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Molecular Pathways: Targeting *NRAS* in Melanoma and Acute Myelogenous Leukemia

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Abstract

Successful targeting of specific oncogenic "driver" mutations with small-molecule inhibitors has represented a major advance in cancer therapeutics over the last 10–15 years. The most common activating oncogene in human malignancy, *RAS* (rat sarcoma), has proved to be an elusive target. Activating mutations in *RAS* induce mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase–AKT pathway signaling and drive malignant progression in up to 30% of cancers. Oncogenic *NRAS* mutations occur in several cancer types, notably melanoma, acute myeloid leukemia (AML), and less commonly, colon adenocarcinoma, thyroid carcinoma, and other hematologic malignancies. Although *NRAS*-mutant tumors have been recalcitrant to targeted therapeutic strategies historically, newer agents targeting MAPK/extracellular signal–regulated kinase kinase 1 (MEK1)/2 have recently shown signs of clinical efficacy as monotherapy. Combination strategies of MEK inhibitors with other targeted agents have strong preclinical support and are being evaluated in clinical trials. This review discusses the recent preclinical and clinical studies regarding the role of *NRAS* in cancer, with a focus on melanoma and AML.

Background

Wild-type *NRAS*

Three *RAS* (rat sarcoma) family members are frequently mutated across the spectrum of malignancy: *NRAS* (neuroblastoma RAS), *KRAS* (Kirsten RAS), and *HRAS* (Harvey RAS). Ras proteins comprise a family of low-molecular-weight GTPases. Wild-type RAS serves a critical role in cellular proliferation; *KRAS* knockout mice are characterized by embryonic lethality due to liver insufficiency and anemia (1). NRAS and HRAS appear to be more

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dispensable; knockout mice have mildly immune-deficient and normal phenotypes, respectively, suggesting that expression of these genes is less ubiquitous (2).

RAS proteins function as a conduit for signals received from receptor tyrosine kinases (RTKs) on the cell surface through downstream cell signaling partners to nuclear transcription factors regulating cell growth and cell cycling proteins. Under physiological conditions, RAS activation is initiated by binding of an upstream RTK to its ligand (see Figure 1). This interaction induces RTK autophosphorylation, dimerization, and activation. Adaptor molecule recruitment is triggered (such as growth factor receptor-bound protein 2 [grb2]), which subsequently recruits one of a family of guanine nucleotide exchange factors (GEFs). These GEFs catalyze the rate-limiting step of RAS activation: the exchange of a GDP for a GTP and include son of sevenless homolog 1 (SOS1), SOS2, and Ras protein-specific guanine nucleotide-releasing factor (3). A number of GTPase-activating proteins (GAPs), notably including neurofibromin 1 (NF1), function as RAS suppressors and oppose this activation step. Once activated, RAS signals through a variety of downstream targets, most notably the mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K)–AKT, and Ral–guanine nucleotide dissociation stimulator (GDS) pathways to induce cell growth and proliferation. Many other RAS targets have also been described, including AF-6, Ras and Rab interactor 1 (RIN-1), and phospholipase C, but their role in normal and aberrant signaling is unknown.

Mutant *NRAS*

Oncogenic activation of *RAS* has been described in 20%–30% of human cancers (4–8). RAS is named for a retrovirus that induced murine sarcomas that were later found to have activating *RAS* mutations (9). *NRAS* alterations were initially identified in 1983 on chromosome 1 in neuroblastoma, closely following the identification of *KRAS* and *HRAS* (10, 11). Constitutive activation in the setting of malignancy is caused by a single point mutation, almost exclusively occurring in codons 12, 13, and 61. Mutations in codon 61 induce *RAS* activation by disrupting GTPase activity and thereby locking RAS into its active conformation. Codon 12 and 13 mutations produce the same overall effect by decreasing sensitivity to the GAPs (12). Oncogenic mutations in codons 12 and 13 predominate in *KRAS* and *HRAS* across the spectrum of malignancies. *NRAS* mutations occur much more often in codon 61 in both melanoma and acute myeloid leukemia (AML) and most frequently involve an arginine for glutamine substitution (Q61R) (13). Notably, inactivating mutations or deletions in *NF1*, a GAP, dysregulates *NRAS* and induces similar pathway activation as mutant *NRAS*.

NRAS mutations are present in the majority of congenital melanocytic nevi but occur only rarely in other benign melanocytic nevi. By contrast, mutations in *BRAF* are identified in the large majority of benign nevi (14, 15). This suggests that *NRAS* mutations are an early, critical oncogenic event in melanomagenesis but are not sufficient to induce invasive melanoma without cooperating genetic events (such as cyclin-dependent kinase [CDK]/retinoblastoma [Rb] pathway alterations or loss of p53) (16). The role of *NRAS* in oncogenic progression in AML is less well understood. An analysis of samples from patients with myelodysplastic syndromes (MDS) and AML arising from MDS identified only a modest

increase in the frequency of *NRAS* mutations in the secondary AML cohort compared with the MDS group (11% vs 5.7%), suggesting that *NRAS* mutations may be an early event in MDS (17). By contrast, mutations in other genes frequently altered in AML (such as *NPM1*, *FLT3*, *RUNX1*, and *MLL*) were present much more often in the AML samples compared with MDS. NRAS in colon adenocarcinoma may play a different pathophysiological role than KRAS. In a mouse model, KRAS G12D caused hyperproliferation and induced invasive adenocarcinoma in conjunction with adenomatous polyposis coli loss, whereas NRAS G12D conferred resistance to apoptosis but did not induce cellular proliferation (18).

Constitutively activated NRAS signals through several pathways to mediate oncogenic effects, notably the MAPK, PI3K-AKT, and Ral-GDS pathways (3). Under physiological conditions in normal melanocytes, wild-type activated NRAS signals through dimers of BRAF rather than CRAF. In *NRAS*-mutant melanoma, extracellular signal–regulated kinase (ERK)–mediated feedback inactivates BRAF, RAF isoform switching occurs, and mutant NRAS mediates downstream signaling through CRAF (19). NRAS-induced MAPK signaling leads to cyclin D1 expression and cell cycle dysregulation and promotion of prosurvival pathways (20, 21). Although the effects of mutant NRAS on PI3K-AKT and Ral-GDS signaling are less well characterized, there is evidence that these pathways have nonredundant functions in oncogenic transformation (22). PI3K, AKT3, or PTEN alterations rarely co-occur with NRAS mutations, suggesting that activated NRAS is sufficient to promote activation of this pathway (6). Oncogenic RAS also appears to promote metastases, immune evasion, metabolic reprogramming, and microenvironment remodeling (3, 13). Recent studies have shown *NRAS*-mutant melanomas to display a greater dependency upon the expression of the prosurvival protein Mcl-1 than their *BRAF*-mutant counterparts (23).

Clinical implications of *NRAS* mutations

NRAS mutations are present in 15%–20% of melanomas, 10% of AMLs, 1%–2% of colon cancers, and 8%–10% of thyroid cancers (13). *NRAS* mutations are also present in a variety of other hematologic malignancies, including acute lymphocytic leukemia (11%), multiple myeloma (18%), MDS (5%), and chronic myelomonocytic leukemia (19%) (24–26). In melanoma, *NRAS* confers distinct prognostic and histopathologic characteristics when compared with other genetic subtypes. *NRAS*-mutant melanoma has been associated with poor prognosis compared with *BRAF*-mutant and *NRAS*/*BRAF* wild-type melanomas. Furthermore, compared with *BRAF*-mutant melanomas, primary tumors are thicker with more mitoses but are less often ulcerated. In childhood AML, activating *NRAS* mutations commonly co-occur with *NPM1* mutations and occur frequently in the favorable-risk population (27). No association with cytogenetic alterations or clinical outcomes has been identified. In colon adenocarcinoma, *NRAS* mutations do not appear to confer specific clinical features, although most series have assessed < 10 *NRAS*-mutant colon cancers (28).

Currently, it is unclear whether all activating *NRAS* mutations induce a common oncogenic phenotype or if particular amino acid substitutions in *NRAS* confer distinct clinical and prognostic features. Two small studies have suggested that $KRAS^{G12V}$ mutations conferred a worse prognosis compared with $KRAS^{G12D}$ in both lung and colon adenocarcinoma (29, 30). However, in hematologic malignancies (ie, childhood acute lymphocytic leukemia), specific

mutations in *NRAS* did not appear to induce differing clinical or prognostic features (31). In a retrospective study of colon adenocarcinoma, mutations in *NRAS* (any codon) or *KRAS* (codons 61, 117, and 146) appeared to confer resistance to the anti–epidermal growth factor receptor (EGFR) monoclonal antibody panitumumab in a similar fashion when compared with colon adenocarcinoma with *KRAS* codon 12 or 13 mutation (32).

Acquired mutations in *NRAS* (G12D/R, G13R, and Q61K/R/L) and *KRAS* (G12C, G12R, and Q61H) have emerged as resistance mechanisms to BRAF inhibitors. In one study, resistance to dabrafenib and vemurafenib occurred in 18% and 7% of patients with progressing *BRAF*-mutant melanoma, whereas another population demonstrated 18% of resistant tumors with *NRAS* mutations but none with *KRAS* mutations (33, 34). With so few tumors analyzed, it remains to be determined whether the combination of BRAF and MEK inhibitors will suppress the development of *NRAS*-mutant, BRAF inhibitor–resistant tumors.

Clinical-Translational Advances

Direct RAS targeting

Attempts to directly target NRAS with small-molecule inhibitors have been largely unsuccessful. The major class of directed RAS-targeted therapeutics investigated has been farnesyltransferase inhibitors (FTIs). Farnesylation of a cysteine residue on the RAS oncoprotein prior to its insertion into the cell membrane is the primary translational modification essential for transforming activity of RAS (35). Although preclinical activity was observed in *RAS*-mutant cell lines and animal models, clinical activity with this class of agents has been disappointing (36–38). In an unselected melanoma population, no responses were observed in 14 patients, despite potent inhibition of phosphorylated ERK (39). A single phase 2 trial in AML did demonstrate activity with FTI therapy (tipifarnib) with occasional complete responses; the mutational status of those responding patients was not reported (40). Off-target side effects were observed in these trials as numerous additional proteins crucial for cellular function were also likely inhibited.

RNA interference (RNAi) may be another potential approach to directly target NRAS. Rather than targeting the mutated protein, RNAi involves antisense oligonucleotides/small interfering RNAs (siRNA) that interfere with mRNA, inhibiting production of the oncoprotein (41). RNAi is a useful technique in preclinical models to thoroughly inhibit gene activity across a spectrum of mutations but has remained a challenge to incorporate clinically from a drug-delivery standpoint (42). These molecules lack stability in the circulation and require molecular modification for delivery of these nucleotides to tumors. A recent phase 1 study provided evidence that this approach may be feasible in clinical practice (43). This approach used nanoparticles packaged with siRNA designed to reduce the expression of ribonucleotide reductase M2 (*RRM2*) and demonstrated effective nanoparticle delivery to melanoma cells and decreased expression of *RRM2*. Despite the incredible promise of this technique, there remain some technical hurdles prior to clinical development.

MEK inhibition

Because direct targeting of GTPases has proved difficult, many efforts have shifted to inhibiting downstream mediators of NRAS. In *BRAF*-mutant melanoma, inhibition of MEK is an effective therapeutic strategy that has improved overall survival (44). Thus far, MEK inhibitors also appear to be the most active class of agents against *NRAS*-mutant melanoma (44). Preclinical models demonstrated activity of MEK inhibition in *NRAS*-mutant melanoma (45). Despite strong mechanistic and preclinical rationale for using MEK inhibitors in *RAS*-mutated malignances, their activity has been modest at best, with the exception of a single phase 2 trial (discussed below). Structural and functional analyses have identified a possible explanation for the differential sensitivity for *BRAF*- and *RAS*-mutant malignancies. An allosteric MEK inhibitor in development (cobimetinib; GDC-0973) that has demonstrated activity in *BRAF*-mutant melanoma potently inhibits phosphorylated MEK, which appears to be required to block MAPK signaling in *BRAF*-driven malignancies (46). Conversely, 2 MEK inhibitors earlier in the development process with more preclinical activity in *KRAS*-mutant cancers (GDC-0623 and G-573) induce a hydrogen bond interaction with the S212 codon of MEK, which inhibits phosphorylation by wild-type RAF. Other novel MEK inhibitors such as CH5126766 (RO5126766) function similarly and block the phosphorylation of MEK by reactivated CRAF, thereby inhibiting ERK signaling (47). Therefore, *RAS*-specific and *RAF*-specific MEK inhibitors may be distinguished for future development.

Binimetinib (MEK162), a selective MEK1/2 inhibitor, appears to be the most active small-molecule inhibitor for *NRAS*-mutant melanoma currently in development. A phase 2 trial assessed this compound in both *BRAF*- and *NRAS*-mutant melanoma; objective responses (confirmed and unconfirmed) were observed in 20% of patients in the *NRAS* group, and an additional 43% had stable disease as their best response (48). The progression-free survival in this study was similar for both the *NRAS*-mutant cohort (3.7 months) and the *BRAF* mutant-group (3.6 months). Currently, an *NRAS* mutant-specific phase 3 trial (NRAS Melanoma and MEK Inhibitor [NEMO]) comparing binimetinib with dacarbazine is ongoing (NCT01763164). Other MEK inhibitors have been less well studied but may also have some activity in *NRAS*-mutant melanoma. Although no responses have been observed with selumetinib, trametinib induced temporary stable disease in 2 of 7 treated patients in a phase 1 study (49, 50). RO5126766 demonstrated a single objective response in a patient with *NRAS*-mutant melanoma in a phase 1 trial (51). Because single-agent MEK inhibitor therapies may have some activity and are generally well tolerated, there is great enthusiasm for clinical trials assessing MEK inhibitors as a component of rationally chosen combination regimens.

Fewer preclinical or clinical studies have been conducted with MEK inhibitors in hematologic malignancies. Nevertheless, there is preclinical rationale to target MAPK signaling in *NRAS*- or *NF1*-mutated leukemia (52, 53). In AML, a phase 2 trial of selumetinib was conducted, with 7% of patients harboring *NRAS* mutations. None of these patients had an objective response, and modest activity was observed in the overall cohort (54). A genotype-unselected trial of trametinib in AML has recently completed accrual,

although results have not yet been reported. Additionally, a trial combining idarubicin, cytarabine, and binimetinib is planned for relapsed AML (NCT02049801).

Other inhibitors of MAPK signaling

Several other targeted agents are rational for use in *NRAS*-mutant melanoma on the basis of preclinical studies. Although the currently available RAF inhibitors induce paradoxical hyperactivation of ERK signaling in *BRAF* wild-type cells that promotes cancer growth, a new class of inhibitors that does not cause this phenomenon has been developed. One of these novel agents (PLX7904) demonstrated activity in vemurafenib-resistant cell lines that harbored a secondary *NRAS* Q61K mutation (55). SCH772984, an inhibitor of ERK1/2, the final common signaling component in the MAPK pathway, showed activity in xenograft models of *BRAF*- and *NRAS*-mutant melanomas (56). This agent has not entered clinical development, but other ERK inhibitors are in early-phase trials.

Combination therapy

Because *NRAS* activates multiple cell signaling pathways, single-agent MEK inhibition is likely insufficient to induce apoptosis and restrain tumor growth in most tumors. *NRAS* promotes both the MAPK and PI3K-AKT pathways; therefore, one obvious approach would be combining MEK inhibitors with agents blocking the PI3K-AKT pathway. See Figure 2 for a summary of NRAS-targeted therapies. Preclinically, there is significant rationale for dual pathway inhibition; additive activity was observed in *NRAS*-mutant cell lines with inhibition of both MAPK and PI3K-AKT signaling (57, 58). Currently, no clinical trials are recruiting that are restricted to *RAS*-mutant tumors, but several early-phase studies for advanced cancers are underway. These include the combination of trametinib and an AKT inhibitor (uprosertib; GSK2141795) in *BRAF* wild-type melanoma (NCT01941927) and in AML (NCT01907815). Binimetinib and several different PI3K/AKT pathway inhibitors are also being evaluated in early-stage trials (NCT01363232, NCT01337765, NCT01449058).

Recent preclinical observations have also generated a great deal of interest in combining MEK inhibitors with CDK4/6 inhibitors. Full review of the CDK4/6 pathway is outside the scope of this review but is briefly discussed here and reviewed elsewhere (59). The *CDKN2A* gene transcription product, $p16^{INK4A}$, inhibits CDK4 and CDK6. CDK4/6 are serine/threonine kinases that phosphorylate Rb1, diminishing its ability to regulate the cell cycle. *CDKN2A* loss or CDK4/6-activating mutations/amplifications therefore inhibit Rb1 function and thus promote cell cycle progression.

MAPK signaling and the cell cycle regulatory pathways are dysregulated in nearly all melanomas, suggesting that co-targeting these pathways may be an attractive treatment strategy (6). In an inducible mouse model with an *NRAS* Q61K mutation on the background of *CDKN2A* loss, MEK inhibition with trametinib induced apoptosis but did not induce cell cycle arrest or tumor regression. By contrast, extinction of *NRAS* by RNAi induced major tumor regression (60). When these molecular phenotypes were compared, CDK4 was identified as key to this differential effect. Subsequently, treatment with MEK and CDK4/6 inhibitors (trametinib and palbociclib, respectively) induced tumor regression, apoptosis, and cell cycle arrest. Based on these preclinical results, a clinical trial assessing MEK and

CDK4/6 inhibition with binimetinib and LEE011 (CDK4/6 inhibitor) in *NRAS*-mutant melanoma is now enrolling (NCT01781572). An additional phase I trial is being planned that combines trametinib and palbociclib (NCT02065063). If these agents prove effective, further trials will be needed to determine whether this combination is effective in other *RAS*-mutant malignancies or whether the effect is specific to melanoma.

Other combinations have demonstrated clinical or preclinical efficacy in *NRAS*-mutant melanoma. Sorafenib and tivantinib, a MET tyrosine kinase receptor inhibitor, were used in 8 patients with *NRAS*-mutant melanoma, with 2 patients achieving a complete or partial response and 2 additional patients experiencing best responses of stable disease (61). Preclinical data also suggest that combining inhibitors of MEK and WNT signaling, as well as AKT/nuclear factor κB inhibition, may have value in *NRAS*-mutant melanoma (62, 63). Targeting the molecular chaperone heat shock protein 90 (HSP90) is one strategy that allows the simultaneous suppression of multiple downstream targets of RAS signaling. In a recent preclinical study, the HSP90 inhibitor XL888 was noted to have promising antitumor activity in a panel of *NRAS*-mutant melanoma cell lines, in part through suppression of *CDK4*, *AKT*, and *WEE1* expression (23).

Immune-based therapy

The immune-based therapies (including high-dose interleukin 2 [IL-2], ipilimumab, and novel agents targeting the programmed cell death 1/ligand [PD-1/PD-L1] axis) are currently utilized in melanoma irrespective of genotype. Recent studies have suggested that immune therapy may confer increased benefit to the *NRAS*-mutant cohort. In a retrospective study, patients with *NRAS*-mutant melanoma had greater response rates to high dose IL-2 compared with other genetic subgroups (64). Additionally, we evaluated whether this observation extends to ipilimumab and anti–PD-1/PD-L1. Patients with *NRAS*-mutant melanoma had higher response rates to all immune therapy compared with those with *NRAS*/*BRAF* wild-type melanoma (32% vs 18%), with an especially marked benefit with anti–PD-1/PD-L1 in a small sample (65). No mechanism has yet been identified, although *NRAS*-induced up-regulation of melanoma lineage antigens or PD-L1 may explain this finding. These studies will need to be confirmed prospectively with increased patient numbers. In AML and other hematologic malignancies, trials of anti–PD-1/PD-L1 are in early phases; no data yet exist for a genotype-specific effect in these settings.

Conclusions

Activating *NRAS* mutations play a critical role in oncogenesis in a large percentage of melanomas and hematologic malignancies. Mutated *NRAS* promotes oncogenesis through activated MAPK, PI3K, and Ral-GDS signaling and confers specific clinical and pathological characteristics in melanoma; its phenotypic effects are less clear in other cancers. Targeting *NRAS* has been a challenge clinically. Single-agent MEK inhibitors are showing early signs of clinical efficacy. Strategies combining MEK inhibitors with agents targeting the CDK4/6 or PI3K-AKT pathway members are promising approaches to more effectively treat patients with melanoma, whereas *NRAS* targeting has lagged behind in the

other malignancies. Rational combination strategies with a MEK inhibitor backbone may improve therapy for patients with *RAS*-mutated malignancies.

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References

1. Johnson L, Greenbaum D, Cichowski K, Mercer K, Murphy E, Schmitt E, et al. K-ras is an essential gene in the mouse with partial functional overlap with N-ras. Genes Dev. 1997; 11:2468–81. [PubMed: 9334313]
2. Esteban LM, Vicario-Abejón C, Fernández-Salguero P, Fernández-Medarde A, Swaminathan N, Yienger K, et al. Targeted genomic disruption of H-ras and N-ras, individually or in combination, reveals the dispensability of both loci for mouse growth and development. Mol Cell Biol. 2001; 21:1444–52. [PubMed: 11238881]
3. Fedorenko IV, Gibney GT, Smalley KS. NRAS mutant melanoma: biological behavior and future strategies for therapeutic management. Oncogene. 2013; 32:3009–18. [PubMed: 23069660]
4. Cancer Genome Atlas Network. Comprehensive molecular characterization of human colon and rectal cancer. Nature. 2012; 487:330–7. [PubMed: 22810696]
5. Cancer Genome Atlas Network. Comprehensive molecular characterization of urothelial bladder carcinoma. Nature. 2014; 507:315–22. [PubMed: 24476821]
6. Hodis E, Watson IR, Kryukov GV, Arold ST, Imielinski M, Theurillat JP, et al. A landscape of driver mutations in melanoma. Cell. 2012; 150:251–63. [PubMed: 22817889]
7. Cancer Genome Atlas Network. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. N Engl J Med. 2013; 368:2059–74. [PubMed: 23634996]
8. Ding L, Getz G, Wheeler DA, Mardis ER, McLellan MD, Cibulskis K, et al. Somatic mutations affect key pathways in lung adenocarcinoma. Nature. 2008; 455:1069–75. [PubMed: 18948947]
9. Malumbres M, Barbacid M. RAS oncogenes: the first 30 years. Nat Rev Cancer. 2003; 3:459–65. [PubMed: 12778136]
10. Shimizu K, Goldfarb M, Suard Y, Perucho M, Li Y, Kamata T, et al. Three human transforming genes are related to the viral ras oncogenes. Proc Natl Acad Sci U S A. 1983; 80:2112–6. [PubMed: 6572964]
11. Hall A, Marshall CJ, Spurr NK, Weiss RA. Identification of transforming gene in two human sarcoma cell lines as a new member of the ras gene family located on chromosome 1. Nature. 1983; 303:396–400. [PubMed: 6304521]
12. Colicelli J. Human RAS superfamily proteins and related GTPases. Sci STKE. 2004; 2004:RE13. [PubMed: 15367757]
13. Pylayeva-Gupta Y, Grabocka E, Bar-Sagi D. RAS oncogenes: weaving a tumorigenic web. Nat Rev Cancer. 2011; 11:761–74. [PubMed: 21993244]
14. Poynter JN, Elder JT, Fullen DR, Nair RP, Soengas MS, Johnson TM, et al. BRAF and NRAS mutations in melanoma and melanocytic nevi. Melanoma Res. 2006; 16:267–73. [PubMed: 16845322]
15. Botton T, Yeh I, Nelson T, Vemula SS, Sparatta A, Garrido MC, et al. Recurrent BRAF kinase fusions in melanocytic tumors offer an opportunity for targeted therapy. Pigment Cell Melanoma Res. 2013; 26:845–51. [PubMed: 23890088]

16. Bardeesy N, Bastian BC, Hezel A, Pinkel D, DePinho RA, Chin L. Dual inactivation of RB and p53 pathways in RAS-induced melanomas. Mol Cell Biol. 2001; 21:2144–53. [PubMed: 11238948]

17. Dicker F, Haferlach C, Sundermann J, Wendland N, Weiss T, Kern W, et al. Mutation analysis for RUNX1, MLL-PTD, FLT3-ITD, NPM1 and NRAS in 269 patients with MDS or secondary AML. Leukemia. 2010; 24:1528–32. [PubMed: 20520634]

18. Haigis KM, Kendall KR, Wang Y, Cheung A, Haigis MC, Glickman JN, et al. Differential effects of oncogenic K-Ras and N-Ras on proliferation, differentiation and tumor progression in the colon. Nat Genet. 2008; 40:600–8. [PubMed: 18372904]

19. Dumaz N, Hayward R, Martin J, Ogilvie L, Hedley D, Curtin JA, et al. In melanoma, RAS mutations are accompanied by switching signaling from BRAF to CRAF and disrupted cyclic AMP signaling. Cancer Res. 2006; 66:9483–91. [PubMed: 17018604]

20. Filmus J, Robles AI, Shi W, Wong MJ, Colombo LL, Conti CJ. Induction of cyclin D1 overexpression by activated ras. Oncogene. 1994; 9:3627–33. [PubMed: 7970723]

21. Boisvert-Adamo K, Aplin AE. Mutant B-RAF mediates resistance to anoikis via Bad and Bim. Oncogene. 2008; 27:3301–12. [PubMed: 18246127]

22. Mishra PJ, Ha L, Rieker J, Sviderskaya EV, Bennett DC, Oberst MD, et al. Dissection of RAS downstream pathways in melanomagenesis: a role for Ral in transformation. Oncogene. 2010; 29:2449–56. [PubMed: 20118982]

23. Haarberg HE, Paraiso KH, Wood E, Rebecca VW, Sondak VK, Koomen JM, et al. Inhibition of Wee1, AKT, and CDK4 underlies the efficacy of the HSP90 inhibitor XL888 in an in vivo model of NRAS-mutant melanoma. Mol Cancer Ther. 2013; 12:901–12. [PubMed: 23538902]

24. Ward AF, Braun BS, Shannon KM. Targeting oncogenic Ras signaling in hematologic malignancies. Blood. 2012; 120:3397–406. [PubMed: 22898602]

25. Curtin JA, Fridlyand J, Kageshita T, Patel HN, Busam KJ, Kutzner H, et al. Distinct sets of genetic alterations in melanoma. N Engl J Med. 2005; 353:2135–47. [PubMed: 16291983]

26. Irahara N, Baba Y, Nosho K, Shima K, Yan L, Dias-Santagata D, et al. NRAS mutations are rare in colorectal cancer. Diagn Mol Pathol. 2010; 19:157–63. [PubMed: 20736745]

27. Berman JN, Gerbing RB, Alonzo TA, Ho PA, Miller K, Hurwitz C, et al. Prevalence and clinical implications of NRAS mutations in childhood AML: a report from the Children's Oncology Group. Leukemia. 2011; 25:1039–42. [PubMed: 21358716]

28. Mouradov D, Domingo E, Gibbs P, Jorissen RN, Li S, Soo PY, et al. Survival in stage II/III colorectal cancer is independently predicted by chromosomal and microsatellite instability, but not by specific driver mutations. Am J Gastroenterol. 2013; 108:1785–93. [PubMed: 24042191]

29. Keohavong P, DeMichele MA, Melacrinos AC, Landreneau RJ, Weyant RJ, Siegfried JM. Detection of K-ras mutations in lung carcinomas: relationship to prognosis. Clin Cancer Res. 1996; 2:411–8. [PubMed: 9816185]

30. Andreyev HJ, Norman AR, Cunningham D, Oates J, Dix BR, Iacopetta BJ, et al. Kirsten ras mutations in patients with colorectal cancer: the 'RASCAL II' study. Br J Cancer. 2001; 85:692–6. [PubMed: 11531254]

31. Perentesis JP, Bhatia S, Boyle E, Shao Y, Shu XO, Steinbuch M, et al. RAS oncogene mutations and outcome of therapy for childhood acute lymphoblastic leukemia. Leukemia. 2004; 18:685–92. [PubMed: 14990973]

32. Douillard JY, Oliner KS, Siena S, Tabernero J, Burkes R, Barugel M, et al. Panitumumab-FOLFOX4 treatment and RAS mutations in colorectal cancer. N Engl J Med. 2013; 369:1023–34. [PubMed: 24024839]

33. Shi H, Hugo W, Kong X, Hong A, Koya RC, Moriceau G, et al. Acquired resistance and clonal evolution in melanoma during BRAF inhibitor therapy. Cancer Discov. 2014; 4:80–93. [PubMed: 24265155]

34. Van Allen EM, Wagle N, Sucker A, Treacy DJ, Johannessen CM, Goetz EM, et al. The genetic landscape of clinical resistance to RAF inhibition in metastatic melanoma. Cancer Discov. 2014; 4:94–109. [PubMed: 24265153]

35. Kohl NE, Wilson FR, Mosser SD, Giuliani E, deSolms SJ, Conner MW, et al. Protein farnesyltransferase inhibitors block the growth of ras-dependent tumors in nude mice. Proc Natl Acad Sci U S A. 1994; 91:9141–5. [PubMed: 8090782]
36. Rao S, Cunningham D, de Gramont A, Scheithauer W, Smakal M, Humblet Y, et al. Phase III double-blind placebo-controlled study of farnesyl transferase inhibitor R115777 in patients with refractory advanced colorectal cancer. J Clin Oncol. 2004; 22:3950–7. [PubMed: 15459217]
37. Van Cutsem E, van de Velde H, Karasek P, Oettle H, Vervenne WL, Szawlowski A, et al. Phase III trial of gemcitabine plus tipifarnib compared with gemcitabine plus placebo in advanced pancreatic cancer. J Clin Oncol. 2004; 22:1430–8. [PubMed: 15084616]
38. Konstantinopoulos PA, Karamouzis MV, Papavassiliou AG. Post-translational modifications and regulation of the RAS superfamily of GTPases as anticancer targets. Nat Rev Drug Discov. 2007; 6:541–55. [PubMed: 17585331]
39. Gajewski TF, Salama AK, Niedzwiecki D, Johnson J, Linette G, Bucher C, et al. Phase II study of the farnesyltransferase inhibitor R115777 in advanced melanoma (CALGB 500104). J Transl Med. 2012; 10:246. [PubMed: 23228035]
40. Kirschbaum MH, Synold T, Stein AS, Tuscano J, Zain JM, Popplewell L, et al. A phase 1 trial dose-escalation study of tipifarnib on a week-on, week-off schedule in relapsed, refractory or high-risk myeloid leukemia. Leukemia. 2011; 25:1543–7. [PubMed: 21625235]
41. Eskandarpour M, Kiaii S, Zhu C, Castro J, Sakko AJ, Hansson J. Suppression of oncogenic NRAS by RNA interference induces apoptosis of human melanoma cells. Int J Cancer. 2005; 115:65–73. [PubMed: 15688405]
42. Kelleher FC, McArthur GA. Targeting NRAS in melanoma. Cancer J. 2012; 18:132–6. [PubMed: 22453013]
43. Davis ME, Zuckerman JE, Choi CH, Seligson D, Tolcher A, Alabi CA, et al. Evidence of RNAi in humans from systemically administered siRNA via targeted nanoparticles. Nature. 2010; 464:1067–70. [PubMed: 20305636]
44. Flaherty KT, Robert C, Hersey P, Nathan P, Garbe C, Milhem M, et al. Improved survival with MEK inhibition in BRAF-mutated melanoma. N Engl J Med. 2012; 367:107–14. [PubMed: 22663011]
45. Davies BR, Logie A, McKay JS, Martin P, Steele S, Jenkins R, 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; 6:2209–19. [PubMed: 17699718]
46. Hatzivassiliou G, Haling JR, Chen H, Song K, Price S, Heald R, et al. Mechanism of MEK inhibition determines efficacy in mutant KRAS- versus BRAF-driven cancers. Nature. 2013; 501:232–6. [PubMed: 23934108]
47. Ishii N, Harada N, Joseph EW, Ohara K, Miura T, Sakamoto H, et al. Enhanced inhibition of ERK signaling by a novel allosteric MEK inhibitor, CH5126766, that suppresses feedback reactivation of RAF activity. Cancer Res. 2013; 73:4050–60. [PubMed: 23667175]
48. Ascierto PA, Schadendorf D, Berking C, Agarwala SS, van Herpen CM, Queirolo P, et al. MEK162 for patients with advanced melanoma harbouring NRAS or Val600 BRAF mutations: a non-randomised, open-label phase 2 study. Lancet Oncol. 2013; 14:249–56. [PubMed: 23414587]
49. Falchook GS, Lewis KD, Infante JR, Gordon MS, Vogelzang NJ, DeMarini DJ, et al. Activity of the oral MEK inhibitor trametinib in patients with advanced melanoma: a phase 1 dose-escalation trial. Lancet Oncol. 2012; 13:782–9. [PubMed: 22805292]
50. Patel SP, Lazar AJ, Papadopoulos NE, Liu P, Infante JR, Glass MR, et al. Clinical responses to selumetinib (AZD6244; ARRY-142886)-based combination therapy stratified by gene mutations in patients with metastatic melanoma. Cancer. 2013; 119:799–805. [PubMed: 22972589]
51. Martinez-Garcia M, Banerji U, Albanell J, Bahleda R, Dolly S, Kraeber-Bodere F, et al. First-inhuman, phase I dose-escalation study of the safety, pharmacokinetics, and pharmacodynamics of RO5126766, a first-in-class dual MEK/RAF inhibitor in patients with solid tumors. Clin Cancer Res. 2012; 18:4806–19. [PubMed: 22761467]

52. Lauchle JO, Kim D, Le DT, Akagi K, Crone M, Krisman K, et al. Response and resistance to MEK inhibition in leukaemias initiated by hyperactive Ras. Nature. 2009; 461:411–4. [PubMed: 19727076]
53. Lyubynska N, Gorman MF, Lauchle JO, Hong WX, Akutagawa JK, Shannon K, et al. A MEK inhibitor abrogates myeloproliferative disease in Kras mutant mice. Sci Transl Med. 2011; 3:76ra27.
54. Jain N, Curran E, Iyengar NM, Diaz-Flores E, Kunnavakkam R, Popplewell L, et al. Phase II study of the oral MEK inhibitor selumetinib in advanced acute myeloid leukemia (AML): a University of Chicago Phase II Consortium Trial. Clin Cancer Res. 2014; 20:490–8. [PubMed: 24178622]
55. Le K, Blomain ES, Rodeck U, Aplin AE. Selective RAF inhibitor impairs ERK1/2 phosphorylation and growth in mutant NRAS, vemurafenib-resistant melanoma cells. Pigment Cell Melanoma Res. 2013; 26:509–17. [PubMed: 23490205]
56. Morris EJ, Jha S, Restaino CR, Dayananth P, Zhu H, Cooper A, et al. Discovery of a novel ERK inhibitor with activity in models of acquired resistance to BRAF and MEK inhibitors. Cancer Discov. 2013; 3:742–50. [PubMed: 23614898]
57. Posch C, Moslehi H, Feeney L, Green GA, Ebaee A, Feichtenschlager V, et al. Combined targeting of MEK and PI3K/mTOR effector pathways is necessary to effectively inhibit NRAS mutant melanoma in vitro and in vivo. Proc Natl Acad Sci U S A. 2013; 110:4015–20. [PubMed: 23431193]
58. Jaiswal BS, Janakiraman V, Kljavin NM, Eastham-Anderson J, Cupp JE, Liang Y, et al. Combined targeting of BRAF and CRAF or BRAF and PI3K effector pathways is required for efficacy in NRAS mutant tumors. PLoS One. 2009; 4:e5717. [PubMed: 19492075]
59. Sheppard KE, McArthur GA. The cell-cycle regulator CDK4: an emerging therapeutic target in melanoma. Clin Cancer Res. 2013; 19:5320–8. [PubMed: 24089445]
60. Kwong LN, Costello JC, Liu H, Jiang S, Helms TL, Langsdorf AE, et al. Oncogenic NRAS signaling differentially regulates survival and proliferation in melanoma. Nat Med. 2012; 18:1503–10. [PubMed: 22983396]
61. Means-Powell JA, Adjei AA, Puzanov I, Dy GK, Goff LA, et al. Safety and efficacy of MET inhibitor tivantinib (ARQ 197) combined with sorafenib in patients (pts) with NRAS wild-type or mutant melanoma from a phase I study. J Clin Oncol. 2012; 30(15 suppl):8519.
62. Conrad WH, Swift RD, Biechele TL, Kulikauskas RM, Moon RT, Chien AJ. Regulating the response to targeted MEK inhibition in melanoma: enhancing apoptosis in NRAS- and BRAF-mutant melanoma cells with Wnt/β-catenin activation. Cell Cycle. 2012; 11:3724–30. [PubMed: 22895053]
63. Feng Y, Lau E, Scortegagna M, Ruller C, De SK, Barile E, et al. Inhibition of melanoma development in the $Nras^{(Q61K)}$::$Ink4a^{-/-}$ mouse model by the small molecule BI-69A11. Pigment Cell Melanoma Res. 2013; 26:136–42. [PubMed: 23035722]
64. Joseph RW, Sullivan RJ, Harrell R, Stemke-Hale K, Panka D, Manoukian G, et al. Correlation of NRAS mutations with clinical response to high-dose IL-2 in patients with advanced melanoma. J Immunother. 2012; 35:66–72. [PubMed: 22130161]
65. Johnson DB, Lovly CM, Flavin M, Ayers GD, Zhao Z, Iams WT, et al. NRAS mutation: a potential biomarker of clinical response to immune-based therapies in metastatic melanoma (MM). J Clin Oncol. 2013; 31(15 suppl):9019.

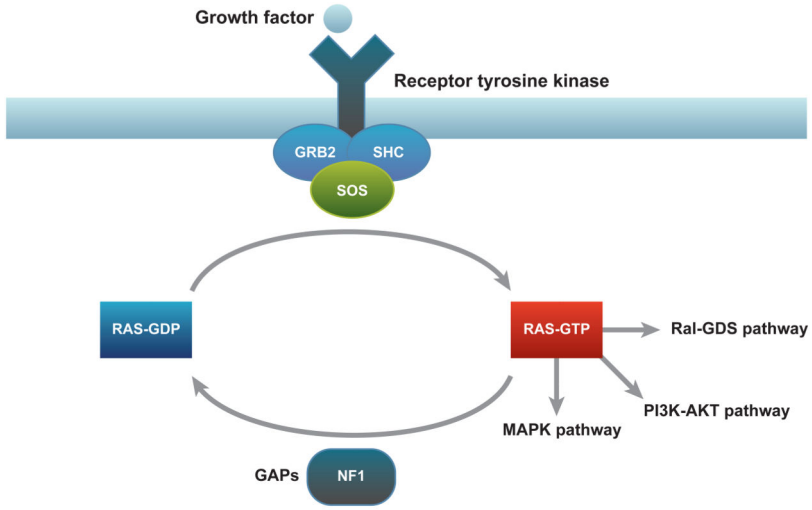

Figure 1.

Wild-type RAS activation in normal cells. The RAS activation process is triggered by interaction between a receptor tyrosine kinase and its ligand. This recruits an adaptor molecule (growth factor receptor-bound protein 2 [GRB2] and others) that subsequently causes activation of son of sevenless homolog (SOS) and other guanine nucleotide exchange factors (GEFs). GEFs catalyze the conversion of RAS-GDP (inactive) to RAS-GTP. GTPase-activating proteins (GAPs, including neurofibromin 1 [NF1]) oppose this activation step. Activated RAS then signals through the mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K)–AKT, and Ral–guanine nucleotide dissociation stimulator (GDS) pathways to induce cell growth and proliferation. SHC, Src homology 2 domain-containing transforming protein.

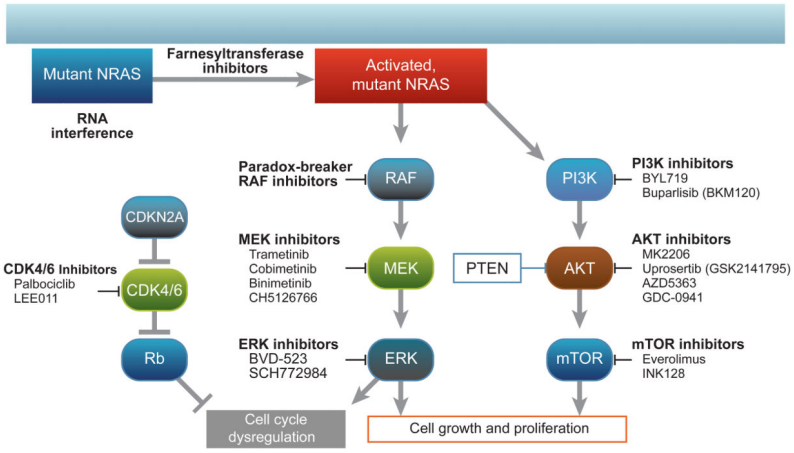

Figure 2.

Therapeutic strategies to target mutant NRAS: Direct targeting strategies include inhibition of *RAS* transcription by RNA interference and blockade of posttranslational modification by farnesyltransferase inhibitors. Agents that block downstream mitogen-activated protein kinase (MAPK) signaling include inhibitors of MAPK/ERK kinase (MEK) and extracellular signal–regulated kinase (ERK) as well as paradox-breaker RAF inhibitors. Agents targeting cell cycle regulation and the phosphoinositide 3-kinase (PI3K)–AKT pathway are also being evaluated in combination with MAPK pathway inhibitors. CDK, cyclin-dependent kinase; mTOR, mammalian target of rapamycin; Rb, retinoblastoma.