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Inhibition of Ras for cancer treatment: the search continues

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Abstract

Background—The *RAS* oncogenes (*HRAS, NRAS* and *KRAS*) comprise the most frequently mutated class of oncogenes in human cancers (33%), stimulating intensive effort in developing anti-Ras inhibitors for cancer treatment.

Discussion—Despite intensive effort, to date no effective anti-Ras strategies have successfully made it to the clinic. We present an overview of past and ongoing strategies to inhibit oncogenic Ras in cancer.

Conclusions—Since approaches to directly target mutant Ras have not been successful, most efforts have focused on indirect approaches to block Ras membrane association or downstream effector signaling. While inhibitors of effector signaling are currently under clinical evaluation, genome-wide unbiased genetic screens have identified novel directions for future anti-Ras drug discovery.

Keywords

Ras; farnesyltransferase inhibitors; Raf; phosphoinositol 3-kinase; oncogene addiction; synthetic lethality

Introduction

Initially identified as retroviral oncogenes transduced from the rodent genome, mutationally activated human *RAS* genes were subsequently linked to human cancer in 1982 [1]. This prompted intensive research to elucidate the structure, biochemistry and biology of wild type and mutant Ras proteins to provide clues for the development of small molecules to block mutant Ras function in cancer. For the purpose of this review, the focus will be exclusively on the “classical” Ras protein family members H-, N-, and K-Ras (isoforms 4A and 4B). We summarize the unsuccessful approaches that have been considered to directly target mutant Ras, the directions taken to block Ras membrane association or downstream effector

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signaling, and more recently unbiased functional screens for synthetic lethal partners of mutant *KRAS*.

RAS gene mutation in human cancer: the focus is now on *KRAS* *KRAS*: the most frequently mutated *RAS* gene in human cancers

The three human *RAS* genes (*HRAS, KRAS* and *NRAS*) encode four highly related (>90% identity) proteins (Fig. 1A). Mutational activation of *RAS* genes is associated with 33% of human cancers, making it one of the most frequent oncogenic mutations [2]. Although *HRAS* was historically the most studied *RAS* gene, ironically, it is the isoform least mutated in human cancers. From data available at the COSMIC database (www.sanger.ac.uk/genetics/CGP/cosmic/), mutations in *KRAS* are associated with the highest percentage of all human cancers (21.6%), followed by *NRAS* (8.0%), and with *HRAS* mutations the least frequently mutated (3.3%). *KRAS* mutations comprise 86% of all *RAS* mutations (Fig. 1B). In particular, *KRAS* is the predominant or exclusive *RAS* gene mutated in three of the top four neoplasms that account for cancer deaths in the US: lung, colon and pancreatic cancer [3]. As described below, there is evidence for distinct functions of *RAS* genes in normal and neoplastic cell biology.

Genome-wide sequencing of human cancers: *KRAS* mutation is the predominant oncogene alteration in lung, colon and pancreatic cancer

Pancreatic ductal adenocarcinoma (PDAC) is the most common cancer of the pancreas, comprising over 85% of all cases [4]. With an estimated 43,140 new cases and 36,800 deaths in 2010, PDAC ranks 4th in cancer-related deaths in the United States and has a relative 1-year survival rate of 20% and a 5-year survival rate of only 4% [3].

A model for pancreatic ductal adenocarcinoma (PDAC) development, where mutational activation of *KRAS* and the mutational loss of *TP53, SMAD4* and *CDKN2A (*encodes p16 INK4A and p19 ARF*)* tumor suppressor function defined key genetic steps in tumor progression [5, 6] (Fig. 1C). In particular, the frequent mutation of *KRAS* has been well-established [7]. With the recent complete exon sequencing of pancreatic cancer, it established that the most frequently mutated genes in this cancer were already known, with no novel and significant genetic lesions found [8]. While many other genes were found to be mutated, their low representation in a majority of pancreatic cancers verified that aberrant K-Ras function remains the most important target for pancreatic cancer treatment.

Prior to exon sequencing of PDAC, the most frequently mutated genes known to be associated with the progression of this cancer were *KRAS* and the *TP53, CDKN2A* and *SMAD4* tumor suppressors [4]. The outcome of sequence analyses of 20,661 genes in 24 pancreatic cancers was that these same four genes remained the top four most frequently mutated genes, with *KRAS* mutations found in 114 of 114 PDAC tumors [8].

With an estimated 142,570 new cases and 51,370 deaths in 2010, colorectal cancer (CRC) ranks 3rd in cancer-related deaths in the United States [3]. Frequent *KRAS* mutations had been established previously for colorectal cancer [9] and comprises an early genetic event in CRC progression [10] (Fig. 1D). A similar picture emerged from exon sequencing of colorectal cancers. In a study which 18,191 genes were sequenced in 11 colorectal tumors, *KRAS* was the most frequently mutated oncogene and second only to *TP53* mutations for all mutated genes [11].

With an estimated 232,520 new cases and 157,300 deaths in 2010, lung cancer ranks 1st in cancer-related deaths in the United States [3]. In a study of 188 primary lung adenocarcinomas where 623 genes with known or potential relationships to cancer were sequenced, *KRAS* was the most frequently mutated oncogene [12]. When taken together,

these sequencing studies verify that *KRAS* remains the most significant target for new therapies for these three deadly cancers.

Mutant *RAS* function is required for tumor maintenance

Since *KRAS* mutation is typically an early event in cancer progression, and since cancer is a multi-step genetic process, there remains debate as to whether targeting aberrant Ras function alone will be a therapeutically-useful approach for the advanced cancer [13, 14] . One of the first studies supporting the importance of mutant *KRAS* for advance tumor cell growth involved homologous recombination ablation of the endogenous *KRAS* allele in HCT-166 and DLC-1 colorectal carcinoma cell lines that harbored additional genetic mutations [15]. Loss of the mutant but not wild type *KRAS* allele greatly impaired anchorage-independent growth and tumor growth in nude mice.

A second key study assessed the importance of activated *RAS* for mouse melanoma tumor formation and maintenance [16]. Using a doxycycline-inducible mutant *HRAS* transgene in a mouse melanoma model null for the *INK4A* tumor suppressor, doxycycline treatment caused primary melanoma tumor formation. Upon withdrawal of doxycycline and downregulation of mutant *HRAS* expression, dramatic tumor regression was seen.

A third key study utilized RNA interference to stably silence mutant *KRAS* expression in CAPAN-1 pancreatic carcinoma cell line, resulting in impaired tumorigenic growth [17]. Similarly, using inducible shRNA to silence mutant *KRAS* in SW480 colorectal or CAPAN-1 pancreatic human tumor cells reduced tumor xenograft growth in mice [18]. These and many similar studies provide compelling evidence that if pharmacologic ablation of mutant Ras function can be achieved in advanced cancers, there will likely be a very significant therapeutic benefit.

Mutant Ras proteins are persistently GTP-bound and active

Ras proteins function as GDP/GTP-regulated binary on-off switches that regulate cytoplasmic signal transduction (Fig. 2A). Wild type Ras proteins cycle between a GTP-bound (active) and GDP-bound (inactive) state, which is regulated by guanine-nucleotide exchange factors (RasGEFs) that promote formation of Ras-GTP and GTPase activating proteins (RasGAPs) that promote formation of inactive Ras-GDP [19].

Mutant Ras proteins contain single amino acid missense mutations (most commonly at residues 12, 13 or 61) that render them GAP-insensitive, and thus persistently GTP-bound and active, leading to chronic stimulus-independent activation of effector signaling (Fig. 2B). Therefore, one of the first considerations for developing anti-Ras inhbitors was based on the successful template of developing small molecule antagonists of ATP binding to protein kinases. The binding of ATP to protein kinases occurs at low micromolar ranges and effective ATP competitive protein kinase inhibitors bind with nanomolar affinities. In contrast, the main reason for the lack of success with GTP antagonists is the high binding affinity at picomolar levels of GTP to Ras. A second strategy for inhibiting Ras included efforts to develop small molecules that can “mimic” RasGAP and restore the GTPase activity of mutant Ras proteins. Unfortunately, despite the discovery of RasGAP to guide these efforts, no success was seen for these endeavors. After these disappointments in developing therapies that directly targeted oncogenic Ras, the focus was shifted to more indirect approaches.

Ras proteins are membrane-associated signal transducers: indirect approaches for targeting Ras

Initially, it was believed that Ras proteins were solely positioned at the inner face of the plasma membrane where they act as signal transducers for cell surface receptors. However, subsequent studies have demonstrated that in addition to the plasma membrane, Ras signaling has now been observed on intracellular membranes such as endosomes, the endoplasmic reticulum, the Golgi apparatus, and mitochondria [20]. This subcellular compartmentalization of signaling helps to explain the role Ras plays in the diversity of cellular processes, including growth, survival and differentiation. Receptors found on these membranes (e.g., receptor tyrosine kinases, G protein-coupled receptors, integrins, etc.) are receptors activated by a diverse spectrum of intracellular and extracellular stimuli. The activated receptors then initiate signaling activities that lead to RasGEF-mediated transient activation of Ras. Activated Ras can then bind to and stimulate a diverse spectrum of functionally diverse downstream effectors, resulting in regulated activation of a complex array of cytoplasmic signaling networks. Ras activation is transient, returning back to the inactive state when the stimulus is terminated. The essential roles of membrane association and downstream effector signaling in Ras-mediated oncogenesis provide the foundation for the two primary indirect approaches that have been pursued for blocking Ras. In the following sections, we highlight the various strategies that have been used.

Inhibitors of Ras membrane association

Post-translational lipid modification and membrane association are key determinants necessary for proper functioning of Ras. The four Ras proteins terminate with a C-terminal CAAX tetrapeptide motif (where C = cysteine, A = aliphatic amino acid, X = terminal amino acid) which is the target for covalent addition of a C15 farnesyl isoprenoid lipid, catalyzed by the enzyme farnesyltransferase (FTase) [21] (Fig. 3). Two subsequent modifications signaled by the farnesylated CAAX motif are endoproteolytic cleavage of the AAX sequence catalyzed by the Ras-converting enzyme-1 (Rce1) and the carboxymethylation of the now terminal isoprenylated cysteine residue by the isoprenylcysteine carboxymethyltransferase-1 (Icmt1). While these CAAX modifications are necessary, they are not sufficient to promote Ras association with the inner face of the plasma membrane. Instead, Ras proteins possess a second C-terminal signal upsteam of the CAAX motif that promotes full plasma membrane recruitment and hence full Ras function. H-Ras, N-Ras and K-Ras4A undergo an additional covalent modification, the addition of palmitate fatty acid to cysteine residues. K-Ras4B contains a polybasic amino acid sequence that serves as a second signal for its association with the plasma membrane. Inhibitors of Ras membrane association involve either inhibitors of FTase or farnesyl moiety-containing molecules that are proposed to function as antagonists of Ras membrane association.

Farnesyltransferase inhibitors (FTIs)

Since the 1989 discovery that Ras proteins are farnesylated, and shown to be essential for Ras membrane association and transformation, much emphasis has been placed on successfully targeting this lipid modification [22, 23]. Structure-function mutagenesis studies of the CAAX motif provided the first evidence that farnesylation were critical for Ras transforming activity. Mutation of the cysteine residue of the CAAX motif prevented farnesylation and all subsequent C-terminal modifications, rendering Ras cytosolic and non-transforming [24-26].

The finding that Ras function was critically dependent on farnesylation stimulated ample excitement towards the possibility of identifying a pharmacologic approach of inhibiting Ras function, especially considering that the farnesyl pyrophosphate contributing this lipid group to proteins was a necessary intermediate component of the mevalonate-cholesterol

biosynthetic pathway, whose synthesis could be blocked by cholesterol-lowering drugs already in clinical use (e.g. lovastatin). Lovostatin, an HMG-CoA reductase inhibitor, was the first FDA-approved statin for lowering cholesterol to prevent cardiovascular disease in patients with hypercholesterolemia. However, since the clinically effective concentration of statins sufficient for lowering cholesterol biosynthesis was much lower than the concentration needed to block Ras farnesylation [27], the search began for the enzyme required for the addition of the farnesyl group to Ras. In 1990 Goldstein, Brown and colleagues isolated and characterized the farnesyltransferase (FTase) enzyme [28]. They also showed that the Ras CAAX tetrapeptide sequence alone was effective in blocking FTase activity.

These findings stimulated a frenzied effort by both pharmaceutical companies and academic researchers to design cell-permeable CAAX peptidomimetics as possible FTase inhibitors (FTIs) [22, 23]. Additionally, with the enzyme in hand, high throughput chemical library screens were initiated to identify small molecule inhibitors of FTase and used to develop potent and selective FTase inhibitors (FTIs). One potential complication in these efforts was the existence of a closely related enzyme, geranylgeranyltransferase type I (GGTase-I) [29]. Like FTase, GGTase-I recognizes C-terminal CAAX motifs. However, GGTase-I preferentially recognizes CAAX motifs where the terminal X residue is leucine, and catalyzes the addition of the more hydrophobic C20 geranylgeranyl isoprenoid. In contrast, FTase preferentially recognizes CAAX motifs where X is methionine, alanine, serine or glutamine.

Numerous chemically-diverse FTIs were developed, including CAAX eptidomimetics, nopeptide peptidomimetics, farnesyl diphosphate analogs, and bisubstrate inhibitors with several advancing into clinical testing for oncology, either alone or in combination with conventional cytotoxic drugs [22]. Generally, these showed potent selectivity for FTase and not the closely related GGTase-I. Of these, two nonpeptide peptidomimetics, tipifarnib (R115777) and lonafarnib (SCH66336), underwent the most significant clinical evaluation (Fig. 3).

FTIs showed impressive anti-H-Ras and anti-tumor activity in preclinical cell culture [30, 31] and mouse models, in particular an H-Ras-driven mammary tumor model [32]. These impressive observations resulted in FTIs entering Phase I studies in 1999, with some progressing to Phase III clinical trials in 2002.

However, two key issues led to the eventual demise of FTIs in the clinic and as anti-Ras inhibitors [23]. First, many of the early preclinical studies focused on models of H-Ras-driven oncogenesis. An early suggestion that such models were not accurate models for FTI evaluation came from a study showed that tumor cell line sensitivity to FTI growth inhibition *in vitro* did not correlate with *RAS* mutation status [33]. While FTIs indeed effectively blocked H-Ras farnesylation and membrane association, and transformation, it was subsequently determined that FTIs did not effectively block N-Ras and K-Ras protein prenylation, membrane association and transforming activity [34-37]. This was due to an unexpected biochemical difference among the three Ras proteins. When FTase activity is blocked, K-Ras4B and N-Ras (the two most common mutated Ras proteins in cancer) can serve as substrates for GGTase-I and undergo alternative prenylation with the addition of a geranylgeranyl isoprenoid which can effectively substitute for the farnesyl group and support Ras membrane association and transforming activity [38, 39]. Therefore, it was not surprising that phase II and III clinical trial analyses with pancreatic cancer, where *KRAS* is mutated in 90% of all pancreatic cancers, resulted in negative findings [40-42].

A second key misconception regarding FTIs was the simple assumption that they acted as "anti-Ras inhibitors", yet Ras proteins are not the only substrates for FTase [29]. There are a number of farnesylated proteins (>50) with various roles in the cell, including growth regulation [43]. For example, the Rheb small GTPase [43] is a farnesylated protein and an activator of mammalian target of rapamycin (mTOR), a pathway commonly deregulated in cancer. Thus, the anti-tumor activities of FTIs very likely involve inhibition of function of other farnesylated proteins. The therapeutic value of FTIs may also be complicated by inhibiting the function of some farnesylated Ras family GTPases that function as tumor suppressors (e.g., Di-Ras1/Rig, ARHI/NOEY2, RRP22/RasL10A) [44-46].

Despite some patients responding to FTI treatment with an unclear understanding of what the therapeutically-important targets are, FTIs are no longer being pursued for oncology. Recently however, FTIs have been considered for the treatment of other diseases. In particular, Hutchinson-Gilford Progeria Syndrome (HGPS; also called progeria) is caused by a mutation in the gene encoding lamin A (*LMNA*), resulting in expression of a defective lamin A protein that retains the farnesyl modification. Promising results with FTI treatment in cell culture [47-50] and mouse models [51] support their clinical value for this disease [52]. Since progeria patients number fewer than 50 worldwide, that FTIs may fortuitously serve as a therapeutic approach for this disorder prompted the first ever clinical trial for this disease in 2007. Completed in 2009, the results from this trial have yet to be reported. A second clinical trial is planned, where lonafarnib will be used in combination with pravastatin (an inhibitor of the mevalonate biosynthetic pathway and hence all protein prenylation) and zoledronic acid, a biphosphonate which is an approved drug used to prevent skeletal fractures in patients with cancers, as well as for treating osteoporosis (http://www.progeriaresearch.org/).

In light of the alternative prenylation seen with K-Ras and N-Ras when FTase activity is blocked, concurrent inhibition of both FTase and GGTase-I have also been considered. However, because there are over 50 known or putative substrates for GGTase-I [29], normal cell toxicity has been a concern with such approaches. Despite this concern, cell culture and tumor xenograft studies [37, 53-55] and additional genetic studies in mouse models of *RAS*-driven oncogenesis [56, 57] support the anti-tumor activity of GGTase-I inhibitors (GGTIs), with one highly selective GGTI (GGTI-2418) currently in Phase I clinical evaluation. Early Phase I results found GGTI-2418 well-tolerated with minimal toxicity, supporting expansion of the trial (http://www.tigrispharma.com). One FTI evaluated in clinical trials, L-778,123, also possessed dual inhibitory activity for GGTase-I (FTase IC_{50} = 2 nM, GGTase-I IC_{50} = 98 nM) and inhibited GGTase-I activity in the patient, but nevertheless still failed to block K-Ras prenylation [58].

Inhibitors of Rce1 and Icmt

In addition to FTases, the two less explored CAAX-signaled modifications have also been considered as targets for anti-Ras inhibitors [59]. Compared to FTIs, there was only a 50% reduction in K-Ras4B membrane association and transforming activity when the Rce1 and Icmt modifications were blocked [60]. These observations suggested limited clinical value in targeting these two enzymes. However, recent studies provide evidence for the potential usefulness of inhibitors of Rce1 and Icmt inhibitors for blocking Ras oncogenicity. Their effectiveness may be due to the concurrent impairment of function of other CAAX-terminating small GTPases (e.g., Rac and Ral) that have been shown to be required for Ras-mediated growth transformation.

In one group of studies, mouse embryo fibroblasts deficient in Rce1 revealed that Ras proteins were incompletely processed and membrane-associated [61, 62]. Cre-mediated loss of *RCE1* in fibroblasts generated from mice with a conditional *RCE1* allele resulted in a loss

of endoproteolytic processing and methylation of the Ras protein. Additionally, excision of *RCE1* reduced anchorage-independent growth in Ras-mediated transformation. In another study, excision of *RCE1* in a skin carcinoma cell line greatly reduced their growth [62]. Loss of *ICMT* resulted in inhibition of K-Ras-mediated anchorage-independent growth in soft agar assays and tumor growth in nude mice. Finally, in a recent study, an *ICMT* deficiency reduced lung tumor development in a mouse model of *KRAS*-induced cancer [63]. However, this issue may be highly context dependent, since an Rce1 deficiency was found to accelerate mutant *KRAS*-induced myeloproliferative disease [64].

In reference to Rce1 as a target for anti-Ras inhibition, only limited development of Rce1 inhibitors has been described. In one study, several compounds were found to be effective at a low micromolar range for both yeast and human Rce1 in a compound library screen and were identified as possible tools for design of future Rce1 inhibitors [65]. An additional study showed that peptidyl (acyloxy)methyl ketones could inhibit Rce1 enzyme activity *in vitro* [66].

From a chemical library screen, a small molecular inhibitor of Icmt named cysmethynil (2-[5-(3-methylphenyl)-1-octyl-1H-indol-3-yl]acetamide) was identified by Casey and colleagues [59]. Cysmethynil treatment inhibited cell growth in an Icmt-dependent fashion and resulted in mislocalization of Ras in cancer cells. In addition, the anchorage-independent growth of a colon cancer cell line was blocked by cysmethynil, and this effect was reversed by ectopic overexpression of Icmt, indicating that the inhibition was target-based. Additionally, treatment of PC3 human prostate cell-derived xenograft tumors with cysmethynil resulted in markedly reduced tumor size [67].

Other small molecules with Icmt inhibitory activity have also been described. The anti-folate compound methotrexate has been shown to inhibit Icmt function. In a colon cancer cell line, methotrexate treatment resulted in a decrease in methylation of the Ras protein by nearly 90%, and mislocalization of Ras to the cytoplasm [68]. Several natural product inhibitors of Icmt have been discovered in a high-throughput screen campaign [69-71].

Finally, palmitoylation have also been considered as a potential anti-Ras target [72, 73]. However, the enzymology of Ras palmitoylation is complex and a better understanding of the specificity of the DHHC domain proteins that function as *S*-palmitoyltransferases remains to be achieved.

Farnesyl-containing small molecule inhibitors of Ras membrane association

As discussed earlier in the review, C-terminal farnesylation of Ras is critical for localization to the plasma membrane, and this localization is necessary for Ras binding to effector molecules in the various downstream signaling pathways. Studies have shown that insertion of the lipophilic prenyl moiety into the plasma (or other lipid bilayer) membrane is not a simple random event, but that specific “prenyl receptors” facilitate prenylated protein binding [74]. These and other studies [38, 75-78] lend support to the hypothesis that prenylation also provides specificity for interaction partners. Therefore, ongoing work is focused on inhibiting the binding of farnesylated Ras to sites on the inner surface of the plasma membrane.

Two farnesyl isoprenoid-containing small molecules have been described, salirasib (also called FTS; *S*-trans,trans-farnesylthiosalicylic acid) and TLN-4601 [79-82], that are proposed to antagonize Ras function by competition for membrane-bound farnesyl-binding docking proteins. Whereas clinical trial analyses of TLN-4601 have recently been terminated, there are continued preclinical and clinical analyses of salirasib.

Salirasib, a farnesylcysteine mimetic, selectively disrupts the association of chronically active Ras proteins with the plasma membrane [83]. The proposed mechanism of Salirasib is compete with Ras for binding to membrane-associated Ras escort proteins (galectins), which possess putative farnesyl-binding domains, thereby dislodging Ras from the plasma membrane and disrupting effector signaling. Studies show that galectin-1 interacts with mutant H-Ras and K-Ras, and that this interaction is required for membrane localization of the GTPases and subsequent transforming activity in human and rat epithelial cells [84, 85].

Salirasib blocks the membrane association of H-, K-, and N-Ras proteins in both transformed cells and cancer cells with oncogenic mutant Ras or hyperactivated wild type Ras, including pancreatic, melanoma, glioblastoma, neuroblastoma and neurofibromatosis cancer cells [80, 86-92]. Additionally, Kloog and colleagues demonstrated that signaling from three of the most-studied effector pathways downstream of Ras, Raf-MEK-ERK [88], RalGEF-Ral [92], and PI3K-AKT [90-93], could be suppressed by treatment with salirasib. Inhibition of aberrant Ras activation in cells by salirasib may alter a variety of cellular properties, including cell survival [94], proliferation [80, 86], and migration [93, 95]. In another study, tumor growth was inhibited by salirasib and was associated with a reduction of the abundance of Ras in the tumor tissue in pancreatic and neurofibromatosis xenograft tumor models [92, 96]. However, salirasib has also been shown to block mTOR activity directly [97-99], an unexpected activity in light of the fact that mTOR is not farnesylated. Therefore, salirasib may possess multiple mechanisms of action that can contribute to inhibition of tumor growth. Finally, Phase I clinical trials have shown that salirasib was well-tolerated and several Phase I/II trials are ongoing [100].

Targeting downstream Ras effector signaling pathways

Activated Ras binds preferentially to a spectrum of functionally diverse downstream effectors in which most are characterized by Ras binding (RBD) or Ras association (RA) domains that directly interact with Ras [101]. The Raf kinases are the best characterized of all the effectors of Ras [102] (Fig. 4). However, there exist at least 10 functionally distinct classes of Ras effectors, with evidence for Raf and four non-Raf effectors in Ras transformation. The frequent mutational activation of B-Raf (20%) and the *PIK3CA* gene product (12%), the p110α catalytic subunit of phosphoinositide 3-kinase (PI3K) in human cancers, together with the well-established role of these pathways in signaling networks that regulate cell growth [102, 103], have provided strong validation of the importance of these two effectors in oncogenic Ras function.

Cell culture [104] and mouse model [105-107] studies support the importance of the Ral GTPase-specific guanine nucleotide exchange factor (RalGEFs), phospholipase C epsilon and Tiam1 effectors in Ras-mediated oncogenesis. The involvement of multiple effectors in Ras-mediated oncogenesis prompts several questions. First, is there one “right” effector pathway for targeting or will concurrent inhibition of multiple effector pathways be required? Second, will mutant K-Ras utilize the same effector pathways in lung, colon and pancreatic cancer, or will cancer type-specific approaches been required? Below we summarize the validation and status of the development of inhibitors of the three best validated Ras effector signaling networks.

Inhibitors of the Raf-MEK-ERK mitogen-activated protein kinase (MAPK) cascade

The best understood and most heavily studied Ras effector pathway is the Raf-MEK-ERK MAPK cascade [102]. Raf serine/threonine protein kinases (A-Raf, B-Raf and c-Raf-1) phosphorylate and activate the substrates MEK1 and MEK2 dual specificity protein kinases, and MEK1/2 in turn phosphorylate and activate the ERK1 and ERK2 MAPKs. Activated

ERKs then phosphorylate and regulate the activities of a diverse spectrum of substrates that are estimated to comprise over 160 proteins [108].

The non-overlapping occurrence of *BRAF* and *RAS* mutations in melanoma and CRC cancer suggests functionally equivalent roles in Ras-mediated oncogenesis [109]. It is this phenomenon that has made the Raf-MEK-ERK MAPK pathway an attractive target for therapeutics against cancers harboring *RAS* mutations. Currently, multiple inhibitors of Raf and MEK kinases are in preclinical and clinical development [110] (Fig. 5 and Table 1). Below we focus on two Raf inhibitors (sorafenib and PLX4032) and one MEK inhibitor (AZD) that have undergone significant clinical evaluation.

Originally developed as an inhibitor of Raf-1 [111], sorafenib (BAY 43-9006) is a potent inhibitor of both wild type and mutant B-Raf kinases *in vitro*. From crystallographic analyses, it was determined that the inhibitor bound to the ATP-binding pocket and prevented kinase activation, preventing substrate binding and phosphorylation [112]. However, it was later reported that sorafenib is a potent kinase inhibitor of multiple cell surface receptors involved in tumor angiogenesis including VEGFR-2, VEGFR-3, PDGFR-β, Flt-3, c-Kit and FGFR-1[113].

Sorafenib, was approved in 2005 for the treatment of advanced renal cell carcinomas (RCC) and in 2007 for unresectable hepatocellular carcinoma (HCC). Since the frequency of *BRAF* and *RAS* mutations in these cancers is low [2, 114, 115], it is unclear whether Raf inhibition is the mechanism for antitumor activity of sorafenib. Instead, the anti-angiogenesis activity of sorafenib is most likely the basis for its efficacy in these cancers.

PLX4032 (now called Vemurafenib/RO5185426), a potent and selective inhibitor of mutant B-Raf, is currently in Phase I/II clinical evaluation. *In vitro* analysis against a panel of 65 non-Raf kinase showed PLX4032 is a highly selective inhibitor of B-Raf kinase activity, with an IC_{50} of 44 nM against V600E-mutant B-Raf [116]. Most of the kinases tested showed >100-fold higher IC_{50} than mutant Raf. In addition, cell culture experiments showed PLX4032 potently inhibited cell proliferation and MEK activation in melanoma and thyroid carcinoma cell lines harboring mutant B-Raf.

Recent cell culture and mouse model studies with PLX4032 found that it is effective against *BRAF* mutant tumor cell lines, but paradoxically, led to Raf activation in *RAS* mutant cell lines [117-119]. For *BRAF* mutant tumor cells, inhibition of ERK activation and growth were seen. In contrast, ERK activation rather than inactivation was seen in *RAS* mutant cell lines. The mechanistic explanation for this unexpected activity is based on earlier observations of a role for dimerization formation in Raf activation [119]. These studies found that paradoxical Raf pathway activation by PLX4032 and other Raf inhibitors requires Raf binding to mutationally activated Ras, but only when Raf activation is dependent on Ras. These findings potentially argue against the use of Raf inhibitors in *RAS* mutant tumors.

Consistent with these preclinical findings, recent Phase I/II evaluation of PLX4032 have shown dramatic anti-tumor activity with mutant *BRAF* melanomas. In a Phase I/II clinical trial, it was found that treatment of *BRAF* mutant metastatic melanoma with PLX4032 resulted in complete or partial tumor regression in the majority of patients [120]. However, only 52% of patients with the *BRAF* mutation responded to PLX4032 and for those patients who responded, drug resistance developed quickly, from 2-18 months and an average duration of response of only 6.2 months. Hence, while dramatic initial tumor regression is seen, which is far superior to what is seen with the standard of care (dasatinib), it remains to be determined whether overall patient survival time is improved with PLX4032 in ongoing Phase III clinical trials. Nevertheless, the significant initial tumor regression seen in a

majority of treated patients has stimulated debate regarding the necessity and ethics of randomized clinical trial design where the experimental arm is clearly showing more significant tumor response [121].

Additional studies of PLX4032 provide further insight into the mechanism of action of PLX4032. First, Bollag and colleagues determined that a near-complete suppression of ERK activation is apparently required for a clinical response [122]. They also observed that inhibition of cytosolic and not nuclear ERK better correlated with clinical efficacy. Second, two studies addressed possible mechanisms of tumor resistance [123, 124]. In contrast to the resistance mechanisms seen with BCR-Abl and the epidermal growth factor receptor, where mutations in the drug target impair drug binding, indirect mechanisms (e.g., *NRAS* mutation) were seen for PLX4032 resistance.

A number of potent and selective MEK1 and MEK2 inhibitors have been developed and are currently under clinical evaluation (Fig. 5). With being the only known catalytic substrates of Raf kinases, MEK1 and MEK2 are closely related dual-specificity kinases, capable of phosphorylating both serine/threonine and tyrosine residues of their substrates, p44 ERK1 and p42 ERK2 (Fig. 3). The fact that ERK1/2 are the only known substrates of MEK1/2, has led to perhaps an oversimplified perception of this signaling pathway, as a simply unidirectional linear signaling pathway. Often depicted as such a simple pathway downstream of Ras, it prompts the logical assumption that inhibition of this pathway at the level of Raf or MEK should be equivalent in blocking ERK activation by mutant Ras.

Of the many MEK1/2 inhibitors under development, there has been significant preclinical study of selumetinib (AZD6244). Selumetinib is an orally bioavailable benzimidazole derivative known to potently inhibit MEK1/2 *in vitro* and in cell-based assays [6, 125-127]. Like other MEK inhibitors, selumetinib is an ATP, non-competitive inhibitor, contributing to their very selective properties. Preclinical evaluation of selumetinib showed antitumor activity in several human xenograft models including colon, pancreas, breast, NSCLC and melanoma and has moved into clinical development. Cell culture studies suggest that MEK inhibitors may be effective against *BRAF* but not *RAS* mutant cancer cells [126-128]. These studies also reveal compensatory feedback mechanisms that may allow tumor cells to overcome the growth inhibitory consequences of MEK inhibition [129].

Recently, initial results of a first in human dose-ranging study to assess the pharmacokinetics, pharmacodynamics and toxicities of AZD6244 in patients with advanced solid tumors concluded that AZD6244 was well tolerated [130]. Currently, there are up to 43 completed and ongoing Phase I/II clinical trials evaluating AZD6244 as monotherapy or in combination with conventional cytotoxic drugs (clinicaltrials.gov).

Inhibitors of the PI3K-AKT-mTOR pathway

The second best-characterized Ras effectors are the catalytic subunits of the class I PI3Ks (p110α, β, δ and γ) which has been shown to be required for Ras transformation [131]. The PI3K-Akt-mTOR pathway is one of the most frequently altered signal transduction pathways in human cancers [103]. It has been implicated in multiple cellular functions such as proliferation and survival. PI3K converts phosphoinositides (4,5) bisphosphate to phosphoinositide (3,4,5)bisphosphate (PIP3). Membrane-associated PIP3 promotes the activation of diverse cytoplasmic signaling proteins, in particular, the Akt serine/threonine kinases, as well as other signaling proteins. In addition to activation by Ras, the PI3K-AKT pathway is deregulated by a variety of mechanisms in human cancers. This can include the loss of phosphatase and tensin homolog deleted from chromosome ten (*PTEN*), a dual specificity phosphatase and tumor suppressor gene, and is the primary negative regulator of this pathway. Hence, the components of this pathway have been attractive targets for anti-

cancer drug discovery, with many inhibitors of PI3K, AKT and mTOR currently under clinical trial analyses [132, 133] (Fig. 5 and Table 2). Some PI3K inhibitors are pan-class I PI3K inhibitors, others are isoform specific, and a number of PI3K inhibitors also have activity for the structurally similar catalytic domain of mTOR. Two mTOR inhibitors have already been approved for use for advanced renal cell cancer (temsirolimus and everolimus), which interestingly is a cancer with infrequent *RAS* mutational activation.

The importance of PI3K in Ras-initiated oncogenesis was shown in mouse models where a Ras binding impaired mutant of p110α impaired mutant HRAS-associated skin carcinoma formation and mutant KRAS-induced lung tumor formation [134]. However, there is limited evidence from cell culture and model studies that concurrent inhibition of the Raf-MEK-ERK and PI3K-AKT-mTOR pathways may be required for pharmacologic inhibition of mutant RAS-driven cancer growth. For example, in one study, mutant PIK3CA but not KRAS-driven lung tumor formation was responsive to NVP-BEZ235, a dual pan-PI3K and mammalian target of rapamycin (mTOR) inhibitor [135]. However, concurrent treatment with selumetinib did impair KRAS-induced tumor formation.

RalGEF-Ral pathway

Past studies have demonstrated that in a subset of tumors there is no correlation between *KRAS* mutation status and ERK activation [18, 136], suggesting that a Raf-independent function of Ras is important. Recent studies have demonstrated that additional effector pathways may play significant roles in Ras-mediated oncogenesis [101, 137, 138]. In particular, RalGEFs are activators of the highly related Ras-like RalA and RalB small GTPases (82% sequence identity) [139]. Similar to Ras, Ral GTPases function as GDP/GTP-regulated switches in signal transduction. Although there has been no evidence of mutations in the various components of this pathway, there is substantial evidence validating a role for Ral GTPases in multiple human cancers.

The RalGEF-Ral pathway was characterized initially to play a relatively minor role in Ras transformation of rodent fibroblasts [140]. However, subsequent studies by Counter and colleagues established a very significant role for this effector pathway in Ras transformation of human cells [141]. In particular, a significant role for Ral GTPases in pancreatic cancer has been established [18, 142]. Additionally, studies of bladder and prostate cancer support the role of RalGEF-Ral signaling in tumor invasion and metastasis [143, 144]. Finally mouse model studies showed that homozygous deletion of RalGDS (a RalGEF) caused resistance to Ras-induced skin tumor formation [107]. One RalGEF, Rgl2, was found overexpressed in pancreatic tumors and important for pancreatic cancer cell line growth and invasion *in vitro* [104]. Consequently, there is increasing interest in targeting this pathway for novel anti-Ras strategies for cancer treatment [145].

Recent studies support the possibility that inhibitors of GGTase-I (GGTI) can be effective inhibitors of Ral GTPases in oncogenesis [146]. Similar to Ras, Ral-GTPases terminate with a carboxyl-terminal CAAX motif. GGTaseI catalyzes addition of a geranylgeranyl isoprenoid to the cysteine residue of the CAAX motif, followed by modifications by the same Rce1 and Icmt enzymes involved in Ras processing. However, as with FTIs, since other GGTI substrates (e.g., RhoA, RhoC, Rac) are involved in oncogenesis, GTTI anti-tumor activity may also involve inhibition of non-Ral targets. Finally, a recent study identified RalA as a substrate for Aurora-A [147]. Since Aurora-A phosphorylation of RalA was important for Aurora-A-induced cellular motility and transformation. Additionally, the Aurora-A phosphorylation site (Ser-194) was shown to be essential for RalA-mediated anchorage-independent growth and tumor formation [148]. These studies suggest that inhibitors of Aurora-A, currently in Phase I clinical trial analyses may be effective inhibitors of RalA function.

Combination therapy

With only a few exceptions, conventional cytoxic cancer chemotherapy is most effective when applied as concurrent treatment with a cocktail of drugs with different mechanisms of activation. This approach is based on the fact that tumors are comprised of a genetically heterogeneous population where different subpopulations will exhibit resistance to different therapeutic approaches. Therefore, it is not surprising that an emerging paradigm is that molecularly targeted therapies will also be most effective when applied in combination. Finally, a second trend is that molecularly targeted therapies can enhance the effectiveness of cytotoxic drugs as well as radiation treatment. Below we summarize representative examples of these combination approaches. Other examples are summarized in Tables 1-3.

Concurrent inhibition of the Raf-MEK-ERK and the PI3K-AKT-mTOR pathways

That Ras can drive oncogenesis through multiple effectors suggests that effective inhibition of Ras will require concurrent inhibition of different effector networks. Consistent with this situation, several preclinical studies have found more effective anti-tumor activity with concurrent inhibition of Raf-MEK-ERK and PI3K-AKT-mTOR. For example, mutant *KRAS*-driven lung tumor formation in mice was inhibited only with concurrent treatment with the ARRY-142886 MEK inhibitor and the BEZ235 dual specificity pan-PI3K and mTOR inhibitor [135]. Pre-clinical studies have demonstrated synergistic inhibition with co-targeting Raf-MEK-ERK MAPK and PI3K-AKT-mTOR pathways with Raf and AKT/mTOR inhibitors in human melanoma cells [149]. Also, synergistic inhibition of proliferation have been observed with *in vitro* and *in vivo* models of hepatocellular carcinoma and non-small cell lung cancer using combinations of MEK and mTOR inhibitors [150, 151]. These and other observations provide the rationale for planned or ongoing clinical trials with combination inhibition of specific components of each of these two key Ras effector pathways (Table 3).

Another basis for the requirement for combination approaches is the induction of compensatory signaling mechanisms that overcome inhibition of a signaling pathway at a specific point. Such mechanisms appear to account for the resistance to Raf inhibition. As previously discussed, Raf inhibitors such as PLX4032 have been used in treating melanoma with the disappointing observation of drug resistance from 2-18 months after initial treatment [118]. One study found that resistance can occur through mutational activation of *NRAS* or upregulated expression of the PDGFRβ receptor tyrosine kinase [124]. Another study described upregulation of the Cot/Tpl2 serine/threonine kinase [123]. These mechanisms bypass PLX4032 inhibition by activating MEK-ERK signaling by alternative routes. These resistance mechanisms may then be overcome by concurrent treatment with inhibitors of these mechanisms, for example, by MEK inhibition. One clinical trial is utilizing the combined treatment with GSK2118436 and GSK1120212 for patients having *BRAF* mutant tumors treated previously with GSK2118436 alone and with no evidence for progression (Table 3).

Inhibition of the Raf-MEK-ERK MAPK and the PI3K-AKT-mTOR pathways with chemotherapy

Chemotherapy remains as the prime treatment strategy for combating many different types of cancers [152]. Chemotherapeutic drugs target various biological processes such as DNA replication and cell division in the cell (normal and tumor) which can result in numerous side effects [153]. Additionally, drug resistance to chemotherapy can develop over prolonged use as has been seen with doxorubicin and taxol [153]. It is this combination of side effects and drug resistance to chemotherapy that argues for the need to identify better and alternative strategies for treating cancer.

Although drug resistance occurs with chemotherapeutic drugs as well as small molecule inhibitors in cancer, studies have been conducted combining both types of drugs for determining potential synergistic growth inhibition effects against tumor cells with less toxicity to the patient. In a pre-clinical study combining paclitaxel (taxol) and MEK inhibitors in ovarian carcinoma cell lines, results demonstrated enhanced apoptosis and growth inhibition [154]. In a phase II clinical trial conducted in patients with advanced hepatocellular carcinoma, the combination of sorafenib (Raf inhibitor) and doxorubicin improved progression-free and overall survival [155]. In a completed second phase II trial, the progression-free survival of sorafenib and tegafur/uracil (UFUR) for the treatment of advanced or metastatic hepatocellular carcinoma was studied (http://clinicaltrials.gov).

In addition to the advantanges of combining chemotherapy and small molecule inhibitors for treating cancer, there are also challenges. Combinations of MEK inhibitors and chemotherapy can have antagonistic results. Studies have shown that chemotherapeutic drugs can activate the Raf-MEK-ERK MAPK pathway through diverse mechanisms. Doxorubicin has been shown to activate both p53 and calcium calmodulin kinase which can activate this pathway [153]. Also, taxol has been shown in studies to stimulate activation of this pathway [156]. MEK inhibitors in combination with betulinic acid, a drug toxic for melanoma cells, prevented an increase in betunlinic acid-induced apoptosis *in vitro* [157]. Another challenge with combining chemotherapy and inhibitors is the time schedule for adding each drug regiment. The order of administration of the chemotherapeutic drugs and inhibitors can determine a synergistic or antagonistic outcome.

Inhibition of the Raf-MEK-ERK MAPK and the PI3K-AKT-mTOR pathways with radiotherapy

Although radiation is one of the common methods for treating cancers, many advanced cancers are radioresistant. Various inhibitors have been evaluated for their potential to serve as a radiosensitizer. In one study, selumetinib (MEK inhibitor) pre-treatment radiosensitized lung, prostate, and pancreatic cancer cells *in vitro* and *in vivo* [158]. A mitotic catastrophe event (due to radiation-induced G2 cell cycle checkpoint activation abrogation) was found to be increased in cells receiving both the MEK inhibitor and radiation versus the inhibitor alone. In addition to the the Raf-MEK-ERK MAPK pathway, PI3K-AKT-mTOR inhibitors have been demonstrated to radiosensitize the tumor vasculature both *in vitro* and *in vivo* [159, 160]. Also, mTOR and radiation have been shown to be instrumental for the regulation of autophagy [160, 161]. The combination of mTOR inhibitors and radiation may be beneficial inducing autophagy as it relates to cancer treatment.

Oncogene addition and synthetic lethality: unbiased searchs for novel anti-Ras therapies

In light of the current lack of success in developing clinically useful anti-Ras drugs, recent studies have taken advantage of *KRAS* oncogene addiction to search for synthetic lethal partners of mutant *KRAS*. Utilizing RNA interference (RNAi) technologies, large-scale interfering RNA screens have been applied to take a functional and unbiased approach to identify therapeutic targets for anti-Ras inhibition [162-164]. Perturbation of these genes may result in oncogene-specific “synthetic lethal” genetic interactions that could provide new therapeutic opportunities.

These screens are based on the concept of synthetic lethality, in which two genes are defined as synthetically lethal if mutation of either gene alone is compatible with viability but the simultaneous mutation of both genes leads to death [165]. Mutationally-activated *RAS* genes thus represent one gene and RNAi-mediated ablation in cancer cells of the expression of a second gene provides the second hit. Synthetic lethal interactions can involve genes within the same pathway, genes within parallel pathways that cooperate with respect to an essential function, or genes within distant pathways that become functionally connected

because of the response of the cell to a specific perturbation. Since normal cells lack mutant *RAS*, genes identified in this manner should in principle be selectively lethal for tumors but not normal cells.

In one study which included a limited RNAi library targeting 1,011 genes with a focus on protein kinases, it was found that cells that were dependent on mutant *KRAS* genetically interacted with the STK33 serine/threonine kinase as a synthetic lethal partner irrespective of the tissue of origin, whereas STK33 was not required by *KRAS*-independent cells [163]. STK33 promotes cancer cell viability in a kinase activity-dependent manner by regulating the suppression of mitochondrial apoptosis mediated through S6K1-induced inactivation of the death agonist BAD selectively in mutant *KRAS*-dependent cells. The synthetic lethality functional screen was important, since there was no alteration in *STK33* expression, no mutations, and no transforming activity of *STK33* was detected. Hence, with the classical analyses of cancer-causing genes, *STK33* would have not been identified. In a second study that included a genome-wide RNAi screen, identification of synthetic lethal interaction partners with the *KRAS* oncogene was done targeting 32,293 unique human transcripts [162]. The genes identified encode a functionally diverse set of proteins that regulate several biological processes, especially mitotic functions. One of these genes that was characterized in this study was Polo-like kinase 1 (PLK1), a serine/threonine kinase that plays a key role in mitosis. PLK1 is a component of the anaphase-promoting complex/cyclosome, and the proteasome that, when inhibited, results in prometaphase accumulation and the subsequent death of Ras mutant cells. Results from this study demonstrated that reduced expression of genes in this pathway correlated with increased survival of patients bearing tumors with a Ras transcriptional signature. Pharmacological inhibitors of PLK1 and other mitotic proteins can selectively impair the viability of Ras mutant cells and be exploited fro therapeutic purposes.

A third study of a limited RNAi screen to identify synthetic lethal partners of mutant *KRAS* found the non-canonical IκB kinase, TANK-binding kinase 1 (TBK1) [164]. TBK1 is a serine/threonine kinase that can activate the NF-kappaB transcription factor and support cell survival. TBK1 was selectively essential in cells that harbor mutant *KRAS*. Interestingly, TBK1 was identified previously as a key downstream effector of RalB-dependent tumor cell survival [166]. Suppression of TBK1 induced apoptosis specifically in human cancer cell lines that depend on oncogenic *KRAS* expression. In conclusion, the synthetic lethal screening identified TBK1 and NF-κB signaling essential in *KRAS* mutant tumors.

In a fourth study, instead of using RNAi screening to identify synthetic lethal screening partners with mutant *KRAS* as described in the previous three studies, the focus was to identify a gene signature for *KRAS* dependency [167]. Comparing two classes of cancer cells that do or do not require K-Ras to maintain viability revealed a gene expression signature in K-Ras-dependent cells. Two of the genes that were found to encode pharmacologically tractable proteins were the Syk and Ron tyrosine kinases. To validate this screen, the study demonstrated that *KRAS* mutant tumor cell lines were more sensitive to induction of apoptosis by treatment with a small molecule inhibitor of Syk.

While further validation of these synthetic lethal partners of mutant *KRAS* are needed, these studies support the potential usefulness of synthetic lethality screens in identifying novel targets and directions for anti-Ras drug discovery.However, caution for this approach is also raised by a recent study that utilized both genetic and pharmacologic inhibition of STK33 and reached a conclusion which conflicts with the earlier library screening study [163]. Instead, they concluded that STK33 function is not essential for *KRAS* mutant-dependent human tumor cells [168].

Future Perspectives

Despite the limited success from almost three decades of anti-Ras research and drug discovery, substantial progress has been made in understanding Ras biology and function that will shorten the final path to clinically effective anti-Ras drugs. First, a bitter lesson learned from the development of farnesyltransferase inhibitors is the fact that the three *RAS* genes do not encode functionally identical proteins. This has resulted in a shift in research and drug discovery efforts which are now focused on K-Ras. Second, with the unexpected findings made with Raf and MEK inhibitors, a better appreciation for the complex and dynamic nature of signaling networks has been made, where the Raf-MEK-ERK cascade is not a simple linear pathway. Understanding how the cancer cell can adapt to inhibition of one specific signaling protein will help focus future efforts on approaches that target specific signaling networks at multiple levels. Third, while the limitations of the classical tumor cell line xenograft tumor models have long been appreciated, early observations made with newer mouse models will accelerate the transition to greater reliance on genetically-engineered mouse models of cancer to more accurately predict drug response in the patient. Finally, the continued development and application of genome-wide unbiased functional screening efforts will lead to novel and unexpected new directions for anti-Ras drug discovery. The fact that these efforts have identified protein kinases may render Ras a more tractable target. As we stay optimistic about Ras becoming a “tractable” druggable target in the future, one has to keep in the mind the well known adage, “Nothing worth having comes easy”.

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Bibliography

1. Cox A, Der CJ. Ras history:the saga continues. Small GTPases. 2010; 1:1–27. [PubMed: 21686116]
2. Karnoub AE, Weinberg RA. Ras oncogenes: split personalities. Nat Rev Mol Cell Biol. 2008; 9(7): 517–531. [PubMed: 18568040]
3. Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. CA Cancer J Clin. 2010; 60(5):277–300. [PubMed: 20610543]
4. Hruban RH, Wilentz RE, Kern SE. Genetic progression in the pancreatic ducts. Am J Pathol. 2000; 156(6):1821–1825. [PubMed: 10854204]
5. Hezel AF, Kimmelman AC, Stanger BZ, Bardeesy N, Depinho RA. Genetics and biology of pancreatic ductal adenocarcinoma. Genes Dev. 2006; 20(10):1218–1249. [PubMed: 16702400]
6. Yeh JJ, Der CJ. Targeting signal transduction in pancreatic cancer treatment. Expert Opin Ther Targets. 2007; 11(5):673–694. [PubMed: 17465725]
7. Almoguera C, Shibata D, Forrester K, Martin J, Arnheim N, Perucho M. Most human carcinomas of the exocrine pancreas contain mutant c-K-ras genes. Cell. 1988; 53(4):549–554. [PubMed: 2453289]
8. Jones S, Zhang X, Parsons DW, et al. Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. Science. 2008; 321(5897):1801–1806. [PubMed: 18772397] [K-Ras signaling was found to be genetically altered in 100% of pancreatic tumors analyzed, confirming the importance of K-Ras as a potential therapeutic target in pancreatic cancer.]
9. Forrester K, Almoguera C, Han K, Grizzle WE, Perucho M. Detection of high incidence of K-ras oncogenes during human colon tumorigenesis. Nature. 1987; 327(6120):298–303. [PubMed: 2438556]
10. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. Cell. 1990; 61(5):759–767. [PubMed: 2188735]

11. Wood LD, Parsons DW, Jones S, et al. The genomic landscapes of human breast and colorectal cancers. Science. 2007; 318(5853):1108–1113. [PubMed: 17932254]
12. Ding L, Getz G, Wheeler DA, et al. Somatic mutations affect key pathways in lung adenocarcinoma. Nature. 2008; 455(7216):1069–1075. [PubMed: 18948947]
13. Vogelstein B, Fearon ER, Hamilton SR, et al. Genetic alterations during colorectal-tumor development. N Engl J Med. 1988; 319(9):525–532. [PubMed: 2841597]
14. Schaeffer BK, Glasner S, Kuhlmann E, Myles JL, Longnecker DS. Mutated c-K-ras in small pancreatic adenocarcinomas. Pancreas. 1994; 9(2):161–165. [PubMed: 8190717]
15. Shirasawa S, Furuse M, Yokoyama N, Sasazuki T. Altered growth of human colon cancer cell lines disrupted at activated Ki-ras. Science. 1993; 260(5104):85–88. [PubMed: 8465203]
16. Chin L, Tam A, Pomerantz J, et al. Essential role for oncogenic Ras in tumour maintenance. Nature. 1999; 400(6743):468–472. [PubMed: 10440378]
17. Brummelkamp TR, Bernards R, Agami R. Stable suppression of tumorigenicity by virus-mediated RNA interference. Cancer Cell. 2002; 2(3):243–247. [PubMed: 12242156]
18. Lim KH, Baines AT, Fiordalisi JJ, et al. Activation of RalA is critical for Ras-induced tumorigenesis of human cells. Cancer Cell. 2005; 7(6):533–545. [PubMed: 15950903]
19. Herrmann C, Horn G, Spaargaren M, Wittinghofer A. Differential interaction of the ras family GTP-binding proteins H-Ras, Rap1A, and R-Ras with the putative effector molecules Raf kinase and Ral-guanine nucleotide exchange factor. J Biol Chem. 1996; 271(12):6794–6800. [PubMed: 8636102]
20. Fehrenbacher N, Bar-Sagi D, Philips M. Ras/MAPK signaling from endomembranes. Mol Oncol. 2009; 3(4):297–307. [PubMed: 19615955]
21. Sebti SM, Der CJ. Opinion: Searching for the elusive targets of farnesyltransferase inhibitors. Nat Rev Cancer. 2003; 3(12):945–951. [PubMed: 14737124]
22. Basso AD, Kirschmeier P, Bishop WR. Lipid posttranslational modifications. Farnesyl transferase inhibitors. J Lipid Res. 2006; 47(1):15–31. [PubMed: 16278491]
23. Rowinsky EK. Lately, it occurs to me what a long, strange trip it's been for the farnesyltransferase inhibitors. J Clin Oncol. 2006; 24(19):2981–2984. [PubMed: 16769983]
24. Willumsen BM, Christensen A, Hubbert NL. Papageorge AG and Lowy DR: The p21 ras C-terminus is required for transformation and membrane association. Nature. 1984; 310(5978):583–586. [PubMed: 6087162]
25. Hancock JF, Magee AI, Childs JE, Marshall CJ. All ras proteins are polyisoprenylated but only some are palmitoylated. Cell. 1989; 57(7):1167–1177. [PubMed: 2661017]
26. Jackson JH, Cochrane CG, Bourne JR, Solski PA, Buss JE, Der CJ. Farnesol modification of Kirsten-ras exon 4B protein is essential for transformation. Proc Natl Acad Sci U S A. 1990; 87(8):3042–3046. [PubMed: 2183224]
27. Sinensky M, Beck LA, Leonard S, Evans R. Differential inhibitory effects of lovastatin on protein isoprenylation and sterol synthesis. J Biol Chem. 1990; 265(32):19937–19941. [PubMed: 2246270]
28. Reiss Y, Goldstein JL, Seabra MC, Casey PJ, Brown MS. Inhibition of purified p21ras farnesyl:protein transferase by Cys-AAX tetrapeptides. Cell. 1990; 62(1):81–88. [PubMed: 2194674]
29. Reid TS, Long SB, Beese LS. Crystallographic analysis reveals that anticancer clinical candidate L-778,123 inhibits protein farnesyltransferase and geranylgeranyltransferase-I by different binding modes. Biochemistry. 2004; 43(28):9000–9008. [PubMed: 15248757]
30. James GL, Goldstein JL, Brown MS, et al. Benzodiazepine peptidomimetics: potent inhibitors of Ras farnesylation in animal cells. Science. 1993; 260(5116):1937–1942. [PubMed: 8316834]
31. Kohl NE, Mosser SD, deSolms SJ, et al. Selective inhibition of ras-dependent transformation by a farnesyltransferase inhibitor. Science. 1993; 260(5116):1934–1937. [PubMed: 8316833]
32. Kohl NE, Omer CA, Conner MW, et al. Inhibition of farnesyltransferase induces regression of mammary and salivary carcinomas in ras transgenic mice. Nat Med. 1995; 1(8):792–797. [PubMed: 7585182]

33. Sepp-Lorenzino L, Ma Z, Rands E, et al. A peptidomimetic inhibitor of farnesyl:protein transferase blocks the anchorage-dependent and -independent growth of human tumor cell lines. Cancer Res. 1995; 55(22):5302–5309. [PubMed: 7585592]
34. James G, Goldstein JL, Brown MS. Resistance of K-RasBV12 proteins to farnesyltransferase inhibitors in Rat1 cells. Proc Natl Acad Sci U S A. 1996; 93(9):4454–4458. [PubMed: 8633088]
35. Whyte DB, Kirschmeier P, Hockenberry TN, et al. K- and N-Ras are geranylgeranylated in cells treated with farnesyl protein transferase inhibitors. J Biol Chem. 1997; 272(22):14459–14464. [PubMed: 9162087]
36. Rowell CA, Kowalczyk JJ, Lewis MD, Garcia AM. Direct demonstration of geranylgeranylation and farnesylation of Ki-Ras in vivo. J Biol Chem. 1997; 272(22):14093–14097. [PubMed: 9162034] [Provided evidence why farnesyltransferase inhibitors were not effective against cancers dependent on K-ras signaling.]
37. Lerner EC, Zhang TT, Knowles DB, Qian Y, Hamilton AD, Sebti SM. Inhibition of the prenylation of K-Ras, but not H- or N-Ras, is highly resistant to CAAX peptidomimetics and requires both a farnesyltransferase and a geranylgeranyltransferase I inhibitor in human tumor cell lines. Oncogene. 1997; 15(11):1283–1288. [PubMed: 9315095]
38. Cox AD, Hisaka MM, Buss JE, Der CJ. Specific isoprenoid modification is required for function of normal, but not oncogenic, Ras protein. Mol Cell Biol. 1992; 12(6):2606–2615. [PubMed: 1375323]
39. Hancock JF, Cadwallader K, Marshall CJ. Methylation and proteolysis are essential for efficient membrane binding of prenylated p21K-ras(B). Embo J. 1991; 10(3):641–646. [PubMed: 2001678]
40. Cohen SJ, Ho L, Ranganathan S, et al. Phase II and pharmacodynamic study of the farnesyltransferase inhibitor R115777 as initial therapy in patients with metastatic pancreatic adenocarcinoma. J Clin Oncol. 2003; 21(7):1301–1306. [PubMed: 12663718]
41. Van Cutsem E, van de Velde H, Karasek P, et al. Phase III trial of gemcitabine plus tipifarnib compared with gemcitabine plus placebo in advanced pancreatic cancer. J Clin Oncol. 2004; 22(8):1430–1438. [PubMed: 15084616]
42. Macdonald JS, McCoy S, Whitehead RP, et al. A phase II study of farnesyl transferase inhibitor R115777 in pancreatic cancer: a Southwest oncology group (SWOG 9924) study. Invest New Drugs. 2005; 23(5):485–487. [PubMed: 16133800]
43. Basso AD, Mirza A, Liu G, Long BJ, Bishop WR, Kirschmeier P. The farnesyl transferase inhibitor (FTI) SCH66336 (lonafarnib) inhibits Rheb farnesylation and mTOR signaling. Role in FTI enhancement of taxane and tamoxifen anti-tumor activity. J Biol Chem. 2005; 280(35):31101–31108. [PubMed: 16006564]
44. Ellis CA, Vos MD, Howell H, Vallecorsa T, Fults DW, Clark GJ. Rig is a novel Ras-related protein and potential neural tumor suppressor. Proc Natl Acad Sci U S A. 2002; 99(15):9876–9881. [PubMed: 12107278]
45. Luo RZ, Fang X, Marquez R, et al. ARHI is a Ras-related small G-protein with a novel N-terminal extension that inhibits growth of ovarian and breast cancers. Oncogene. 2003; 22(19):2897–2909. [PubMed: 12771940]
46. Elam C, Hesson L, Vos MD, et al. RRP22 is a farnesylated, nucleolar, Ras-related protein with tumor suppressor potential. Cancer Res. 2005; 65(8):3117–3125. [PubMed: 15833841]
47. Yang SH, Bergo MO, Toth JI, et al. Blocking protein farnesyltransferase improves nuclear blebbing in mouse fibroblasts with a targeted Hutchinson-Gilford progeria syndrome mutation. Proc Natl Acad Sci U S A. 2005; 102(29):10291–10296. [PubMed: 16014412]
48. Mallampalli MP, Huyer G, Bendale P, Gelb MH, Michaelis S. Inhibiting farnesylation reverses the nuclear morphology defect in a HeLa cell model for Hutchinson-Gilford progeria syndrome. Proc Natl Acad Sci U S A. 2005; 102(40):14416–14421. [PubMed: 16186497]
49. Capell BC, Erdos MR, Madigan JP, et al. Inhibiting farnesylation of progerin prevents the characteristic nuclear blebbing of Hutchinson-Gilford progeria syndrome. Proc Natl Acad Sci U S A. 2005; 102(36):12879–12884. [PubMed: 16129833]
50. Glynn MW, Glover TW. Incomplete processing of mutant lamin A in Hutchinson-Gilford progeria leads to nuclear abnormalities, which are reversed by farnesyltransferase inhibition. Hum Mol Genet. 2005; 14(20):2959–2969. [PubMed: 16126733]

51. Fong LG, Frost D, Meta M, et al. A protein farnesyltransferase inhibitor ameliorates disease in a mouse model of progeria. Science. 2006; 311(5767):1621–1623. [PubMed: 16484451]
52. Davies BS, Fong LG, Yang SH, Coffinier C, Young SG. The posttranslational processing of prelamin A and disease. Annu Rev Genomics Hum Genet. 2009; 10:153–174. [PubMed: 19453251]
53. Lerner EC, Qian Y, Blaskovich MA, et al. Ras CAAX peptidomimetic FTI-277 selectively blocks oncogenic Ras signaling by inducing cytoplasmic accumulation of inactive Ras-Raf complexes. J Biol Chem. 1995; 270(45):26802–26806. [PubMed: 7592920]
54. Miquel K, Pradines A, Sun J, et al. GGTI-298 induces G0-G1 block and apoptosis whereas FTI-277 causes G2-M enrichment in A549 cells. Cancer Res. 1997; 57(10):1846–1850. [PubMed: 9157972]
55. Vogt A, Sun J, Qian Y, Hamilton AD, Sebti SM. The geranylgeranyltransferase-I inhibitor GGTI-298 arrests human tumor cells in G0/G1 and induces p21(WAF1/CIP1/SDI1) in a p53-independent manner. J Biol Chem. 1997; 272(43):27224–27229. [PubMed: 9341167]
56. Liu M, Sjogren AK, Karlsson C, et al. Targeting the protein prenyltransferases efficiently reduces tumor development in mice with K-RAS-induced lung cancer. Proc Natl Acad Sci U S A. 2010; 107(14):6471–6476. [PubMed: 20308544]
57. Sjogren AK, Andersson KM, Khan O, Olofsson FJ, Karlsson C, Bergo MO. Inactivating GGTase-I reduces disease phenotypes in a mouse model of K-RAS-induced myeloproliferative disease. Leukemia. 2011; 25(1):186–189. [PubMed: 20975663]
58. Lobell RB, Liu D, Buser CA, et al. Preclinical and clinical pharmacodynamic assessment of L-778,123, a dual inhibitor of farnesyl:protein transferase and geranylgeranyl:protein transferase type-I. Mol Cancer Ther. 2002; 1(9):747–758. [PubMed: 12479371]
59. Winter-Vann AM, Casey PJ. Post-prenylation-processing enzymes as new targets in oncogenesis. Nat Rev Cancer. 2005; 5(5):405–412. [PubMed: 15864282]
60. Kato K, Der CJ, Buss JE. Prenoids and palmitate: lipids that control the biological activity of Ras proteins. Semin Cancer Biol. 1992; 3(4):179–188. [PubMed: 1421162]
61. Kim E, Ambroziak P, Otto JC, et al. Disruption of the mouse Rce1 gene results in defective Ras processing and mislocalization of Ras within cells. J Biol Chem. 1999; 274(13):8383–8390. [PubMed: 10085069]
62. Bergo MO, Leung GK, Ambroziak P, Otto JC, Casey PJ, Young SG. Targeted inactivation of the isoprenylcysteine carboxyl methyltransferase gene causes mislocalization of K-Ras in mammalian cells. J Biol Chem. 2000; 275(23):17605–17610. [PubMed: 10747846]
63. Wahlstrom AM, Cutts BA, Liu M, et al. Inactivating Icmt ameliorates K-RAS-induced myeloproliferative disease. Blood. 2008; 112(4):1357–1365. [PubMed: 18502828]
64. Wahlstrom AM, Cutts BA, Karlsson C, et al. Rce1 deficiency accelerates the development of K-RAS-induced myeloproliferative disease. Blood. 2007; 109(2):763–768. [PubMed: 16973961]
65. Manandhar SP, Hildebrandt ER, Schmidt WK. Small-molecule inhibitors of the Rce1p CaaX protease. J Biomol Screen. 2007; 12(7):983–993. [PubMed: 17942791]
66. Porter SB, Hildebrandt ER, Breevoort SR, Mokry DZ, Dore TM, Schmidt WK. Inhibition of the CaaX proteases Rce1p and Ste24p by peptidyl (acyloxy)methyl ketones. Biochim Biophys Acta. 2007; 1773(6):853–862. [PubMed: 17467817]
67. Wang M, Tan W, Zhou J, et al. A small molecule inhibitor of isoprenylcysteine carboxymethyltransferase induces autophagic cell death in PC3 prostate cancer cells. J Biol Chem. 2008; 283(27):18678–18684. [PubMed: 18434300]
68. Winter-Vann AM, Kamen BA, Bergo MO, et al. Targeting Ras signaling through inhibition of carboxyl methylation: an unexpected property of methotrexate. Proc Natl Acad Sci U S A. 2003; 100(11):6529–6534. [PubMed: 12750467]
69. Buchanan MS, Carroll AR, Addepalli R, Avery VM, Hooper JN, Quinn RJ. Natural products, stylissadines A and B, specific antagonists of the P2X7 receptor, an important inflammatory target. J Org Chem. 2007; 72(7):2309–2317. [PubMed: 17315930]
70. Buchanan MS, Carroll AR, Fechner GA, et al. Aplysamine 6, an alkaloidal inhibitor of Isoprenylcysteine carboxyl methyltransferase from the sponge Pseudoceratina sp. J Nat Prod. 2008; 71(6):1066–1067. [PubMed: 18393464]

71. Buchanan MS, Carroll AR, Fechner GA, et al. Small-molecule inhibitors of the cancer target, isoprenylcysteine carboxyl methyltransferase, from Hovea parvicalyx. Phytochemistry. 2008; 69(9):1886–1889. [PubMed: 18466935]
72. Ducker CE, Griffel LK, Smith RA, et al. Discovery and characterization of inhibitors of human palmitoyl acyltransferases. Mol Cancer Ther. 2006; 5(7):1647–1659. [PubMed: 16891450]
73. Draper JM, Smith CD. Palmitoyl acyltransferase assays and inhibitors (Review). Mol Membr Biol. 2009; 26(1):5–13. [PubMed: 19152182]
74. Marshall CJ. Protein prenylation: a mediator of protein-protein interactions. Science. 1993; 259(5103):1865–1866. [PubMed: 8456312]
75. Drugan JK, Khosravi-Far R, White MA, et al. Ras interaction with two distinct binding domains in Raf-1 may be required for Ras transformation. J Biol Chem. 1996; 271(1):233–237. [PubMed: 8550565]
76. Niv H, Gutman O, Henis YI, Kloog Y. Membrane interactions of a constitutively active GFP-Ki-Ras 4B and their role in signaling. Evidence from lateral mobility studies. J Biol Chem. 1999; 274(3):1606–1613. [PubMed: 9880539]
77. Luo Z, Diaz B, Marshall MS, Avruch J. An intact Raf zinc finger is required for optimal binding to processed Ras and for ras-dependent Raf activation in situ. Mol Cell Biol. 1997; 17(1):46–53. [PubMed: 8972184]
78. Williams JG, Drugan JK, Yi GS, Clark GJ, Der CJ, Campbell SL. Elucidation of binding determinants and functional consequences of Ras/Raf-cysteine-rich domain interactions. J Biol Chem. 2000; 275(29):22172–22179. [PubMed: 10777480]
79. Marciano D, Ben-Baruch G, Marom M, Egozi Y, Haklai R, Kloog Y. Farnesyl derivatives of rigid carboxylic acids-inhibitors of ras-dependent cell growth. J Med Chem. 1995; 38(8):1267–1272. [PubMed: 7731012]
80. Marom M, Haklai R, Ben-Baruch G, Marciano D, Egozi Y, Kloog Y. Selective inhibition of Ras-dependent cell growth by farnesylthiosalisylic acid. J Biol Chem. 1995; 270(38):22263–22270. [PubMed: 7673206]
81. Boufaied N, Wioland MA, Falardeau P, Gourdeau H. TLN-4601, a novel anticancer agent, inhibits Ras signaling post Ras prenylation and before MEK activation. Anticancer Drugs. 2010; 21(5): 543–552. [PubMed: 20220516]
82. Campbell PM, Boufaied N, Fiordalisi JJ, et al. TLN-4601 suppresses growth and induces apoptosis of pancreatic carcinoma cells through inhibition of Ras-ERK MAPK signaling. J Mol Signal. 2010; 5:18. [PubMed: 21044336]
83. Blum R, Cox AD, Kloog Y. Inhibitors of chronically active ras: potential for treatment of human malignancies. Recent Pat Anticancer Drug Discov. 2008; 3(1):31–47. [PubMed: 18289122]
84. Paz A, Haklai R, Elad-Sfadia G, Ballan E, Kloog Y. Galectin-1 binds oncogenic H-Ras to mediate Ras membrane anchorage and cell transformation. Oncogene. 2001; 20(51):7486–7493. [PubMed: 11709720]
85. Elad-Sfadia G, Haklai R, Ballan E, Gabius HJ, Kloog Y. Galectin-1 augments Ras activation and diverts Ras signals to Raf-1 at the expense of phosphoinositide 3-kinase. J Biol Chem. 2002; 277(40):37169–37175. [PubMed: 12149263]
86. Haklai R, Weisz MG, Elad G, et al. Dislodgment and accelerated degradation of Ras. Biochemistry. 1998; 37(5):1306–1314. [PubMed: 9477957]
87. Jansen B, Schlagbauer-Wadl H, Kahr H, et al. Novel Ras antagonist blocks human melanoma growth. Proc Natl Acad Sci U S A. 1999; 96(24):14019–14024. [PubMed: 10570191]
88. Gana-Weisz M, Halaschek-Wiener J, Jansen B, Elad G, Haklai R, Kloog Y. The Ras inhibitor S-trans,trans-farnesylthiosalicylic acid chemosensitizes human tumor cells without causing resistance. Clin Cancer Res. 2002; 8(2):555–565. [PubMed: 11839677]
89. Weisz B, Giehl K, Gana-Weisz M, et al. A new functional Ras antagonist inhibits human pancreatic tumor growth in nude mice. Oncogene. 1999; 18(16):2579–2588. [PubMed: 10353601]
90. Blum R, Jacob-Hirsch J, Amariglio N, Rechavi G, Kloog Y. Ras inhibition in glioblastoma down-regulates hypoxia-inducible factor-1alpha, causing glycolysis shutdown and cell death. Cancer Res. 2005; 65(3):999–1006. [PubMed: 15705901]

91. Yaari S, Jacob-Hirsch J, Amariglio N, Haklai R, Rechavi G, Kloog Y. Disruption of cooperation between Ras and MycN in human neuroblastoma cells promotes growth arrest. Clin Cancer Res. 2005; 11(12):4321–4330. [PubMed: 15958613]
92. Barkan B, Starinsky S, Friedman E, Stein R, Kloog Y. The Ras inhibitor farnesylthiosalicylic acid as a potential therapy for neurofibromatosis type 1. Clin Cancer Res. 2006; 12(18):5533–5542. [PubMed: 17000690]
93. Goldberg L, Kloog Y. A Ras inhibitor tilts the balance between Rac and Rho and blocks phosphatidylinositol 3-kinase-dependent glioblastoma cell migration. Cancer Res. 2006; 66(24): 11709–11717. [PubMed: 17178866]
94. Shalom-Feuerstein R, Lindenboim L, Stein R, Cox AD, Kloog Y. Restoration of sensitivity to anoikis in Ras-transformed rat intestinal epithelial cells by a Ras inhibitor. Cell Death Differ. 2004; 11(2):244–247. [PubMed: 14576773]
95. Reif S, Weis B, Aeed H, et al. The Ras antagonist, farnesylthiosalicylic acid (FTS), inhibits experimentally-induced liver cirrhosis in rats. J Hepatol. 1999; 31(6):1053–1061. [PubMed: 10604579]
96. Haklai R, Elad-Sfadia G, Egozi Y, Kloog Y. Orally administered FTS (salirasib) inhibits human pancreatic tumor growth in nude mice. Cancer Chemother Pharmacol. 2008; 61(1):89–96. [PubMed: 17909812]
97. McMahon LP, Yue W, Santen RJ, Lawrence JC Jr. Farnesylthiosalicylic acid inhibits mammalian target of rapamycin (mTOR) activity both in cells and in vitro by promoting dissociation of the mTOR-raptor complex. Mol Endocrinol. 2005; 19(1):175–183. [PubMed: 15459249]
98. Yue W, Wang J, Li Y, Fan P, Santen RJ. Farnesylthiosalicylic acid blocks mammalian target of rapamycin signaling in breast cancer cells. Int J Cancer. 2005; 117(5):746–754. [PubMed: 15957161]
99. Hanker AB, Mitin N, Wilder RS, et al. Differential requirement of CAAX-mediated posttranslational processing for Rheb localization and signaling. Oncogene. 2009; 29(3):380–391. [PubMed: 19838215]
100. Bustinza-Linares E, Kurzrock R, Tsimberidou AM. Salirasib in the treatment of pancreatic cancer. Future Oncol. 2010; 6(6):885–891. [PubMed: 20528225]
101. Repasky GA, Chenette EJ, Der CJ. Renewing the conspiracy theory debate: does Raf function alone to mediate Ras oncogenesis? Trends Cell Biol. 2004; 14(11):639–647. [PubMed: 15519853]
102. Roberts PJ, Der CJ. Targeting the Raf-MEK-ERK mitogen-activated protein kinase cascade for the treatment of cancer. Oncogene. 2007; 26(22):3291–3310. [PubMed: 17496923]
103. Wong KK, Engelman JA, Cantley LC. Targeting the PI3K signaling pathway in cancer. Curr Opin Genet Dev. 2010; 20(1):87–90. [PubMed: 20006486]
104. Vigil D, Martin TD, Williams F, Yeh JJ, Campbell SL, Der CJ. Aberrant overexpression of the Rgl2 Ral small GTPase-specific guanine nucleotide exchange factor promotes pancreatic cancer growth through Ral-dependent and Ral-independent mechanisms. J Biol Chem. 2010; 285(45): 34729–34740. [PubMed: 20801877]
105. Malliri A, van der Kammen RA, Clark K, van der Valk M, Michiels F, Collard JG. Mice deficient in the Rac activator Tiam1 are resistant to Ras-induced skin tumours. Nature. 2002; 417(6891): 867–871. [PubMed: 12075356]
106. Bai Y, Edamatsu H, Maeda S, et al. Crucial role of phospholipase Cepsilon in chemical carcinogen-induced skin tumor development. Cancer Res. 2004; 64(24):8808–8810. [PubMed: 15604236]
107. Gonzalez-Garcia A, Pritchard CA, Paterson HF, Mavria G, Stamp G, Marshall CJ. RalGDS is required for tumor formation in a model of skin carcinogenesis. Cancer Cell. 2005; 7(3):219–226. [PubMed: 15766660]
108. Yoon S, Seger R. The extracellular signal-regulated kinase: multiple substrates regulate diverse cellular functions. Growth Factors. 2006; 24(1):21–44. [PubMed: 16393692]
109. Dhomen N, Marais R. New insight into BRAF mutations in cancer. Curr Opin Genet Dev. 2007; 17(1):31–39. [PubMed: 17208430]

110. Smith RA, Dumas J, Adnane L, Wilhelm SM. Recent advances in the research and development of RAF kinase inhibitors. Curr Top Med Chem. 2006; 6(11):1071–1089. [PubMed: 16842147]
111. Lyons JF, Wilhelm S, Hibner B, Bollag G. Discovery of a novel Raf kinase inhibitor. Endocr Relat Cancer. 2001; 8(3):219–225. [PubMed: 11566613]
112. Wan PT, Garnett MJ, Roe SM, et al. Mechanism of activation of the RAF-ERK signaling pathway by oncogenic mutations of B-RAF. Cell. 2004; 116(6):855–867. [PubMed: 15035987]
113. Wilhelm SM, Carter C, Tang L, et al. BAY 43-9006 exhibits broad spectrum oral antitumor activity and targets the RAF/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis. Cancer Res. 2004; 64(19):7099–7109. [PubMed: 15466206]
114. Gattenlohner S, Etschmann B, Riedmiller H, Muller-Hermelink HK. Lack of KRAS and BRAF mutation in renal cell carcinoma. Eur Urol. 2009; 55(6):1490–1491. [PubMed: 19282104]
115. Tannapfel A, Sommerer F, Benicke M, et al. Mutations of the BRAF gene in cholangiocarcinoma but not in hepatocellular carcinoma. Gut. 2003; 52(5):706–712. [PubMed: 12692057]
116. Sala E, Mologni L, Truffa S, Gaetano C, Bollag GE, Gambacorti-Passerini C. BRAF silencing by short hairpin RNA or chemical blockade by PLX4032 leads to different responses in melanoma and thyroid carcinoma cells. Mol Cancer Res. 2008; 6(5):751–759. [PubMed: 18458053]
117. Hatzivassiliou G, Song K, Yen I, et al. RAF inhibitors prime wild-type RAF to activate the MAPK pathway and enhance growth. Nature. 2010; 464(7287):431–435. [PubMed: 20130576]
118. Heidorn SJ, Milagre C, Whittaker S, et al. Kinase-dead BRAF and oncogenic RAS cooperate to drive tumor progression through CRAF. Cell. 2010; 140(2):209–221. [PubMed: 20141835]
119. Rajakulendran T, Sahmi M, Lefrancois M, Sicheri F, Therrien M. A dimerization-dependent mechanism drives RAF catalytic activation. Nature. 2009; 461(7263):542–545. [PubMed: 19727074]
120. Flaherty KT, Puzanov I, Kim KB, et al. Inhibition of mutated, activated BRAF in metastatic melanoma. N Engl J Med. 2010; 363(9):809–819. [PubMed: 20818844]
121. Miller FG, Joffe S. Equipoise and the dilemma of randomized clinical trials. N Engl J Med. 2011; 364(5):476–480. [PubMed: 21288100]
122. Bollag G, Hirth P, Tsai J, et al. Clinical efficacy of a RAF inhibitor needs broad target blockade in BRAF-mutant melanoma. Nature. 2010; 467(7315):596–599. [PubMed: 20823850]
123. Johannessen CM, Boehm JS, Kim SY, et al. COT drives resistance to RAF inhibition through MAP kinase pathway reactivation. Nature. 2010; 468(7326):968–972. [PubMed: 21107320]
124. Nazarian R, Shi H, Wang Q, et al. Melanomas acquire resistance to BRAF(V600E) inhibition by RTK or N-RAS upregulation. Nature. 2010; 468(7326):973–977. [PubMed: 21107323] [Diversity in mechanisms of resistance in melanomas treated with a BRAF inhibitor.]
125. Davies BR, Logie A, McKay JS, et al. AZD6244 (ARRY-142886), a potent inhibitor of mitogen-activated protein kinase/extracellular signal-regulated kinase kinase 1/2 kinases: mechanism of action in vivo, pharmacokinetic/pharmacodynamic relationship, and potential for combination in preclinical models. Mol Cancer Ther. 2007; 6(8):2209–2219. [PubMed: 17699718]
126. Balmanno K, Chell SD, Gillings AS, Hayat S, Cook SJ. Intrinsic resistance to the MEK1/2 inhibitor AZD6244 (ARRY-142886) is associated with weak ERK1/2 signalling and/or strong PI3K signalling in colorectal cancer cell lines. Int J Cancer. 2009; 125(10):2332–2341. [PubMed: 19637312]
127. Martin TD, Samuel JC, Routh ED, Der CJ, Yeh JJ. Activation and involvement of Ral GTPases in colorectal cancer. Cancer Res. 2011; 71(1):206–215. [PubMed: 21199803]
128. Solit DB, Garraway LA, Pratilas CA, et al. BRAF mutation predicts sensitivity to MEK inhibition. Nature. 2006; 439(7074):358–362. [PubMed: 16273091] [Cell dependency of MEK signaling is influenced by mutations in BRAF and KRas.]
129. Pratilas CA, Taylor BS, Ye Q, et al. (V600E)BRAF is associated with disabled feedback inhibition of RAF-MEK signaling and elevated transcriptional output of the pathway. Proc Natl Acad Sci U S A. 2009; 106(11):4519–4524. [PubMed: 19251651]
130. Adjei AA, Cohen RB, Franklin W, et al. Phase I pharmacokinetic and pharmacodynamic study of the oral, small-molecule mitogen-activated protein kinase kinase 1/2 inhibitor AZD6244 (ARRY-142886) in patients with advanced cancers. J Clin Oncol. 2008; 26(13):2139–2146. [PubMed: 18390968]

131. Castellano E, Downward J. Role of RAS in the regulation of PI 3-kinase. Curr Top Microbiol Immunol. 2011; 346:143–169. [PubMed: 20563706]

132. Garcia-Echeverria C, Sellers WR. Drug discovery approaches targeting the PI3K/Akt pathway in cancer. Oncogene. 2008; 27(41):5511–5526. [PubMed: 18794885]

133. Fasolo A, Sessa C. mTOR inhibitors in the treatment of cancer. Expert Opin Investig Drugs. 2008; 17(11):1717–1734.

134. Gupta S, Ramjaun AR, Haiko P, et al. Binding of ras to phosphoinositide 3-kinase p110alpha is required for ras-driven tumorigenesis in mice. Cell. 2007; 129(5):957–968. [PubMed: 17540175]

135. Engelman JA, Chen L, Tan X, et al. Effective use of PI3K and MEK inhibitors to treat mutant Kras G12D and PIK3CA H1047R murine lung cancers. Nat Med. 2008; 14(12):1351–1356. [PubMed: 19029981]

136. Yip-Schneider MT, Lin A, Barnard D, Sweeney CJ, Marshall MS. Lack of elevated MAP kinase (Erk) activity in pancreatic carcinomas despite oncogenic K-ras expression. Int J Oncol. 1999; 15(2):271–279. [PubMed: 10402237]

137. Rodriguez-Viciana P, McCormick F. RalGDS comes of age. Cancer Cell. 2005; 7(3):205–206. [PubMed: 15766656]

138. Feig LA. Ral-GTPases: approaching their 15 minutes of fame. Trends Cell Biol. 2003; 13(8):419–425. [PubMed: 12888294]

139. Bodemann BO, White MA. Ral GTPases and cancer: linchpin support of the tumorigenic platform. Nat Rev Cancer. 2008; 8(2):133–140. [PubMed: 18219307]

140. Urano T, Emkey R, Feig LA. Ral-GTPases mediate a distinct downstream signaling pathway from Ras that facilitates cellular transformation. Embo J. 1996; 15(4):810–816. [PubMed: 8631302]

141. Hamad NM, Elconin JH, Karnoub AE, et al. Distinct requirements for Ras oncogenesis in human versus mouse cells. Genes Dev. 2002; 16(16):2045–2057. [PubMed: 12183360]

142. Lim KH, O'Hayer K, Adam SJ, et al. Divergent roles for RalA and RalB in malignant growth of human pancreatic carcinoma cells. Curr Biol. 2006; 16(24):2385–2394. [PubMed: 17174914]

143. Smith SC, Oxford G, Wu Z, et al. The metastasis-associated gene CD24 is regulated by Ral GTPase and is a mediator of cell proliferation and survival in human cancer. Cancer Res. 2006; 66(4):1917–1922. [PubMed: 16488989]

144. Yin J, Pollock C, Tracy K, et al. Activation of the RalGEF/Ral pathway promotes prostate cancer metastasis to bone. Mol Cell Biol. 2007; 27(21):7538–7550. [PubMed: 17709381]

145. Vigil D, Cherfils J, Rossman KL, Der CJ. Ras superfamily GEFs and GAPs: validated and tractable targets for cancer therapy? Nat Rev Cancer. 2010; 10(12):842–857. [PubMed: 21102635]

146. Falsetti SC, Wang DA, Peng H, et al. Geranylgeranyltransferase I inhibitors target RalB to inhibit anchorage-dependent growth and induce apoptosis and RalA to inhibit anchorage-independent growth. Mol Cell Biol. 2007; 27(22):8003–8014. [PubMed: 17875936]

147. Wu JC, Chen TY, Yu CT, et al. Identification of V23RalA-Ser194 as a critical mediator for Aurora-A-induced cellular motility and transformation by small pool expression screening. J Biol Chem. 2005; 280(10):9013–9022. [PubMed: 15637052]

148. Lim KH, Brady DC, Kashatus DF, et al. Aurora-A phosphorylates, activates, and relocalizes the small GTPase RalA. Mol Cell Biol. 2010; 30(2):508–523. [PubMed: 19901077]

149. Molhoek KR, Brautigan DL, Slingluff CL Jr. Synergistic inhibition of human melanoma proliferation by combination treatment with B-Raf inhibitor BAY43-9006 and mTOR inhibitor Rapamycin. J Transl Med. 2005; 3:39. [PubMed: 16255777]

150. Wang Z, Zhou J, Fan J, et al. Effect of rapamycin alone and in combination with sorafenib in an orthotopic model of human hepatocellular carcinoma. Clin Cancer Res. 2008; 14(16):5124–5130. [PubMed: 18698030]

151. Legrier ME, Yang CP, Yan HG, et al. Targeting protein translation in human non-small cell lung cancer via combined MEK and mammalian target of rapamycin suppression. Cancer Res. 2007; 67(23):11300–11308. [PubMed: 18056456]

152. Flaherty KT. Chemotherapy and targeted therapy combinations in advanced melanoma. Clin Cancer Res. 2006; 12(7 Pt 2):2366s–2370s. [PubMed: 16609060]

153. McCubrey JA, Steelman LS, Chappell WH, et al. Roles of the Raf/MEK/ERK pathway in cell growth, malignant transformation and drug resistance. Biochim Biophys Acta. 2007; 1773(8): 1263–1284. [PubMed: 17126425]

154. Mabuchi S, Ohmichi M, Kimura A, et al. Inhibition of phosphorylation of BAD and Raf-1 by Akt sensitizes human ovarian cancer cells to paclitaxel. J Biol Chem. 2002; 277(36):33490–33500. [PubMed: 12087097]

155. Abou-Alfa GK, Johnson P, Knox JJ, et al. Doxorubicin plus sorafenib vs doxorubicin alone in patients with advanced hepatocellular carcinoma: a randomized trial. Jama. 2010; 304(19):2154–2160. [PubMed: 21081728]

156. McDaid HM, Lopez-Barcons L, Grossman A, et al. Enhancement of the therapeutic efficacy of taxol by the mitogen-activated protein kinase kinase inhibitor CI-1040 in nude mice bearing human heterotransplants. Cancer Res. 2005; 65(7):2854–2860. [PubMed: 15805287]

157. Rieber M, Rieber MS. Signaling responses linked to betulinic acid-induced apoptosis are antagonized by MEK inhibitor UO126 in adherent or 3D spheroid melanoma irrespective of p53 status. Int J Cancer. 2006; 118(5):1135–1143. [PubMed: 16152620]

158. Chung EJ, Brown AP, Asano H, et al. In vitro and in vivo radiosensitization with AZD6244 (ARRY-142886), an inhibitor of mitogen-activated protein kinase/extracellular signal-regulated kinase 1/2 kinase. Clin Cancer Res. 2009; 15(9):3050–3057. [PubMed: 19366835]

159. Edwards E, Geng L, Tan J, Onishko H, Donnelly E, Hallahan DE. Phosphatidylinositol 3-kinase/Akt signaling in the response of vascular endothelium to ionizing radiation. Cancer Res. 2002; 62(16):4671–4677. [PubMed: 12183424]

160. Paglin S, Lee NY, Nakar C, et al. Rapamycin-sensitive pathway regulates mitochondrial membrane potential, autophagy, and survival in irradiated MCF-7 cells. Cancer Res. 2005; 65(23):11061–11070. [PubMed: 16322256]

161. Moretti L, Attia A, Kim KW, Lu B. Crosstalk between Bak/Bax and mTOR signaling regulates radiation-induced autophagy. Autophagy. 2007; 3(2):142–144. [PubMed: 17204849]

162. Luo J, Emanuele MJ, Li D, et al. A genome-wide RNAi screen identifies multiple synthetic lethal interactions with the Ras oncogene. Cell. 2009; 137(5):835–848. [PubMed: 19490893]

163. Scholl C, Frohling S, Dunn IF, et al. Synthetic lethal interaction between oncogenic KRAS dependency and STK33 suppression in human cancer cells. Cell. 2009; 137(5):821–834. [PubMed: 19490892]

164. Barbie DA, Tamayo P, Boehm JS, et al. Systematic RNA interference reveals that oncogenic KRAS-driven cancers require TBK1. Nature. 2009; 462(7269):108–112. [PubMed: 19847166]

165. Kaelin WG Jr. Synthetic lethality: a framework for the development of wiser cancer therapeutics. Genome Med. 2009; 1(10):99. [PubMed: 19863774]

166. Chien Y, Kim S, Bumeister R, et al. RalB GTPase-mediated activation of the IkappaB family kinase TBK1 couples innate immune signaling to tumor cell survival. Cell. 2006; 127(1):157–170. [PubMed: 17018283]

167. Singh A, Greninger P, Rhodes D, et al. A gene expression signature associated with “K-Ras addiction” reveals regulators of EMT and tumor cell survival. Cancer Cell. 2009; 15(6):489–500. [PubMed: 19477428]

168. Babij C, Zhang Y, Kurzeja RJ, et al. STK33 Kinase Activity is Non-Essential in KRAS-Dependent Cancer Cells. Cancer Res. Jul 8.2011 published online.

Defined key terms

GTPases

A large family of enzymes that exhibit high affinity binding for guanosine diphosphate (GDP) and guanosine triphosphate (GTP) and catalyze the hydrolysis of the bound GTP to GDP and release of orthophoshpate. GTPases function as molecular switches and timers that cycle between inactive GDP-bound and active GTP-bound states. Guanine nucleotide exchange promotes formation of the GTP-bound state due to the ~10-fold cellular GTP:GDP ratio. The human genome encodes for ~220 GTPases, with the Ras superfamily comprising the largest group (154 members), followed by the heterotrimeric G protein alpha subunits (16 members). GTPases are characterized by short stretches of amino acid sequence similarity that comprise the GDP-GTP binding pocket.

Mitogen-activated protein kinase cascades

These cascades are three component protein kinase modules, beginning with an extracellular stimulus-activated mitogen-activated protein kinase (MAPK) kinase kinase (MKKK/MEKK) serine/threonine kinase, which phosphorylates and activates a MAPK dual specificity MAPK kinase (MKK/MEK), which then phosphorylates and activates a MAPK serine/threonine kinase. There are four conventional mammalian MAPK families: ERK1/ 2, p38 (α, β, γ and δ), JNK1/2/3 and ERK5.

Oncogene addiction

The dependence of a cancer cell on an overactive protein or signaling pathway necessary for cell survival and growth. Usually, this is due to mutational activation or overexpression. This greater dependence is proposed to account for why molecularly targeted therapies (e.g., trastuzumab, imatinib, erlotinib) can preferentially block cancer versus normal cell proliferation and survival.

Protein prenyltransferases

A family of three heterodimeric proteins that catalyzes the posttranslational addition of either a farnesyl isoprenoid (farnesyltransferase) or geranylgeranyl isoprenoid (geranylgeranyltransferase-I and geranylgeranyltransferase-II) to C-terminal cysteine residues. Farnesyltransferase and geranylgeranyltransferase-I are also referred to as CAAX prenyltransferases and geranylgeranyltransferase-II as Rab prenyltransferases.

Synthetic lethality

Two genes are synthetic lethal if mutation of either alone is compatible with viability but mutation of both leads to death. Therefore, targeting a gene (e.g., by RNA interference) that is synthetic lethal to a cancer cell-specific genetic mutation (e.g., *RAS* activation) should kill only cancer cells with that genetic mutation and spare normal cells. Synthetic lethality therefore provides a conceptual framework for the identification of cancer cell-selective targets for anti-cancer drug discovery.

Executive Summary

■ The three *RAS* genes are mutated in 33% of human cancers. *KRAS* is the most commonly mutated *RAS* gene, and the predominant isoform mutated in lung, colorectal and pancreatic cancers. Limited evidence also suggests that the three *RAS* genes are not functionally equivalent. Thus, while *HRAS* was traditionally the most intensely studied, recent studies have now shifted the focus to *KRAS*.

■ To date, efforts to develop direct antagonists of mutant Ras proteins have not been successful. GTP competitive inhibitors are not feasible due to the picomolar binding affinity for GTP binding. However, with new technology and information, it remains possible that such approaches can still be identified.

■ Although all Ras proteins are modified by FTase, the Ras isoforms most commonly mutated in human cancers (K-Ras and N-Ras) can be modified by GGTase-I when FTase activity is blocked, resulting in alternative prenylation by the related geranylgeranyl isoprenoid lipid. Despite this clear biochemical explanation for the failure of farnesytransferase inhibitors, there remains a misconception that these failed efforts suggest that Ras is not a clinically useful anti-cancer target.

■ Currently, the most promising approaches for blocking mutant Ras signaling involve inhibitors of Raf or PI3K effector signaling. Combination approaches that block a single effector signaling network at multiple points, or that block two distinct effector signaling networks, are believed to be the most promising directions for these efforts.

■ Recently, functional RNA interference screens have been performed to identify synthetic lethal genetic partners of mutant *KRAS*. Interestingly, these screens have identified protein kinases, thus identifying potentially more tractable directions for the development of anti-Ras inhibitors.

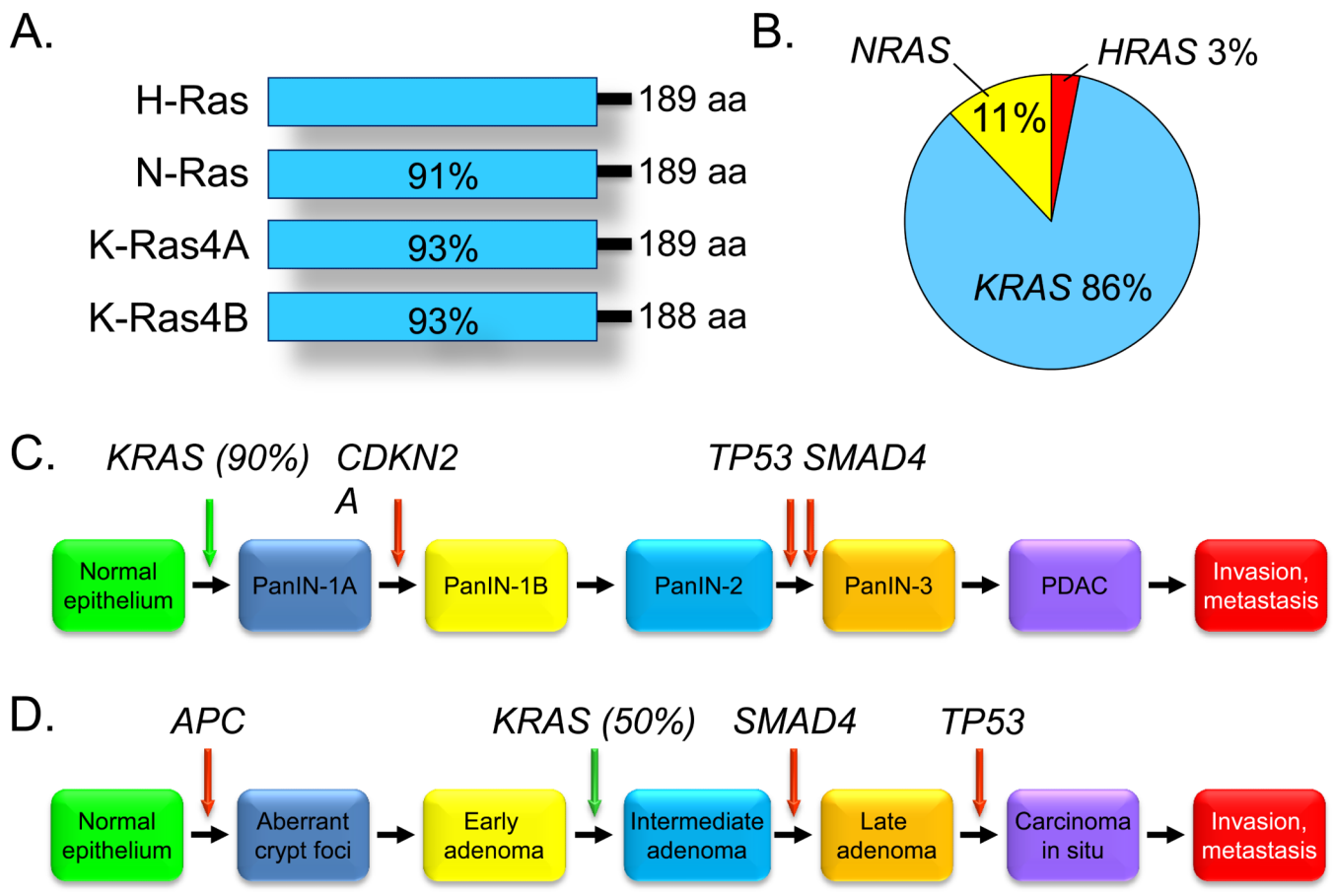

Figure 1. *RAS* mutation in human cancers

A. Human Ras proteins. *RAS* genes encode 188 or 189 amino acid proteins that share the indicated amino acid identity. *KRAS* encodes K-Ras4A or K-Ras4B due to alternative exon four utilization, with *KRAS4B* the predominant transcript. B. Frequency of specific *RAS* mutations. *KRAS* mutations (17,342 unique samples with mutations in a total of 80,140 unique samples) comprise 86% of all *RAS* mutations documented in human tumor cells. Next most frequent are *NRAS* mutations (2,279 mutations in 28,521 samples) and *HRAS* is the least frequent (652 mutations in 19,589 samples). Data are compiled from COSMIC (http://www.sanger.ac.uk/genetics/CGP/cosmic/0. C. Genetic progression of pancreatic ductal adenocarcinoma. D. Genetic progression of colorectal carcinoma.

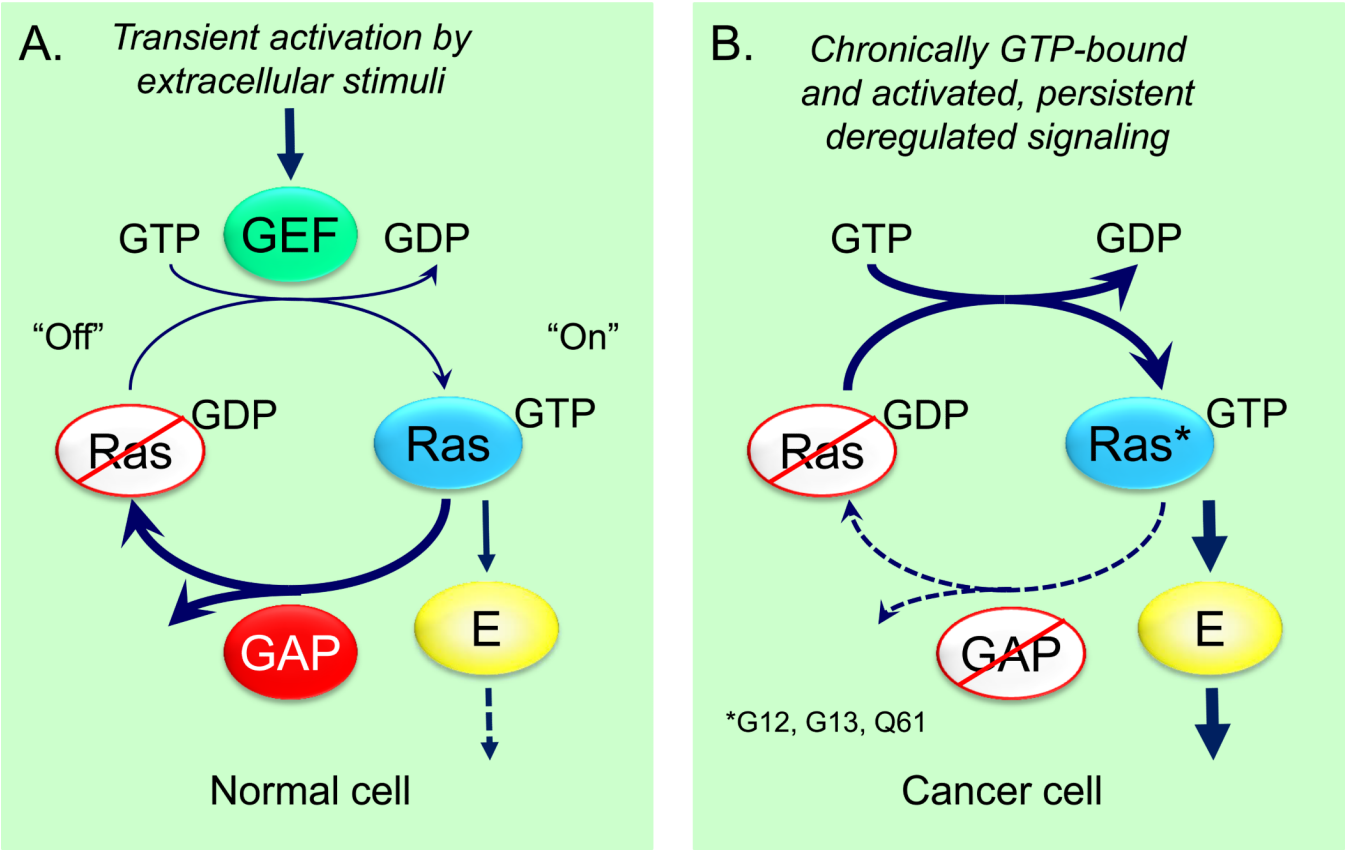

Figure 2. Regulation of the Ras GDP-GTP cycle in normal and neoplastic cells

A. Normal Ras. Wild type Ras proteins cycle between inactive GDP-bound and active GTP-bound states. Growth factors stimulate transient activation of Ras through activation of RasGEFs (e.g., Sos). Ras-GTP binds preferentially to downstream effectors (E). RasGAPs (e.g., neurofibromin) accelerate the intrinsic GTP hydrolysis activity, returning Ras to the inactive state. B. Tumor-associated Ras. Missense mutations primarily at glycine-12, glycine-13 or glutamine-61 impair intrinsic and GAP-stimulated GTP hydrolysis activity, rendering Ras persistently active and GTP-bound.

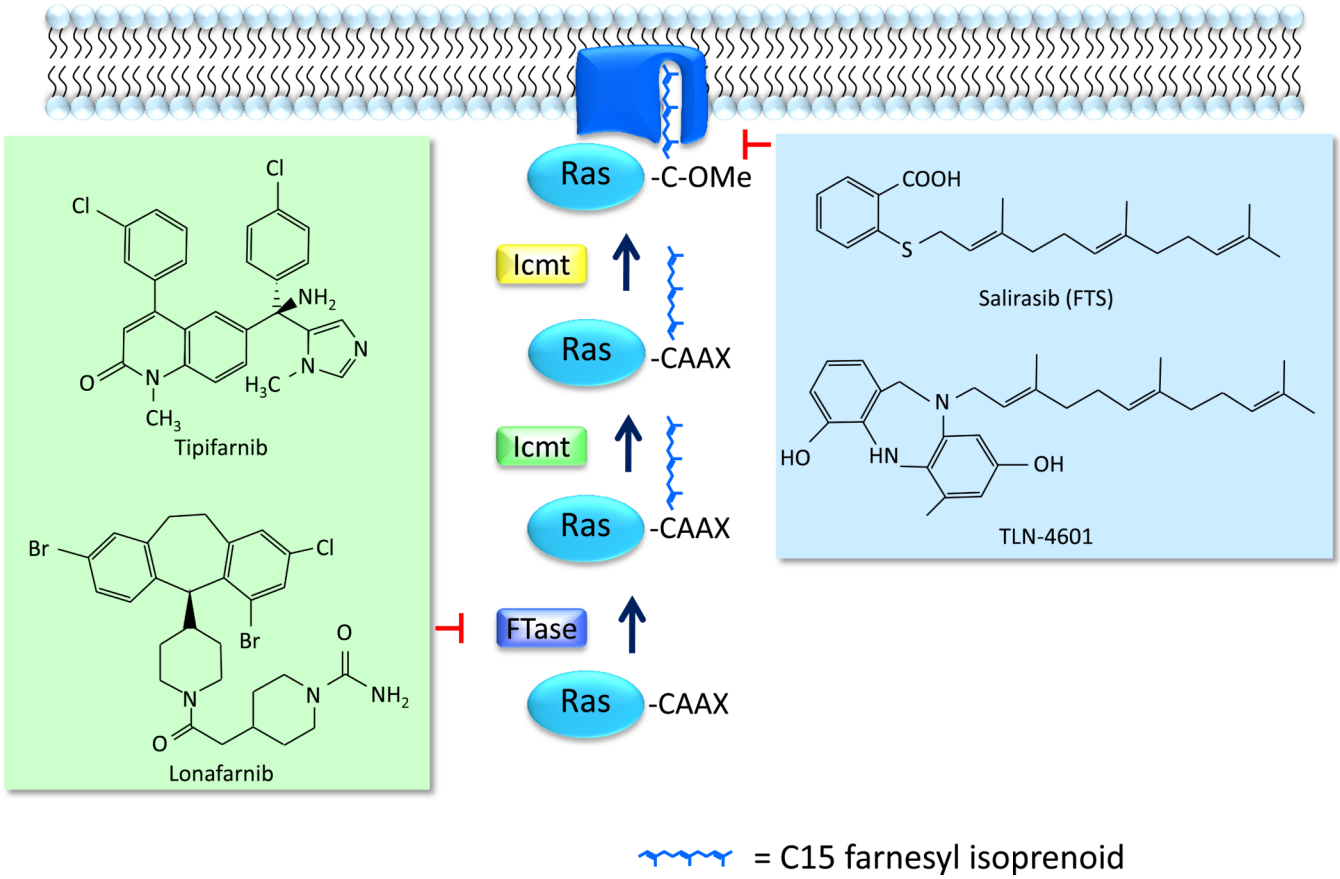

Figure 3. Targeting Ras membrane association for anti-Ras drug discovery

Ras proteins are synthesized initially as cytosolic and inactive proteins. The C-terminal CAAX motif signals for three posttranslational modifications, beginning with cytosolic FTase-catalyzed addition of a C15 farnesyl group and Golgi-associated Rce1 and Icmt catalyzed carboxymethylation of the now terminal farnesylated cysteine residue. Inhibitors of FTase (e.g., tipifarnib and lonafarnib) block all CAAX-signaled modifications. Farnesyl group-containing small molecules (salirasib and TLN-4601) have been evaluated in clinical trials as possible inhibitors of Ras membrane association and oncogenesis.

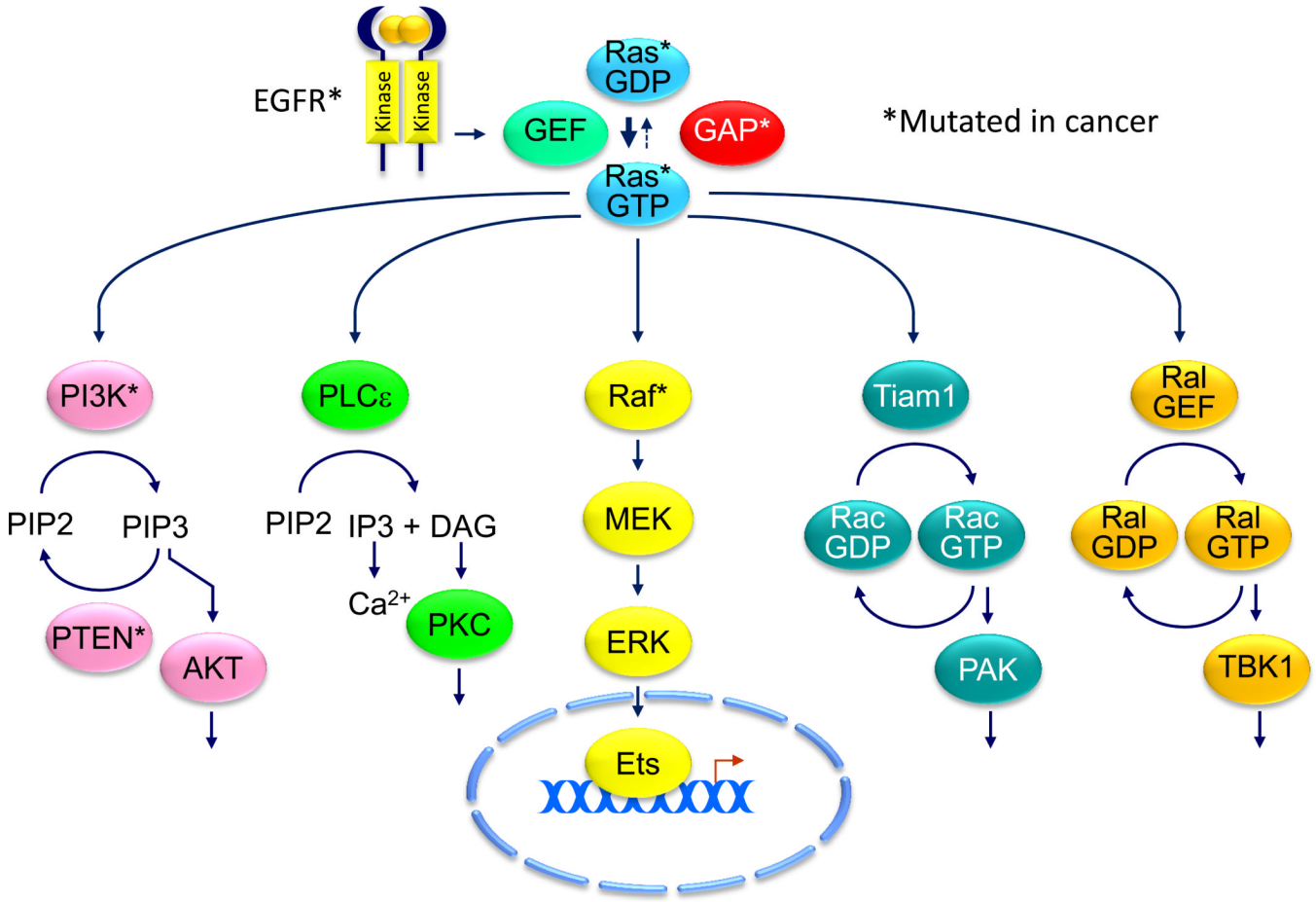

Figure 4. Effectors of Ras-mediated oncogenesis

Ras-GTP binds preferentially to a spectrum of functionally diverse downstream effectors. Of these, five have been validated in cell culture and/or mouse models for their requirement for mutant Ras-induced oncogenesis. In addition to direct mutational activation of Ras, Ras can also be activated indirectly, for example, by mutational inactivation of the neurofibromin RasGAP or by mutational activation of the epidermal growth factor receptor (EGFR).

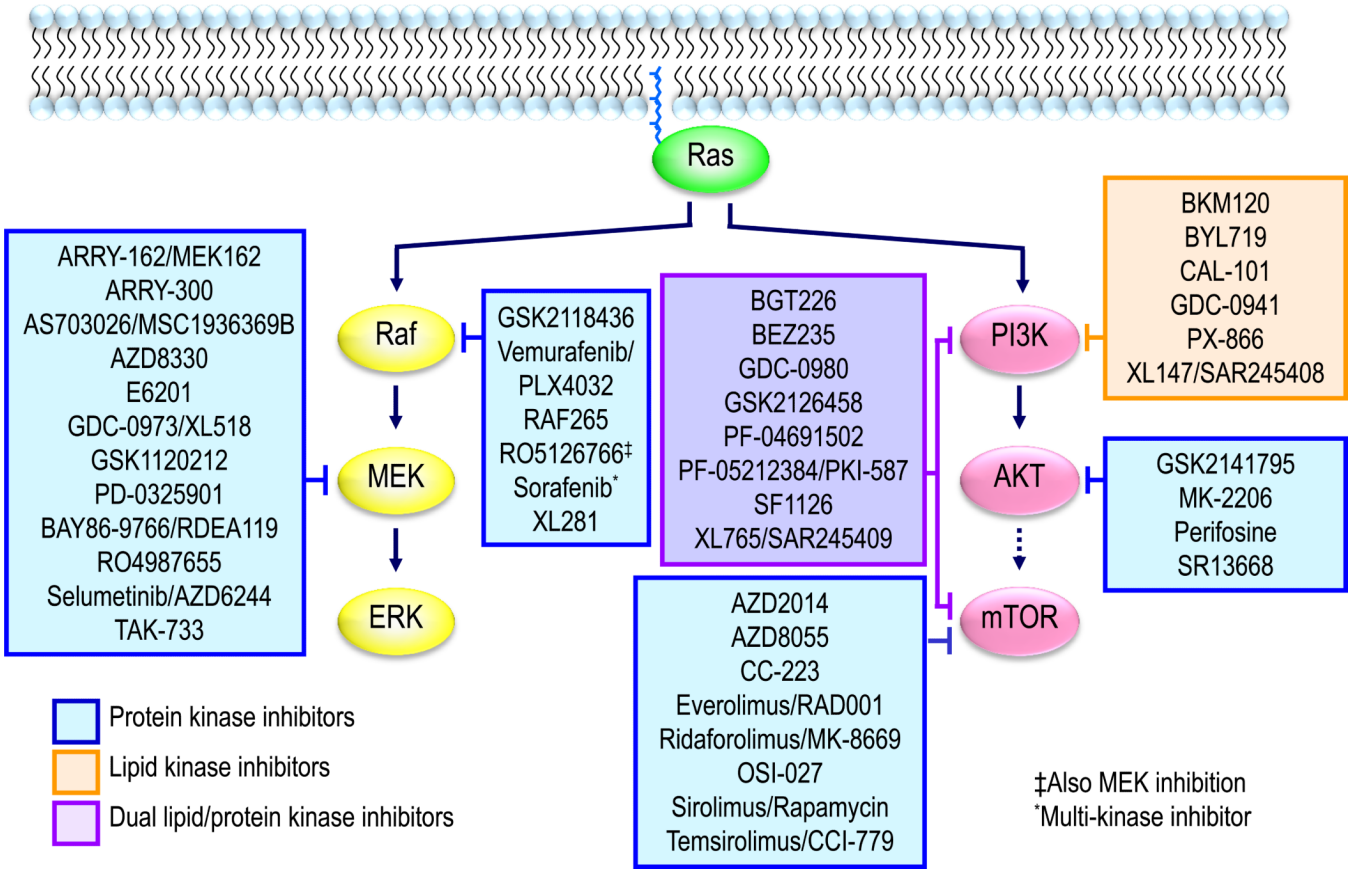

Figure 5. Inhibitors of Raf and PI3K effector signaling under clinical evaluation

Small molecule inhibitors of Raf and MEK, and PI3K, AKT and mTOR are currently being evaluated in Phase I-III clinical trials. Also see Tables 1 and 2. Compiled from http://clinicaltrials.gov

Table 1

Inhibitors of Raf-MEK-ERK signaling under clinical evaluation[a]

Agent	Company	Target	Status	Target population
BMS-908662/XL281	Exexis/Bristol-Myers Squibb	Raf	Phase I-II	Alone or in combination with cetuximab for *KRAS* or *BRAF* mutant advanced or metastatic CRC
			Phase I	In combination with Ipilimumab with unresectable stage III/IV melanoma
GSK2118436	GlaxoSmithKline	Raf	Phase I-II	*BRAF* mutant melanoma, NSCLC and other solid cancers
RAF265	Novartis	Raf, VEGFR-2	Phase I-II	Metastatic melanoma
RO5126766‡	Hoffmann-La Roche	Raf, MEK1/2	Phase I	Advanced tumors
Sorafenib/BAY 43-9006	Bayer	Raf, VEGFR-2, VEGFR-3, PDGFR-β, Flt-3, c-Kit and FGFR-1	Approved; Phase I-III	Approved for advanced RCC and primary liver cancer; in combination with cytotoxic drugs or radiation for various solid tumors and leukemia
Vemurafenib/PLX4032/RG 7204/RO5185426	Plexxicon/Hoffmann-La Roche	Raf	Phase I	Malignant melanoma, CRC
			Phase I-II	Alone or in combination with a drug cocktail for malignant melanoma
			Phase III	Unresectable *BRAF* mutant stage IIIC or IV melanoma
			Phase II	Metastatic or unresectable *BRAF* mutant papillary thyroid cancer and resistant to radioactive iodine
ARRY-438162/MEK162	Array BioPharma/Novartis	MEK1/2	Phase II	*BRAF* or *NRAS* mutant metastatic melanoma, advanced or metastatic biliary cancer, metastatic CRC
ARRY-300	Array BioPharma/Novartis	MEK1/2	Phase I	Pharmacokinetics and pharmacodynamics in healthy subjects
AS703026/MSC1936369B	EMD Serono	MEK1/2	Phase II	In combination with gemcitabine for pancreatic cancer
			Phase I	advanced hematological malignancies
			Phase II	AML
AZD8330	AstraZeneca	MEK1/2	Phase I	Advanced malignancies
BAY86-9766/ RDEA119	Bayer	MEK1/2	Phase I	Advanced or refractory solid tumors
			Phase I-II	In combination with gemcitabine for pancreatic cancer
E6201	Eisai	MEK1/2	Phase I	Advanced solid tumors
GDC-0973/XL518	Exelixis/Genentech	MEK1/2	Phase I	Healthy volunteers
			Phase I	In combination with rabeprazole in healthy volunteers
			Phase I	In solid tumors
			Phase I	In combination with GDC-0941 with locally advanced or metastatic solid tumors
GSK1120212	GlaxoSmithKline	MEK1/2	Phase II	Leukemia-relapsed or refractory
			Phase I	In combination with docetaxel, erlotinib, pemetrexed, pemetrexed +

Agent	Company	Target	Status	Target population
				carboplatin, or nab-paclitaxel in advanced solid tumors
			Phase III	Advanced or metastatic *BRAF* mutant melanoma
			Phase II	In combination with gemcitabine for pancreatic cancer
PD-0325901	Pfizer	MEK1/2	Phase I	In combination with PF-04691502 for advanced cancers
RO4987655	Hoffmann-La Roche	Raf, MEK1/2	Phase I	Advanced cancers
Selumetinib/AZD6244	AstraZeneca/Array BioPharma	MEK1/2	Phase I-II	Alone or in combination with cytotoxic drugs for multiple solid cancers
TAK-733	Millennium Pharmaceuticals	MEK1/2	Phase I	Advanced nonhematologic malignancies

abbreviations: AML, acute myelogenous leukemia; CRC, colorectal cancer; NSCLC, non-small cell lung cancer

[a]Compiled from http://clinicaltrials.gov

Table 2

Inhibitors of PI3K-AKT-mTOR signaling under clinical evaluation[a]

Agent	Company	Target	Status	Target population
BKM120	Novartis	Pan-class I PI3K	Phase I	In combination with carboplatin and paclitaxel with advanced solid tumors
			Phase I	In combination with irinotecan in previously treated advanced CRC
			Phase I	In combination with fulvestrant in estrogen receptor-positive stage IV breast cancer
			Phase I	In combination with BEZ235 and letrozole with hormone receptor and metastatic breast cancer
			Phase I	In combination with GSK1120212 in advanced and selected solid tumors
			Phase I	In combination with BEZ235 in combination with paclitaxel with or without trastuzumab in metastatic or locally advanced solid tumors
			Phase I	In combination with trastuzumab with relapsing HER2 overexpressing metastatic breast cancer
			Phase I-II	Advanced solid tumors and leukemias
			Phase I-II	In combination with bevacizumab in glioblastoma multiforme and RCC
			Phase I-II	In combination with MEK162 in advanced solid tumors
			Phase I	In combination with capecitabine in metastatic breast cancer
BYL719	Novartis	PI3Kα	Phase I	Advanced *PIK3CA* mutant solid tumors
			Phase I	Advanced solid tumors
CAL-101	Calistoga Pharmaceuticals	PI3Kδ	Phase I	In combination with chemotherapeutic agents and CD20 mAb in Non-Hodgkin's lymphoma or chronic lymphyocytic leukemia
			Phase II	In combination with rituximab in leukemia and lymphoma
			Phase I-II	Hematological malignancies
			Phase I	Allergic rhinitis
GDC-0941	Genentech/Piramed	Pan-class I PI3K	Phase I	Non-Hodgkin's lymphoma, solid cancers
			Phase I	In combination with GDC-0973 in locally advanced and metastatic solid tumors
			Phase I	In combination with trastuzumab-MCC-DM1 in metastatic breast cancer

Agent	Company	Target	Status	Target population
			Phase I	In combination with erlotinib in advanced solid tumors
			Phase I	In combination with paclitaxel and bevacizumab in locally recurrent or metastatic breast cancer
			Phase I	In combination with paclitaxel and carboplatin in NSCLC
			Phase I	In combination with ketoconozole in healthy volunteers
			Phase I	In combination with rabeprezole in healthy patients
PX-866	Oncothyreon	Pan-class I PI3K	Phase I-II	Advanced solid tumors
			Phase I-II	In combination with cetuximab in incurable CRC and head and neck cancers
			Phase I-II	In combination with docetaxel in advanced solid tumors
			Phase II	Prostate cancer
XL147/SAR245408	Exelixis/Sanofi-Aventis	Pan-class I PI3K	Phase I-II	In combination with trastuzumab or paclitaxel with breast cancer
			Phase I-II	In combination with letrozole in breast cancer
			Phase I	In combination with paclitaxel and carboplatin in solid tumors
			Phase I	In combination with erlotinib in solid tumors
			Phase I	In combination with MSC1936369B in advanced solid tumors
			Phase I-II	Solid tumors and lymphomas
			Phase I	In combination with XL647 in solid tumors
GSK2141795	GlaxoSmithKline	AKT	Phase I	Solid tumors and lymphoma
			Phase I	In combination with GSK1120212 in cancer
MK-2206	Merck	AKT	Phase I	In combination with standard chemotherapy in locally advanced or metastatic solid tumors
			Phase I	In combination with gefitinib in NSCLC
			Phase I	In combination with trastuzumab and lapatinib in breast cancer and advanced solid tumors
			Phase I	In combination with anastrazole, letrolzole and exemestane in breast cancer
			Phase I	In combination with AZD6244 in advanced solid tumors
			Phase I	In combination with lapatinib in advanced or metastatic solid tumors or breast cancer

Agent	Company	Target	Status	Target population
			Phase I-II	In combination with bendamustine hydrochloride and rituximab in leukemia and lymphoma
			Phase I	In combination with lapatinib with metastatic breast cancer
			Phase I	In combination with dalotumuzab and MK-0752 for advanced cancers
			Phase I	In combination with ridaflorolimus in advanced cancers
			Phase II	In combination with bicalutamide in prostate cancer
			Phase II	In combination with erlotinib in lung cancer
			Phase I-II	In combination with AZD6244 in CRC
			Phase I-II	Advanced solid tumors and hematological cancers
Perifosine	Asta Medica, Zentaris	AKT	Phase II	In combination with single agent chemotherapy in metastatic cancer
			Phase II	In combination with gleevac in GIST
			Phase I-II	In combination with bortezomib and dexamethasone for multiple myeloma
			Phase I	In combination with sorafenib in advance cancers
			Phase I	In combination with docetaxel in relapsed ovarian cancer
			Phase I	In combination with gemcitabine in cancers
			Phase III	In combination with capecitabine in refractory CRC
			Phase I	In combination with sunitinib in advanced cancers
			Phase I-II	In combination with temsirolimus in recurrent and malignant glioma
			Phase I-II	Advanced solid tumors, sarcomas, hematological cancers and Waldenstrom's Macroglobulinemia
			Phase I	In combination with temsirolimus in pediatric solid tumors
			Phase II	In combination with dexamethasone in multiple myeloma
SR13668	SRI international	AKT	Phase I	Healthy volunteers
BGT226	Novartis	PI3K and mTOR	Phase I-II	Advanced solid tumors, breast cancers, Cowden syndrome
			Phase I	Advanced solid tumors
BEZ235	Novartis	PI3K and mTOR	Phase I	In combination with BKM120, in combination with paclitaxel, with or without trastuzumab in locally advanced or metastatic solid tumors

Agent	Company	Target	Status	Target population
			Phase I-II	In combination with MEK162 in advanced solid tumors
			Phase I	In combination with BKM120 and letrozole in metastatic breast cancer
			Phase I-II	Advanced solid tumors
GDC-0980	Genentech	PI3K and mTOR	Phase I	In combination with rabeprazole in healthy volunteers
			Phase I	In combination with paclitaxel, bevacizumab and trastuzumab in locally recurrent or metastatic breast cancer
			Phase I	In combination with fluoropyrimidine, oxaliplatin and bevacizumab in advanced solid tumors
			Phase I	In combination with paclitaxel, carboplatin with or without bevacizumab in solid tumors
			Phase I	Advanced solid tumors or NHL
GSK2126458	GlaxoSmithKline	PI3K and mTOR	Phase I	Solid tumors or lymphoma
			Phase I	In combination with GSK1120212 in cancer
PF-04691502	Pfizer	PI3K and mTOR	Phase I	Advanced malignant solid tumors
			Phase I	In combination with a MEK inhibitor or irinotecan in advanced cancer
PF-05212384/PKI-587	Pfizer	PI3K and mTOR	Phase I	Solid tumors
SF1126	Semafore Pharmaceuticals	PI3K and mTOR	Phase I	Solid tumors
XL765/SAR245409	Exelixis/Sanofi-Aventi	PI3K and mTOR	Phase I-II	In combination with letrozole in breast cancer
			Phase I	In combination with erlotinib in solid tumors
			Phase I	In combination with temozolomide with or without radiation in malignant gliomas
			Phase I	Advanced solid tumors
AZD2014	AstraZeneca	mTOR	Phase I	Advanced solid tumors
AZD8055	AstraZeneca	mTOR	Phase I	Recurrent gliomas
			Phase I-II	Advanced solid tumors
			Phase I-II	Cancer, advanced HCC
CC-223	Celgene	mTOR	Phase I-II	Advanced solid tumors, NHL, or multiple myeloma
Everolimus/RAD001¶	Novartis	mTOR		Approved for RCC
			Phase 1-III	Alone or in combination with cytotoxic drugs for multiple solid and hematological cancers
			Phase II	*NF2* mutant acoustic neuroma
			Phase II	*NF1* mutant and chemotherapy-refractory radiographic progressive low grade gliomas

Agent	Company	Target	Status	Target population
Ridaforolimus/MK-8669/AP23573	Ariad Pharmaceuticals/Merck	mTOR	Phase II	In combination with trastuzumab in metastatic breast cancer
			Phase I	In combination with doxorubicin in cancer and sarcoma
			Phase I	In combination with bevacizumab in solid tumors
			Phase II	In combination with bicalutamide in prostate cancer
			Phase II	In combination with dalotumuzab in breast cancer
			Phase I	In combination with MK-0752 or MK-2206 in advanced neoplasms
			Phase I	In combination with standard chemotherapy in soft tissue sarcoma
			Phase I	In combination with cetuximab in head and neck cancer, NSCLC and CRC
			Phase I	In combination with carboplatin and taxol in endometrial, ovarian and solid tumors
			Phase I	In combination with vironostat in advanced RCC
			Phase I	In combination with dalotuzumab in neoplasms
			Phase I-III	Advanced solid tumors, sarcomas, lymphomas, hepatic insufficiency
			Phase II	*KRAS* mutant NSCLC
OSI-027	OSI Pharmaceuticals	mTOR	Phase I	Advanced solid tumors or lymphoma
Sirolimus/Rapamycin	Wyeth	mTOR		Approved for the prevention of acute renal allograft rejection
			Phase I-III	Alone or in combination with cytotoxic drugs for multiple solid and hematological cancers
Temsirolimus/CCI-779	Wyeth-Ayerst	mTOR		Approved for RCC
			Phase I-III	Alone or in combination with cytotoxic drugs for multiple solid and hematological cancers

[a]Compiled from http://Clinicaltrials.gov. Abbreviations: CRC, colorectal cancer; GIST, gastrointestinal stromal tumor; NF1, neurofibromatosis type 1; NF1, neurofibromatosis type 2; NHL, Non-Hodgkin's lymphoma; NSCLC, non-small cell lung cancer; RCC, renal cell cancer

Table 3

Combination inhibition of Raf and PI3K effector signaling under clinical evaluation[a]

MEK and/or Raf	PI3K and/or mTOR	Status	Patient	Condition
MEK162	BKM162	Not yet recruiting	Phase I	advanced solid tumors
			Phase II	selected solid tumors
MEK162	BEZ235	Not yet recruiting	Phase I	unspecified adult solid tumors
			Phase II	solid tumors
MSC1936369B	SAR245409	Recruiting	Phase I	Locally advanced solid tumors, metastatic solid tumors
PD-0325901	PF-04691502	Not yet recruiting	Phase I	Advanced cancer
GSK1120212	BKM120	Recruiting	Phase I	Advanced and selected solid tumors
BAY86-9766	BAY80-6946	Not yet recruiting	Phase I	Neoplasms
GDC-0941	GDC-0973/XL518	Recruiting	Phase I	Solid cancers
GSK1120212	GSK2126458	Recruiting	Phase I	Cancer
GSK1120212	GSK2141795	Recruiting	Phase I	Cancer
GSK1120212	Everolimus	Active	Phase I	NSCLC, pancreatic cancer and solid cancers
Sorafenib	Everolimus	Recruiting	Phase I	Relapsed and/or refractory solid tumors
			Phase II	Radioactive iodine refractory thyroid cancer
Sorafenib	Temsirolimus	Recruiting	Phase II	Radioactive iodine refractory thyroid cancer
AZD6244	Temsirolimus	Recruiting	Phase II	*BRAF* mutant stage IV melanoma
MEK162 and RAF265	none	Not yet recruiting	Phase I-II	Advanced solid tumors harboring *RAS* or *BRAF* mutations
GSK2118436 and GSK1120212	none	Not yet recruiting	Phase I	*BRAF* mutant metastatic melanoma
		Rollover	Phase II	To provide continued treatment with GSK2118436 to *BRAF* mutant tumors
AZD6244 and sorafenib	none	Recruiting	Phase I-II	Advanced HCC
BAY86-9766 and sorafenib	none	Active	Phase II	HCC

[a]Compiled from http://Clinicaltrials.gov. Abbreviation: HCC, hepatocellular carcinoma