juxtamembrane region of the EGF receptor functions as an activation domain. Mol Cell. 2009; 34:641–51. [PubMed: 19560417]

102. Corcoran RB, et al. EGFR-mediated re-activation of MAPK signaling contributes to insensitivity of BRAF mutant colorectal cancers to RAF inhibition with vemurafenib. Cancer Discov. 2012; 2:227–35. [PubMed: 22448344]

103. Turke AB, et al. MEK inhibition leads to PI3K/AKT activation by relieving a negative feedback on ERBB receptors. Cancer Res. 2012; 72:3228–37. [PubMed: 22552284]

### Outstanding Questions

- What are the key substrates that contribute to ERK dependency in RAS-driven cancers?
- What are the roles of nuclear versus cytosolic ERK substrates in this dependency?
- Which resistance mechanisms will override ERK dependency?
- What biomarkers can accurately and reliably measure effective responses to ERK inhibition?
- How can we predict which patients will respond to ERK inhibitors?
- Can we identify combinations that minimize the normal tissue toxicity induced by ERK inhibition?

**Trends Box**

- Direct inhibitors of RAS, once considered “undruggable”, have recently been developed.
- Both direct and indirect approaches for anti-RAS drug discovery are being pursued.
- ERK inhibitors may overcome limitations of RAF and MEK inhibitors.

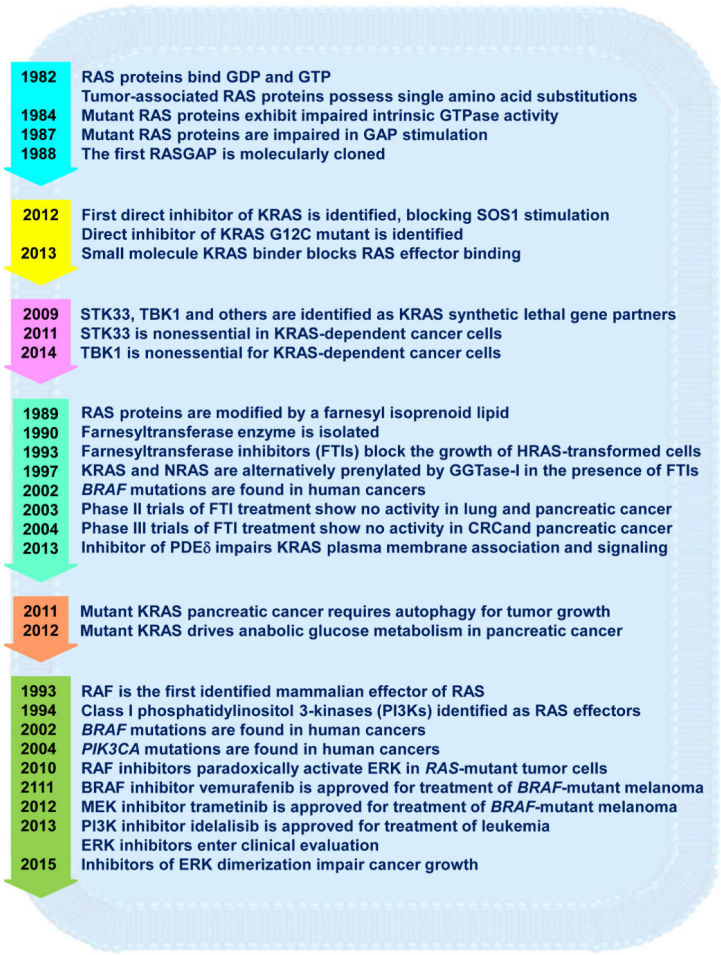


**Figure 1. History of anti-RAS drug discovery**

Summary of key representative events in the search for the still elusive anti-RAS drugs.

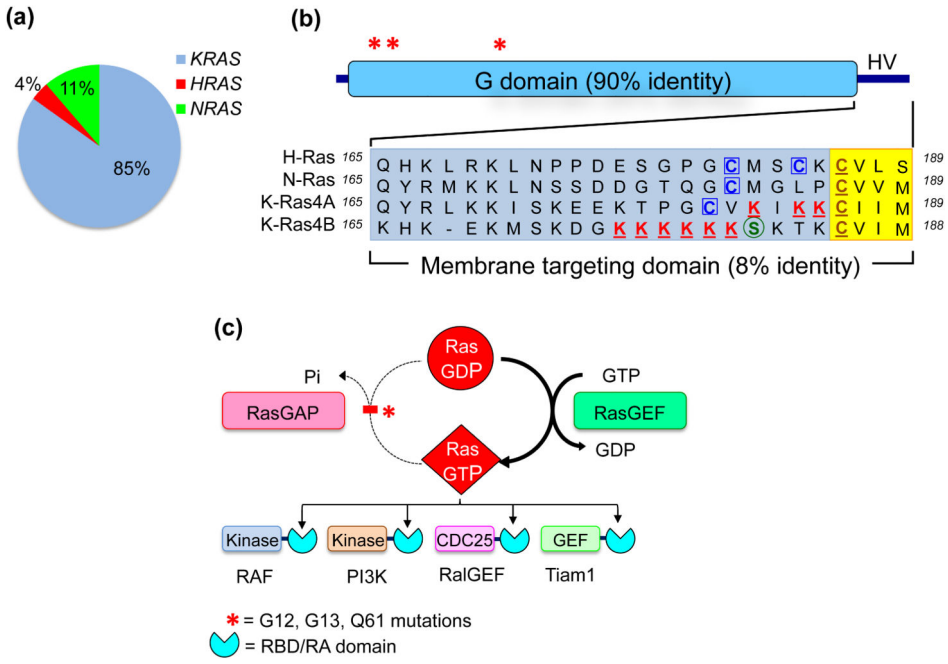


**Figure 2. The RAS proteins**

(a) *RAS* oncogenes (*HRAS*, *NRAS* and *KRAS*) comprise the most frequently mutated gene family in cancer[2,3]. Overall, *RAS* mutations are found in ~25% of human cancers (COSMIC v73). The mutation frequency is not uniform, with frequencies highest in three of the four most deadly cancers in the United States- lung (30%), colorectal (50%) and pancreatic (95%) carcinomas. The frequency of mutation of each *RAS* isoform is also not uniform, with 85% of all *RAS* mutations found in *KRAS*, followed by *NRAS* (11%), whereas *HRAS* is infrequently mutated (4%). (b) The three *RAS* genes encode four highly related proteins of 188-189 amino acids (82-90% sequence identity): HRAS, KRAS4A, KRAS4B and NRAS. RAS proteins are comprised of a highly conserved N-terminal G domain (90% amino acid sequence identity) involved in GTP binding and hydrolysis and a C-terminal membrane-targeting hypervariable (HV) sequence. Underlined C, cysteine of the CAAX motif (highlighted in yellow, the site for farnesylation; see Figure 1). Underlined K, lysine(s) comprising the polybasic domain. Boxed C, site of palmitoylation. Circled S, site of phosphorylation by PKC. (c) RAS proteins function as GDP-GTP regulated binary on-off switches. In normal quiescent cells, RAS is predominantly GDP-bound and inactive. Growth factors activate RAS-selective guanine nucleotide exchange factors (RASGEFs; e.g., SOS1) to promote nucleotide exchange and formation of active RAS-GTP. Once in the active, GTP-bound conformation, RAS can bind to a variety of effector proteins that contain Ras Binding or RAS Association Domains (RBDs/RAs), in order to transmit its downstream signals. RAS-selective GTPase accelerating proteins (RASGAPs; e.g., NF1, neurofibromin) then promote GTP hydrolysis to return RAS to its GDP-bound resting state. Mutated *RAS* genes in cancer harbor missense mutations primarily at three hotspots (G12, G13 and Q61, marked by asterisks); they encode mutant RAS proteins that are GAP-insensitive and are persistently GTP-bound and active.

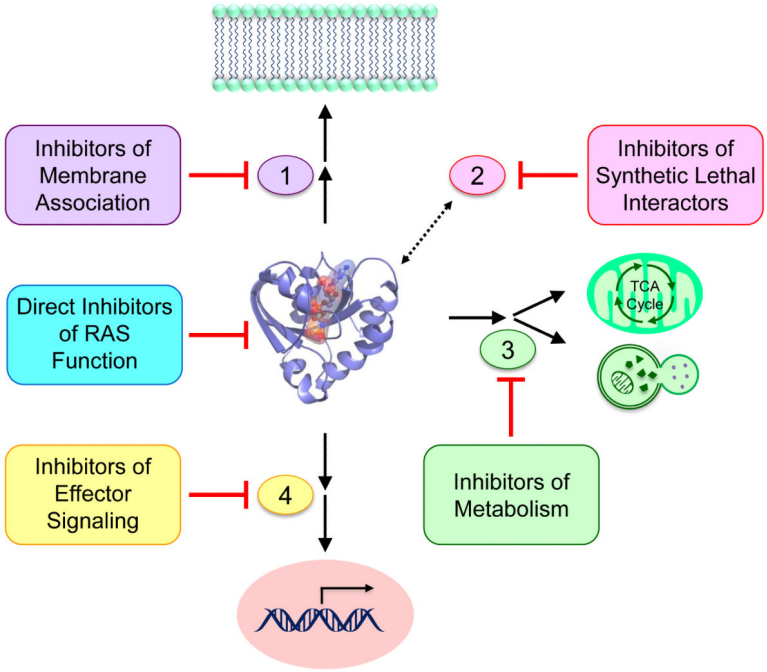


**Figure 3. Pharmacological strategies to inhibit aberrant RAS function**

RAS proteins (center, structure of KRAS4B) must associate with membranes (top) to be biologically active. Once activated, RAS proteins signal to effector cascades that ultimately alter gene transcription (bottom). Shown are one direct and four indirect strategies (1-4) to inhibit the function of RAS in cancer. See text for details.

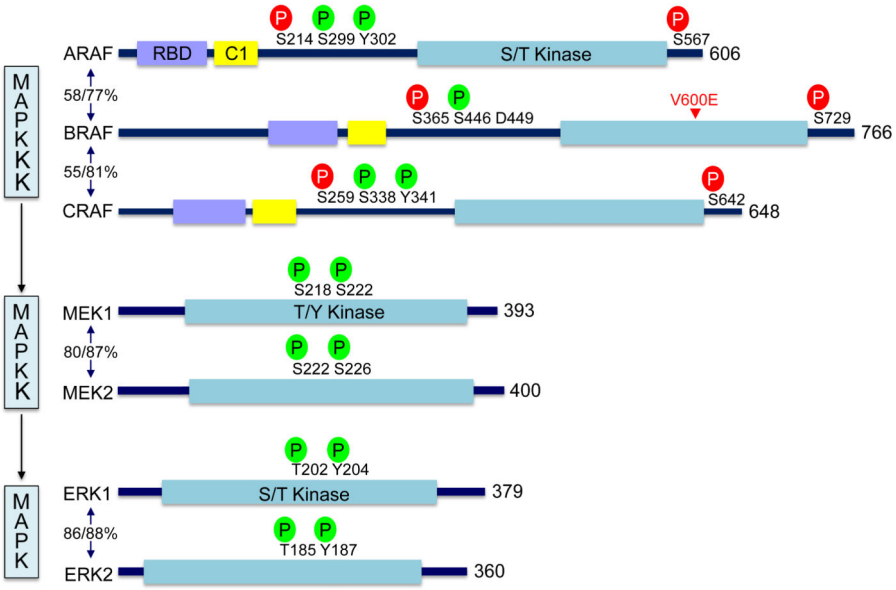


**Figure 4. Components of the RAF-MEK-ERK MAPK cascade**

The RAF-MEK-ERK mitogen-activated protein kinase (MAPK) cascade comprises three sequentially activated protein kinase events: RAF (MAPKKK)→ MEK (MAPKK)→ ERK (MAPK). There are three highly identical human RAF MAPKKK isoforms (ARAF, BRAF, and CRAF), and RAS-mediated homo- or hetero-dimerization of RAF is essential for their full activation[98]. Binding of activated RAS-GTP to the N-terminal RAS-binding domain (RBD) of RAF relieves the N-terminal auto-inhibition of the C-terminal RAF kinase domain and promotes association of the normally cytosolic RAF protein with the plasma membrane, where complex subsequent activation events lead to activation of RAF kinase activity. A still incompletely understood complex set of both negative (red) and positive (green) phosphorylation events regulate RAF catalytic activity[99] (representative sites shown). In the inactive configuration, a 14-3-3 dimer binds to conserved phosphorylation sites in N- and C-terminal residues flanking the kinase domain (ARAF, pS214 and pS576; BRAF, pS365 and p729; CRAF, pS259 and pS621). Protein kinase A and other kinases can phosphorylate these sites. Phosphorylation events that promote kinase activation occur at residues including S338 and Y341 in CRAF (S299 and Y302; ARAF). However, the analogous positions in BRAF are either constitutively phosphorylated (S446) or encode a phosphomimetic residue (D449), explaining why BRAF but not ARAF or RAF can be rendered constitutively activated by a single missense mutation in cancer (V600E). Each activated RAF isoform phosphorylates and activates the highly related MEK1 and MEK2 dual-specificity MAPKKs. Activated MEK1/2 phosphorylate and activate the highly related ERK1 and ERK2 serine/threonine kinases. Total protein and kinase domain sequence identities are indicated (%/%) as determined by CLUSTALW multiple sequence alignment. In stark contrast to the limited substrates of A/B/CRAF and MEK1/2, >200 cytoplasmic and nuclear substrates of ERK1/2 have been described[76].

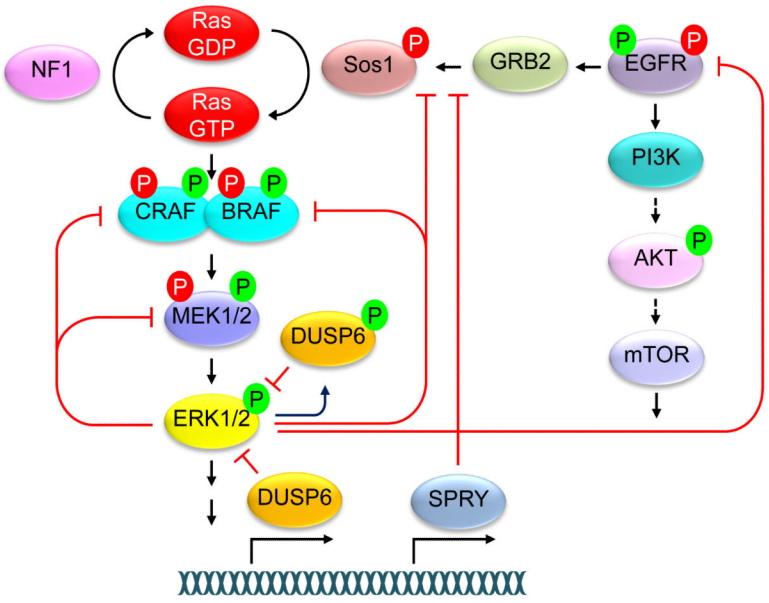


**Figure 5. Mechanisms of ERK negative feedback regulation**

ERK phosphorylation of CRAF disrupts interaction with RAS. ERK phosphorylation of BRAF disrupts dimerization and interaction with RAS. ERK phosphorylation of MEK1 promotes heterodimerization with MEK2. ERK phosphorylation of son of sevenless homolog 1 (SOS1) disrupts interaction with growth factor receptor-bound protein 2 (GRB2). ERK phosphorylation of the dual specificity phosphatase (DUSP6) regulates its protein stability. ERK-activated transcription factors promote expression of DUSP6[100] and the scaffold protein SPRY, with SPRY disrupting SOS1 interaction with GRB2. ERK phosphorylation of T669 in the epidermal growth factor receptor (EGFR) juxtamembrane region is important for EGFR dimerization and activation[101], promoting activation of RAS[102] and PI3K[103].

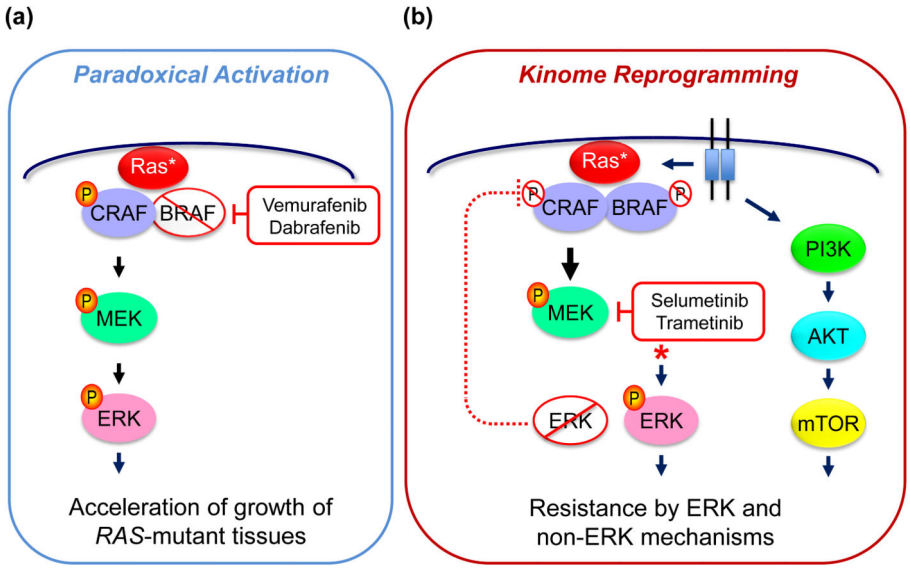


**Figure 6. Mechanisms of resistance to RAF and MEK inhibitors in RAS-mutant cancers**

Second generation RAF inhibitors such as vemurafenib and dabrafenib are BRAF-selective and cause paradoxical activation of ERK (panel (a)). The inhibitor-blocked BRAF forms a heterodimer with active CRAF and complexes with activated RAS. MEK inhibitors transiently block ERK activation. Since high ERK activation can be deleterious for cell proliferation, ERK activation stimulates negative feedback mechanisms that dampen upstream signaling through the pathway (panel (b)). Kinome reprogramming results in rewiring of the signaling networks to increase flux through non-RAF-MEK-ERK pathways such as PI3K-AKT-mTOR.

## Table 1

RAF-MEK-ERK inhibitors under clinical evaluation

| Agent | Other Names | Phase[a] | Targets | Mechanism and properties[e] |
|---|---|---|---|---|
| **RAF** | | | | |
| BGB-283 | | Phase I[b] | RAF, EGFR | Dual RAF dimer and EGFR inhibitor[b] |
| BMS-908662 | XL281 | Phase I/ II[c] | RAF | ATP-competitive, pan-RAF |
| Dabrafenib | GSK2118436 | Approved for BRAF V600E melanoma | RAF | Type I ATP-competitive, BRAF-selective |
| Encorafenib | LGX818 | Phase II | RAF | ATP-competitive, BRAF-selective |
| HM95573 | | Phase I | RAF | Pan-RAF |
| LY3009120 | | Phase I | RAF | ATP-competitive, “paradox breaker” |
| MLN2480 | BIIB-024 | Phase I | RAF | Pan-RAF |
| RAF265 | CHIR-265 | Phase I/ II[c] | RAF, VEGFR | ATP-competitive, multi-kinase |
| Regorafenib | BAY 73-4506 | Approved for metastatic colorectal cancer and advanced gastrointestinal stroma tumors | RAF, KIT, VEGFR | Type II ATP-competitive, multi-kinase |
| Sorafenib | BAY 43-9006 | Approved for unresectable hepatocellular carcinoma, advanced renal cell carcinoma and thyroid cancer | VEGFR2, PDGFRβ, KIT, FLT3, CRAF | Type II ATP-competitive, multi-kinase |
| Vemurafenib | PLX4032, RG7204, RO5185426 | Approved for BRAF V600E melanoma | | Type I ATP-competitive, BRAF-selective |
| **MEK** | | | | |
| ARRY-300 | | Phase I[c] | MEK1/2 | Type III allosteric, non-ATP-competitive; analog of MEK162 |
| AS703988 | MSC2015103B | Phase I[c] | MEK1/2 | Type III allosteric, non-ATP-competitive |
| AZD8330 | ARRY-424704, ARRY-704 | Phase I[c] | MEK1/2 | Type III allosteric, non-ATP-competitive |
| Binimetinib | ARRY-438162, ARRY-162, MEK162 | Phase II | MEK1/2 | Type III allosteric, non-ATP-competitive |
| Cobimetinib | XL-518, GDC-0973, RG7421 | Phase III | MEK1 | Non-ATP competitive, 100-fold selectively for MEK1 over MEK2 |
| E6201 | ER 806201 | Phase I/II | MEK1, MEKK1 FLT3 | Synthetic, fungal metabolite analogue |
| GDC-0623 | RG7420, G-868 | Phase I[c] | MEK1/2 | Type III allosteric, non-ATP-competitive; analog of CI-1040; stabilizes a RAF-MEK complex |
| PD-0325901 | | Phase II | MEK1/2 | Type III allosteric, non-ATP-competitive |
| Pimasertib | AS703026, SAR245509, | Phase II | MEK1/2 | Type III allosteric, non-ATP-competitive |

| Agent | Other Names | Phase[a] | Targets | Mechanism and properties[e] |
|---|---|---|---|---|
| | EMD 1036239, MSC1936369B | | | |
| Refametinib | RDEA119, BAY86-9766 | Phase II | MEK1/2 | Allosteric, non-ATP-competitive |
| RO4987655 | CH4987655, RG7167 | Phase I[c] | MEK1/2 | Allosteric, non-ATP-competitive |
| RO5126766 | CH5126766, RG7304 | Phase I[c] | Raf, MEK1/2 | Type III allosteric, non-ATP-competitive; binds to MEK1/2, forms a stable Raf-MEK-RO5126766 complex, preventing both MEK and ERK phosphorylation |
| Selumetinib | AZD6244, ARRY-142886 | Phase III | MEK1/2 | Type III allosteric, non-ATP-competitive |
| TAK733 | | Phase I[c] | MEK1/2 | Type III allosteric, non-ATP-competitive |
| Trametinib | GSK1120212, JTP-74057 | Approved for BRAF V600E melanoma | MEK1/2 | Type III allosteric, non-ATP-competitive |
| WX-554 | | Phase I/II[d] | | |
| **ERK** | | | | |
| CC-90003 | | Phase I | ERK1/2 | |
| GDC-0994 | RG7842 | Phase I | ERK1/2 | ATP-competitive |
| MK-8353 | SCH900353 | Phase I[d] | ERK1/2 | Allosteric and ATP-competitive |
| Ulixertinib | BVD-523 | Phase I/II | ERK1/2 | ATP-competitive |

[a] Compiled from ClinicalTrials.gov

[b] http://www.beigene.com/

[c] Completed

[d] Terminated

[e] ATP-competitive inhibitors are broadly classified as type I or II, that target the active “in” or inactive “out” conformation of the ATP/$Mg^{2+-}$coordinating three amino acid DFG motif, highly conserved among most protein kinases and located N-terminal to the activation loop. Type III inhibitors bind to a hydrophobic pocket directly adjacent to the ATP-binding site.