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## RAS, wanted dead or alive: Advances in targeting RAS-mutant cancers

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### Abstract

Oncogenic RAS proteins, which are mutated in approximately 24% of all human cancers, have earned a well-deserved reputation as being “undruggable.” However, several studies have challenged that reputation. With the first small molecules that directly target one oncogenic RAS mutant (G12C) undergoing clinical evaluation, there have been substantial advances in finding new anti-RAS therapeutic strategies. Furthermore, new insights have come from the growing appreciation that neither all RAS proteins (HRAS, NRAS, and KRAS4A/KRAS4B) nor all oncogenic RAS mutations (such as at residues Gly<sup>12</sup>, Gly<sup>13</sup>, and Gln<sup>61</sup>) have the same impact on RAS signaling and function. The role of the nonmutated, wild-type RAS proteins in the context of mutant RAS is increasingly considered to be targetable, with reports of strategies that directly disrupt either the RAS interaction with activating guanine nucleotide exchange factors (GEFs) or receptor tyrosine kinase-mediated and GEF-dependent RAS activation (such as by targeting the scaffolding phosphatase SHP2). Lastly, the development of new agents that target downstream effectors of RAS signaling has advanced substantially. In this review, we highlight some important trends in the targeting of RAS proteins in cancer.

### Introduction to RAS

Activating mutations in RAS proteins are found in ~24% of all cancers [as reported in the Catalog of Somatic Mutated in Cancer (COSMIC) database, v89] and are commonly associated with resistance to frontline therapies<sup>1</sup>. The three human *RAS* genes encode four highly identical proteins (83–85% identity): HRAS, NRAS, and KRAS4A and KRAS4B, with *KRAS* encoding splice variants due to alternative exon 4 utilization. Wild-type (WT) RAS proteins are GTP-hydrolyzing proteins (GTPases) that cycle between GTP- and GDP-bound states. In the GDP-bound state, they are unable to engage effector proteins and are considered inactive. RAS is activated by guanine nucleotide exchange factors (GEFs, including SOS1 and SOS2) that promote exchange of GDP for GTP. In the GTP-bound state, RAS engages downstream effectors through its dynamic conformation switch regions (SWI and SWII) to stimulate intracellular signaling. RAS is inactivated by the hydrolysis of bound GTP by GTPase-activating proteins (GAPs; such as neurofibromin) that stimulate the

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otherwise inefficient intrinsic GTPase activity. The activating single missense mutations that have been found in human cancers occur primarily at codons encoding glycines at residues 12 (Gly<sup>12</sup>, commonly referred to clinically and per nomenclature as G12) and 13 (G13) or glutamine at residue 61 (Q61). These single amino acid mutations impair intrinsic and GAP-stimulated GTP hydrolysis, favoring formation of RAS-GTP. Despite the similar functional consequences of these mutations, they are found at disparate frequencies among cancer types and among *RAS* genes. For example, in solid tumors *KRAS* mutations occur most commonly at G12, whereas *NRAS* mutations occur most commonly at Q61<sup>1</sup>. Similarly, *RAS* mutations in pancreatic ductal adenocarcinoma (PDAC) are almost exclusively found in *KRAS* (~98%), whereas in melanoma mutations in *NRAS* predominate (94%)<sup>1</sup>. These findings suggest that the different RAS isoforms, as well as individual mutations within one RAS protein, have distinct properties despite their high sequence similarity and conserved function.

After almost four decades of intensive research, no clinically effective therapies for *RAS*-mutant cancers have been developed, placing RAS at the top of the therapeutic “most-wanted” list. Past challenges in therapeutic targeting of *RAS*-mutant cancers can be ascribed to multiple causes. First, the RAS structure does not display an attractive topology for the design of high affinity and selective small molecule recognition, prompting the widely held perception that RAS itself is “undruggable”. Second, whereas potent and selective inhibitors of the RAF-MEK-ERK protein kinase cascade have been developed to block this key RAS effector pathway, the development and efficacy of MEK inhibitors have been limited by resistance caused by the relief of ERK-mediated feedback inhibition, leading to compensatory reactivation of ERK. Third, inhibitors of farnesyltransferase and thereby of RAS membrane association were rendered ineffective by cells’ unanticipated compensatory use of the related enzyme geranylgeranyltransferase-I, restoring *KRAS* and *NRAS* (but not *HRAS*) membrane association. Fourth, searches for synthetic lethal genetic interactors with mutant RAS proteins were compromised by off-target activities of RNA interference (RNAi) constructs as well as the use of flawed cell models. An additional complicating issue has been an assumption that all *RAS*-mutant cancers share the same therapeutic vulnerabilities. With lessons learned from past failures, recent studies have reinvigorated interest in pursuing directions once left for dead.

## Direct RAS targeting

Recent findings have begun to challenge the perception that RAS is undruggable. A breakthrough that has seen tremendous progress is the development of direct RAS inhibitors that specifically target the glycine to cysteine mutation at residue 12 (G12C) in *KRAS*. This often smoking-associated G12C mutation represents ~12% of all *KRAS* mutations (per COSMIC v89) and is the most prevalent *KRAS* mutation (46%) in non-small cell lung cancer (NSCLC), the most deadly cancer type across both sexes in the US<sup>2</sup>. The first cysteine-directed tethering of a small molecule to *KRAS*<sup>G12C</sup> identified a previously unknown pocket beneath the dynamic SWII region of RAS-GDP, but not RAS-GTP. Therefore, such molecules can lock *KRAS*<sup>G12C</sup> in the inactive GDP state, thus preventing effector engagement<sup>3</sup>. It has been established that substitutions at residues 12, 13, or 61 render RAS insensitive to GAP-catalyzed hydrolysis of GTP to GDP<sup>4</sup>. However, the G12C

mutation does not significantly impair intrinsic GTP hydrolysis compared to other G12/G13 mutations<sup>5</sup>. Consequently, KRAS<sup>G12C</sup> occupies the GDP-bound state more frequently than other mutants and is still dependent on GEF stimulation to achieve full activation. This existence of KRAS<sup>G12C</sup> in the GDP-bound state, together with cysteine-reactive warheads, presented a unique therapeutic vulnerability for G12C-directed inhibitors that recognize only the GDP-bound protein. As a result, first-in-class RAS<sup>G12C</sup>-specific inhibitors have entered phase I/II clinical trials in solid tumors that have a KRAS<sup>G12C</sup> mutation: AMG 510 (NCT03600883, NCT04185883), MRTX-849 (NCT03785249), JNJ-74699157 (NCT03114319), and LY3499446 (NCT04165031) with more in the pipeline. Early clinical trial observations have been promising, in particular for NSCLC<sup>6</sup>.

Nevertheless, treatment-induced acquired resistance will almost certainly reduce the long-term effectiveness of these inhibitors. Lou and colleagues (2019) set out to address this concern by performing a genome-wide CRISPR interference (CRISPRi) screen in search of genes that sensitize cells to KRAS<sup>G12C</sup> inhibition<sup>7</sup> (Figure 1). Using the recently reported G12C inhibitor ARS-1620<sup>8</sup> (G12Ci), they describe genetic dependencies revealed upon KRAS<sup>G12C</sup> inhibition. As expected, they found that G12Ci lethality was enhanced by silencing upstream components of receptor tyrosine kinase (RTK)-mediated signaling that lead to RAS activation, thereby increasing RAS-GDP and making available more SH2 pockets for G12Ci binding. These components included RTKs themselves (such as AXL and FGFR1), scaffolding proteins between RTKs and the RASGEF SOS1 (such as GRB2 and SHP2), as well as SOS1 itself. These results fit with recent studies in preclinical models of mutant KRAS-driven NSCLC and PDAC that found a significant therapeutic benefit from combining the MEK inhibitors selumetinib or trametinib with the SHP2 inhibitor SHP-099<sup>9,10</sup>. Lou *et al.* also observed sensitization to KRAS<sup>G12C</sup> inhibition by loss of genes encoding components of the PI3K-AKT-mTOR pathway (e.g., *PI3KCA*, *AKT1*, *RHEB*, *RPTOR*) and cell cycle regulators (*CDK4* and *CCND1*), consistent with previous findings with RAF and MEK inhibitors<sup>11,12</sup>. Together, these studies suggest that, as KRAS<sup>G12C</sup> inhibitors make their way through the clinic, rational combinations with both upstream and downstream signaling components (such as MEK; NCT04185883) would be beneficial to delay onset of resistance and obtain long-term responses.

This approach to directly targeting KRAS<sup>G12C</sup> is an example of an anti-RAS strategy that cannot be readily applied to other RAS mutants. Although the SWII pocket is present on all GDP-bound RAS proteins, compounds that target KRAS<sup>G12C</sup> rely on the reactive cysteine substitution and the continued dependency of this mutant on GEF activity to achieve its full activated state. Further, the G12C mutation is found at significantly lower frequencies in colorectal (CRC; 8%) and pancreatic ductal (PDAC; 2%) adenocarcinomas<sup>1</sup>, the second and third most common causes of US cancer deaths. Therefore, new strategies need to be considered for the more prevalent KRAS mutations, G12D and G12V. With momentum coming from the first KRAS<sup>G12C</sup>-specific drug entering the clinic, there is hope that the perception of targeting the other RAS mutations becomes widely regarded as “not-yet-druggable”.

Aside from cysteine-tethering screens used to find KRAS<sup>G12C</sup> inhibitors, to date two alternative approaches have been described to identify druggable surfaces on RAS: (1)

fragment library screens<sup>13,14</sup>, and (2) small antibody-like protein screens<sup>15,16</sup>. These methods have yielded promising results, including a new generation of RAS:SOS inhibitors, as well as small molecules and protein binding domains that disrupt effector binding.

In addition to these strategies, a recent discovery has identified a novel approach for directly inhibiting RAS proteins. Biancucci *et al.* (2019) reported the mechanism by which a bacterial endopeptidase, termed the Ras/Rap1-specific endopeptidase (RRSP), disrupts RAS function<sup>17</sup> (Figure 2). RRSP was reported previously to cause proteolytic processing of RAS proteins between residues Tyr-32- and Asp-33 in SWI. Importantly, this cleavage site is present in all RAS proteins and oncogenic mutants, although also found in the related RAPI small GTPase. Proteolytic processing by RRSP did not cause the degradation of RAS or release of nucleotide, but instead locally altered RAS SWI structure that impaired both SOS1 as well as RAF effector binding. While the 494-residue RRSP peptide itself is not a feasible therapeutic, it may be a useful tool reagent for defining a novel RAS vulnerability. That RRSP can disable both WT and mutant RAS proteins can help discern the therapeutic advantages and disadvantages of pan-RAS versus RAS mutant- or isoform-selective inhibitors. Together these strategies have presented clues towards building a growing arsenal of anti-RAS approaches that will inform the development of translatable therapies.

## Targeting upstream regulators

At present, the clinical value of identifying a *RAS* mutation is largely to guide against the use of certain therapies. Thus, while EGFR inhibitors are approved for the treatment of CRC, the FDA recommendation is that patients with *KRAS* mutations should not be candidates for this therapy. In particular, some studies of CRC patients treated with anti-EGFR monoclonal antibodies have shown that patients harboring *KRAS*<sup>G13D</sup> mutations fare better in overall and/or progression free survival than those harboring other RAS mutations<sup>18</sup>. While subsequent studies did not support this premise<sup>20,21</sup>, nevertheless, there is growing appreciation that all *KRAS*-mutant cancers should not be considered one homogeneous patient group<sup>21</sup>. The idea that any *KRAS* mutant cancer would still be responsive to EGFR inhibitor therapy is counterintuitive, given that a key cancer driving consequence of aberrant EGFR signaling is hyperactivation of RAS and ERK MAPK signaling. To address this concept, McFall and colleagues (2019) used computational modeling to identify a mechanism whereby *KRAS*<sup>G13D</sup> mutant CRC may still be uniquely dependent on EGFR signaling<sup>22</sup>. This invokes the active role that the remaining WT RAS isoforms, NRAS and HRAS, play in supporting the cancer driver function of *KRAS*<sup>G13D</sup>. Previous work established that WT RAS can have tumor promoting activity in cells containing mutant (MT) RAS<sup>23–25</sup>. Further, MT RAS can activate WT RAS by binding to the allosteric activation site of the RASGEF SOS1<sup>26</sup> or by sequestration of RASGAPs, both of which lead to increased GTP-bound WT RAS but by different mechanisms. McFall *et al.* report that other *KRAS* mutants are highly effective at sequestering the RASGAP neurofibromin (NF1) and thereby promote formation of WT RAS-GTP independent of upstream RTK activity. In contrast, they report that the *KRAS*<sup>G13D</sup> mutant is much less effective at NF1 sequestration, so only the *KRAS*<sup>G13D</sup> mutant CRC cells remain dependent upon EGFR-stimulated, SOS1-dependent formation of GTP-bound WT RAS (Figure 1). The authors therefore speculate that this provides a mechanistic basis for a therapeutic benefit of

EGFR inhibition in KRAS<sup>G13D</sup> mutant CRC. Another recent study also determined that KRAS<sup>G13D</sup> has distinct structural and biochemical properties, with reduced engagement of RAF and oncogenic potency<sup>27</sup>. These findings with KRAS<sup>G13D</sup> further exemplifies the importance of considering each RAS mutation as distinct.

An early misconception due to the high sequence identity of the four RAS proteins was that they have essentially identical roles as cancer drivers. The focus of early RAS studies on HRAS, based largely on convenience of available reagents, in turn led to additional misconceptions. Beginning with the unexpected finding that farnesyltransferase inhibitors were effective in blocking HRAS, but not KRAS or NRAS, membrane association, the field has refocused on studying the most commonly mutated RAS isoform and its most widely expressed splice variant, KRAS4B. RAS isoform differences in effector utilization have also been described. For example, HRAS, NRAS, and KRAS are reported to have varying specificities for the activation of the downstream effectors PI3K and RAF, with HRAS activating PI3K more strongly than K/NRAS and, conversely, KRAS activating RAF more strongly than H/NRAS<sup>28</sup>. This effector preference could explain tumor promoting roles of WT RAS in the context of MT RAS. Sheffels and colleagues (2018) have shed new light on this topic by reporting that, in a model mouse fibroblast cell system, MT KRAS, but not MT NRAS or HRAS, was more dependent on activation of WT HRAS for growth and oncogenic transformation. They report that MT KRAS dependence on WT RAS activity was due to a requirement for activation of PI3K-AKT, which is accomplished principally by WT HRAS and NRAS. Interestingly, the activation of WT RAS was SOS2-dependent, and did not depend on the feed-forward activity of MT RAS binding to the allosteric region, as has been reported for SOS1<sup>26</sup> (Figure 1). In this model, upstream RTK activation led to the SOS2-dependent activation of PI3K by WT RAS to promote MT KRAS-driven growth. Furthermore, it presents targeting SOS2 as a general strategy for inhibiting WT RAS isozymes in the context of MT KRAS. Targeting SOS2 alone as opposed to both SOS1 and SOS2 might be more therapeutically tractable, as previous reports found double knockout of SOS1 and SOS2 in adult mice to be lethal, whereas mice with single knockout of either SOS1 or SOS2 were viable<sup>29</sup>. This is a timely observation as a new SOS1 therapeutic recently entered phase I clinical trials in KRAS mutant solid tumors (BI-1701963, [NCT04111458](#)).

## Targeting downstream effector signaling

Extensive efforts have been devoted to the targeting of RAS downstream effector signaling, with inhibitors already approved or in advanced clinical candidacy. The RAF-MEK-ERK signaling network, followed by PI3K-AKT-mTOR, are considered the most important mediators of MT RAS-dependent cancer growth. This prominence is supported by findings in mouse models of KRAS-driven cancer as well as by the frequent mutational activation of these RAS effectors (such as PIK3CA<sup>H1047R/E545K</sup> and BRAF<sup>V600E</sup>) in cancer. Fortunately, these signaling components are readily druggable kinases, with inhibitors for both BRAF and PI3K $\alpha$  approved for cancer treatment. However, because of the central roles of these kinase networks in normal cell physiology, and of inhibitor-driven induction of compensatory signaling resistance mechanisms, the clinical advancement of effector inhibitors has been met with both expected and unexpected complications.

Perhaps the most progress has been made in the development of RAF and MEK inhibitors for *BRAF*-mutant melanoma, with three approved inhibitors each for BRAF and for MEK. Mutations in BRAF were identified in 2002 in several tumor types, leading to initial efforts focused on targeting the then lesser-studied RAF isoform, BRAF. The three RAF family proteins (ARAF, BRAF and CRAF/RAF1) are serine/threonine kinases that are activated upon binding to RAS-GTP through translocation to the plasma membrane as well as through relief of N-terminal autoinhibition of the C-terminal kinase domain. Additionally, RAF hetero-/homo-dimerization mediated by association with RAS is required for full activation of RAF catalytic activity towards their only widely accepted substrates, MEK1 and MEK2. MEK1/2 are dual specificity kinases that, like RAF, have only two well-validated substrates, the highly related ERK1 and ERK2 MAPKs. This substrate stringency led to the earlier misconception that the RAF-MEK-ERK kinase cascade acts as a simple linear and unidirectional pathway that culminates in ERK activation, in turn leading the field to focus initially on therapeutics targeting BRAF and MEK, rather than ERK. The first clinically approved BRAF inhibitors, vemurafenib and dabrafenib, were effective in treating *BRAF*-mutant melanoma. However, these inhibitors led to an unexpected paradoxical activation of CRAF through their ability to activate RAS-dependent formation of RAF homo-/hetero-dimers, reviewed elsewhere<sup>30</sup>.

The paradoxical activation of RAF by early BRAF inhibitors prompted the development of RAF inhibitors that do not lead to this unwanted activation, and hence are termed ‘paradox-breakers’. These paradox-breakers are now in clinical development. There is still a concern, however, that even pan-RAF inhibitors will meet challenges like those of BRAF inhibitors, where pathway reactivation occurs through the upregulation of RTKs and relief of negative feedback. To address this, combinations with MEK1/2 inhibitors, such as the clinically approved MEK inhibitors trametinib, cobimetinib and binimetinib, can aid in preventing pathway reactivation that leads to the activation of ERK1/2. The vertical inhibition strategy of targeting BRAF and MEK together has been evaluated in BRAF-mutant melanoma, where combination BRAF and MEK inhibitor treatment is now the standard-of-care<sup>31</sup>. Since ERK1/2 has a broad signaling network of over 1,000 intracellular targets, and since the RAS-RAF-MEK cascade results in the activation of ERK, perhaps inhibiting ERK1/2 would be even more effective. Preclinical models have suggested that this could be the case<sup>32</sup>. Although the development of ERK1/2 inhibitors has lagged behind RAF and MEK inhibitors, appreciation that ERK reactivation is a major basis for acquired resistance to RAF and MEK inhibitors prompted renewed interest. Several selective ERK1/2 inhibitors are now in clinical evaluation ([NCT02857270](#), [NCT03520075](#), [NCT03415126](#) and [NCT03698994](#)).

The PI3K-AKT-mTOR pathway is complementary to the RAF-MEK-ERK cascade and its targeting has been an attractive strategy in RAS mutant cancers. However, in some tissues this pathway seems to play a lesser role in RAS-dependent cancer growth compared to the MAPK pathway. Inhibition of the PI3K pathway, either directly or indirectly, often leads to the upregulation of the MAPK pathway. Disappointingly, the combination of PI3K-AKT-mTOR inhibitors and RAF-MEK inhibitors, despite impressive activities in preclinical mouse models, has been limited by toxicity in cancer patients. Nonetheless, there is hope that some of these challenges will be overcome with more selective, isoform-specific inhibitors. For example, combinations of KRAS<sup>G12C</sup> inhibitors with PI3K-AKT-mTOR

inhibitors should be better tolerated because they would not block activation of ERK MAPK signaling by WT RAS<sup>33</sup>. In a complementary manner, recent reports suggest that RASGEF inhibitors would effectively act as RAS-dependent PI3K and MAPK inhibitors through the impairment of their activation by WT RAS<sup>34</sup>. As progress is made in our understanding of the complex compensatory signaling mechanisms that limit the effectiveness of single agent inhibition of RAS effector signaling, it is hoped that combination inhibitor strategies will be the key to success for therapeutic targeting of the RAF and PI3K effector signaling networks.

In recent years, efforts to find synthetic lethal interactors with mutant KRAS have not uncovered any ‘silver bullets’, as was hoped for at the onset of these studies<sup>35</sup>. In search of genes that selectively kill MT RAS-driven cells, these efforts largely depend on genetic loss-of-function screens, such as RNAi or more recently CRISPRi/CRISPR-KO, in both MT RAS and WT RAS cells. Project DRIVE, collecting perhaps the largest set of cell line dependencies following genome-wide shRNA knock-down, indicated that some of the commonly used MT KRAS cell line models were not dependent on continued expression of KRAS<sup>36</sup>. This observation reminds us that not all MT-RAS driven cells exhibit the same oncogene dependencies, and that synthetic lethal interactions with MT-RAS are highly dependent on specific contexts of tissue, cell type, RAS allele, and RAS mutation.

Unlike the restricted number of substrates of RAF and MEK, hundreds of validated/putative ERK substrates have been identified. Of these substrates, the MYC transcription factor, another ‘undruggable’ cancer target, is arguably the critical component of ERK-dependent cancer growth<sup>37</sup>. The critical interdependency of RAS and MYC in oncogenesis is well-established. While many of the efforts to target MYC have centered on blocking *MYC* gene transcription, a recent study demonstrated the critical role of KRAS in maintaining MYC protein stability, through protein kinases that have yet to be identified<sup>38</sup>. Motivated by this, Blake *et al.* (2019) characterized and applied a MYC degradation screen in PDAC cells to identify protein kinase modulators of MYC protein stability<sup>39</sup>. Using this screen, they identified cyclin-dependent kinase 9 (CDK9) and determined that CDK9 regulated MYC protein expression at both the transcriptional and post-translational levels (Figure 2). With KRAS dependent on MYC to drive cancer growth, disruption of the mechanisms by which KRAS maintains MYC protein stability represents a tractable therapeutic strategy.

As with conventional cytotoxic oncology drugs, where combinations are the rule (e.g., FOLFIRINOX for PDAC), cocktails of signaling inhibitors will likely be required to effectively target RAS effector signaling. Sun *et al.* (2017) identified an initially surprising synergistic combination in NSCLC of MEK inhibition and PARP inhibition<sup>40</sup>. Their finding that inhibition of MEK increased expression and reliance on PARP then enabled rational targeting of this combination. Similarly, Chen *et al.* (2018) reported the synergistic combination of a pan-RAF inhibitor with a CDK4/6 inhibitor, where RAF inhibition could ablate the upregulation of cyclin D1 that occurs upon CDK4/6 inhibition<sup>41</sup>. More recently, two studies independently determined that ERK MAPK inhibition increased the dependency of KRAS-mutant PDAC on autophagy, leading to clinical evaluation of combination MEK and autophagy inhibition for PDAC<sup>42,43</sup>. These and other findings suggest that effective

signaling inhibitor combinations may not always be logically deduced *a priori*, but once identified, can be mechanistically understood and exploited.

## Conclusions and future perspectives

The RAS research field and the pharmaceutical industry have struggled mightily for nearly four decades in search of an elusive anti-RAS therapy. At times, the prospects seemed dim, with past failures creating a perception that RAS is undruggable. However, recent findings reveal promising new directions for these efforts. The advancement of KRAS<sup>G12C</sup>-selective inhibitors to early clinical promise has stimulated considerable excitement that at long last RAS can be targeted. For other RAS mutants, combination strategies that target components upstream or downstream of RAS might open new therapeutic windows. The long history of developing RAS-targeted therapeutics has seen ups and downs, twists and turns, but is ultimately heading towards effective therapeutic strategies.

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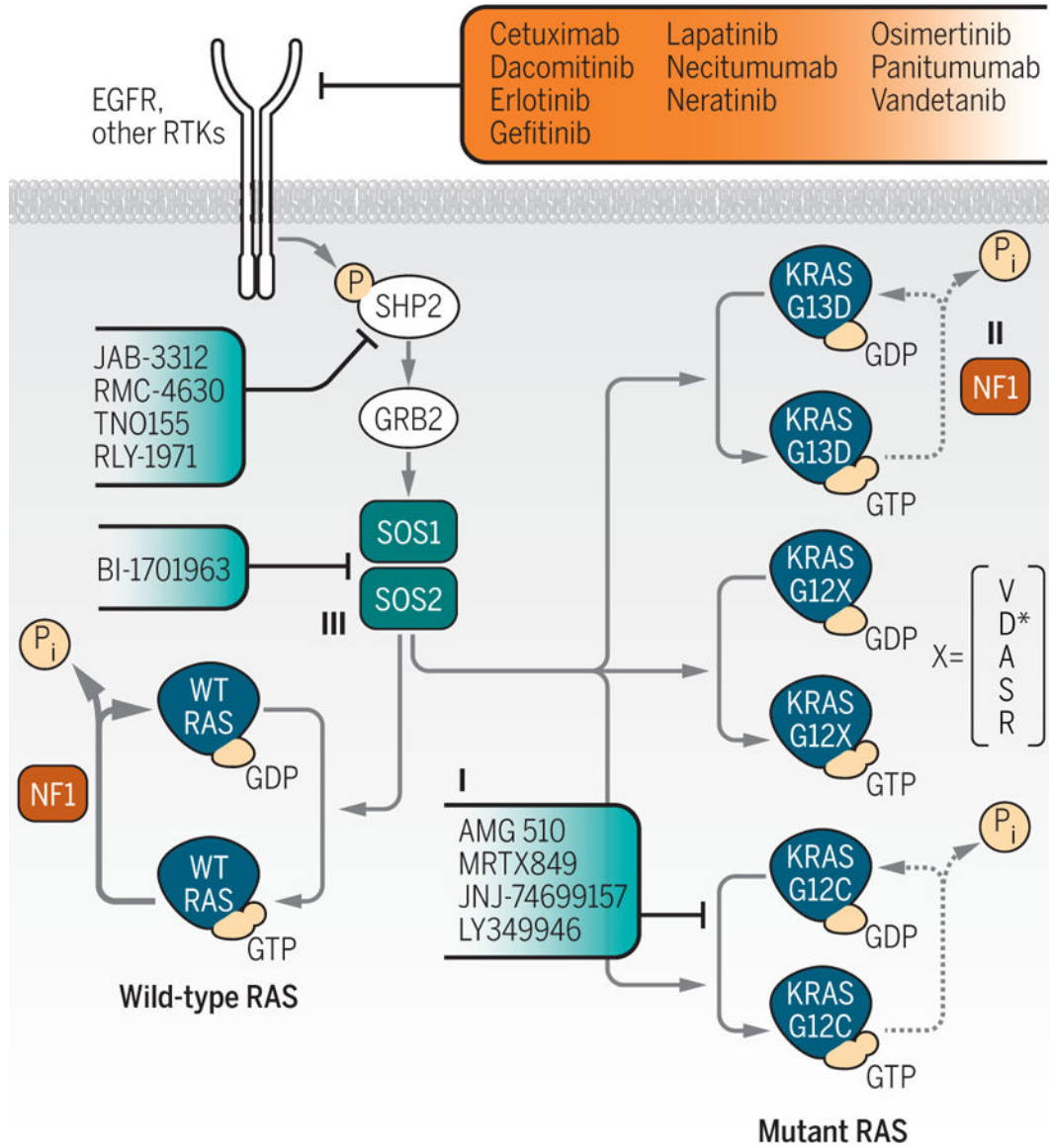
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**Figure 1. Defining novel RAS vulnerabilities for the development of anti-RAS strategies.** A series of recent studies, addressing different aspects of RAS function, have provided potential new clues to targeting RAS for cancer treatment. Where appropriate, clinically approved (red) and investigational (blue) therapeutics indicated for targeting RAS activation and signaling in cancer are noted. Lou *et al.* applied a CRISPR genetic loss-of-function screen to identify signaling modulators of a small molecule inhibitor selective for the KRAS<sup>G12C</sup> mutant (I) found predominantly in lung cancer<sup>7</sup>. In contrast to other G12 mutants, KRAS<sup>G12C</sup> retains intrinsic GTP-hydrolysis (\*with the exception of G12D which retains minor GTP-hydrolysis), thereby enabling its targeting by GDP-KRAS<sup>G12C</sup>-specific small molecules. The findings revealed various pathways to target to enhance the potency and durable efficacy of the inhibitor. McFall *et al.* proposed a mechanism to explain the EGFR-dependence of KRAS<sup>G13D</sup>-mutant colorectal cancer<sup>22</sup>. Decreased affinity for the RAS-GAP NF1 (II) by the G13D mutant protein enables GAP- and EGFR-dependent

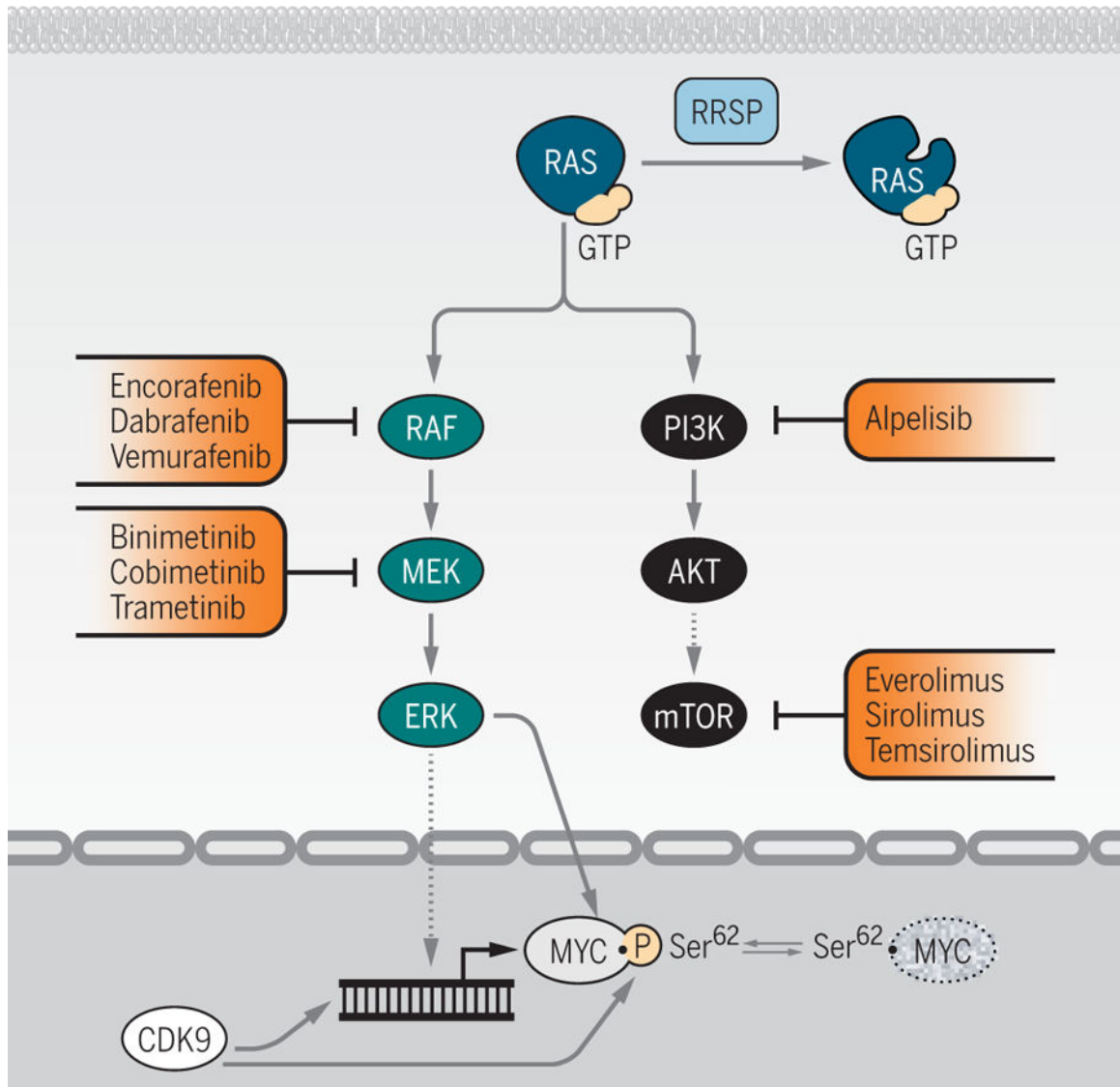
regulation of wild-type RAS, thereby retaining sensitivity to EGFR inhibitors in KRAS<sup>G13D</sup>-mutant cells. And Sheffels *et al.* identified a role for the RAS-GEF SOS2 (III) in promoting WT HRAS activation of AKT to support mutant KRAS-induced transformation of mouse fibroblasts in 3D growth culture conditions.

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**Figure 2. More RAS vulnerabilities, downstream.**

Insight into additional aspects of RAS protein and pathway regulation reveal more ways to potentially target RAS. Biancucci *et al.* showed that endopeptidase RRSP modification of RAS (I) impairs its interaction with downstream kinase RAF<sup>17</sup>. Looking further downstream still, Blake *et al.* applied a MYC degradation screen to identify the kinase CDK9 as a positive regulator of MYC protein stability (II) and, consequently, cell growth and survival<sup>39</sup>. Some clinically approved therapeutics indicated for targeting RAS activation and signaling in cancer are noted (red).