A RAS Renaissance: Emerging Targeted Therapies for KRAS-Mutated Non–Small Cell Lung Cancer

Neil Vasan1, Julie L. Boyer2, and Roy S. Herbst3

Abstract

Of the numerous oncogenes implicated in human cancer, the most common and perhaps the most elusive to target pharmacologically is RAS. Since the discovery of RAS in the 1960s, numerous studies have elucidated the mechanism of activity, regulation, and intracellular trafficking of the RAS gene products, and of its regulatory pathways. These pathways yielded druggable targets, such as farnesyltransferase, during the 1980s to 1990s. Unfortunately, early clinical trials investigating farnesyltransferase inhibitors yielded disappointing results, and subsequent interest by pharmaceutical companies in targeting RAS waned. However, recent advances including the identification of novel regulatory enzymes (e.g., Rce1, Icmt, Pdeδ), siRNA-based synthetic lethality screens, and fragment-based small-molecule screens, have resulted in a “Ras renaissance,” signified by new Ras and Ras pathway–targeted therapies that have led to new clinical trials of patients with Ras-driven cancers. This review gives an overview of KRas signaling pathways with an emphasis on novel targets and targeted therapies, using non–small cell lung cancer as a case example. Clin Cancer Res; 20(15); 3921–30. ©2014 AACR.

Introduction

Three RAS genes encode four proteins: HRas, KRas4a, KRas4b, and NRas (1). These proteins are GTPases, which function as molecular switches: “on” when bound to GTP and “off” when bound to GDP. Ras-GTP can bind to numerous partner proteins, termed “effectors,” and these Ras-effector interactions lead to a cascade of downstream signaling events (2). In normal cells, Ras signaling is crucial for proliferation, differentiation, and survival (3).

The hydrolysis of GTP to GDP by Ras is a slow process, and therefore Ras cycles between these states with the aid of regulatory proteins. GTPase-activating proteins (GAP) catalyze the hydrolysis of GTP to GDP (“on to off”), whereas guanine nucleotide exchange factors (GEF) catalyze the dissociation of GDP, with GTP binding afterward due to its high concentration in cells (“off to on”; ref. 4; Fig. 1A).

However, this pathway is co-opted by oncogenic mutations in Ras. Among the four Ras isoforms, the most common mutations are at amino acid positions G12, G13, and Q61 (5). Crystal structures of Ras proteins have modeled these mutants’ mechanisms of activation. Q61 mutants prevent coordination of a water molecule necessary for GTP hydrolysis (6), whereas G12 and G13 mutants prevent binding of Ras to its GAP and interfere with the orientation of Q61. These mutants result in Ras-GTP in an “on” state, driving oncogenesis (7; Fig. 1B).

The Ras proteins are important mediators of cell signaling. There is a wide range of Ras effector proteins, notably Raf (MAP kinase pathway), PI3K (Akt/mTOR pathway), and RalGDS (Ral pathway). These effectors (which represent only a subset of downstream Ras signaling nodes) are highly complex with numerous redundancies and interactions between pathways (8). Dysregulated Ras signaling results in increased proliferation, decreased apoptosis, disrupted cellular metabolism, and increased angiogenesis, all seminal hallmarks of cancer (9; Fig. 1B).

Ras Mutations: Differences from Isoform to Amino Acid

RAS is the most commonly mutated oncogene in cancer (8), with distinct Ras isoforms detected in various cancers (10). KRas is the most commonly mutated isoform. Listed in order of percentage of cases, KRas mutations are most common in cancers of the pancreas, colon, biliary tract, and lung (the majority of which are adenocarcinomas); NRas mutations are most common in cancers of the skin (malignant melanoma) and hematopoietic system (acute myeloid leukemia, AML); and HRas mutations are most common in cancers of the head and neck (squamous cell carcinoma) and urinary tract (transitional cell carcinoma). Ras mutations are much less common in cancers of the breast, central nervous system, or prostate (5; Fig. 2A). Why certain cancers seem to be driven preferentially by specific isoforms remains an outstanding question.

Another unsettled issue in oncogenesis is the differential role, if any, among different Ras-activating point mutations.

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In lung cancer, the most common mutations are KRas G12C, G12V, and G12D (11). Other KRas-driven cancers have different mutational frequencies: in the colon G12D, G12V, and G13D; in the pancreas G12D, G12V, and G12R; and in the biliary tract G12D, G12V, and G12S (Fig. 2B). Unlike in HRas and NRas, KRas Q61 oncogenic mutations are very rare (7).

In vitro, KRas G12V and G12R have greater transforming ability, as shown by soft agar colony formation (12). Unexpectedly, there is no correlation between the GTPase activity of the mutant and its propensity to transform (13). However, once transformed, certain mutations are more aggressive than others. Mice with KRas G12V, G12R, and G12D had higher-stage lung tumors compared with KRas G12C or wild-type (14). In patients with lung cancer, KRas G12C resulted in increased sensitivity to pemetrexed and paclitaxel compared with G12V and G12D, although G12D patients were more likely to respond to sorafenib (15).

A recent retrospective analysis of the Biomarker-integrated Approaches of Targeted Therapy for Lung Cancer Elimination (BATTLE) clinical trial (16; discussed below) found worse progression-free survival (PFS) for the group of patients with either KRas G12C or G12V, compared with other KRas mutants, or wild-type [1.84 months vs. 3.35 months (P = 0.046), vs. 1.95 months; ref. 17]. KRas G12C and G12V had increased signaling through Ral and decreased signaling through Akt. This study suggests that targeted treatments and clinical trials in non–small cell lung cancer (NSCLC) may need to consider the specific KRas point mutation.

Together, the in vitro, in vivo, and patient data suggest a greater oncogenic potential for KRas G12V (present in ~20% of KRas-mutated lung cancers) compared with other mutations (18).

**Inhibiting Ras Membrane Association**

A series of enzymes (Fig. 3), beginning with farnesyltransferase (FTase), acts posttranslationally on the C-terminal C-A-A-X motif of Ras, resulting in the attachment to membranes through cysteine prenylation (19). Next, Ras traffics to the endoplasmic reticulum, where its last three amino acids are proteolyzed by Ras-converting enzyme (RCE1), and then its C-terminus is methylated by isoprenylcysteine carboxyl methyltransferase (ICMT; ref. 20). In the Golgi, HRas, NRas, and KRas4A are palmitoylated, and the fully processed Ras then traffics to its final plasma membrane location (21). KRas4B is not palmitoylated but rather associates electrostatically with the membrane through a polybasic stretch in its C-terminus (22). Thus, two modes of membrane association poise Ras isoforms for activation and signaling.

**FTase**

Initial attempts to inhibit Ras focused on FTase (23). These FTase inhibitors (e.g., lonafarnib, tipifarnib; ref. 24) were oral medications, well tolerated, specific for FTase, and were effective against HRas-transformed cells and HRas-driven murine tumors (25). However, these drugs did not increase survival in clinical trials of patients with KRas-mutated pancreatic cancer (26). Later studies found that
with inhibition of FTase, KRas could be alternatively prenylated by geranylgeranyltransferase I (GGTase I; ref. 25). Moreover, dual inhibition of FTase and GGTase I did not decrease levels of prenylated KRas (27). Notably, tipifarnib has shown antitumor activity against AML, chronic myelogenous leukemia, and myeloproliferative disorders (28; often driven by NRas) and in breast cancer, (29) which warrants further study.

Another class of FTase inhibitors has been developed (e.g., salirasib; ref. 30) containing farnesylcysteine, thought to compete for membrane-docking proteins that bind farnesyl moieties (Fig. 3). In vitro, salirasib inhibits all Ras isoforms (31). However, it failed to induce radiographic response or increase survival in a phase II NSCLC trial (32).

Together, these studies revealed several inherent problems in targeting Ras prenylation. First, there is alternative prenylation with dissimilarities among isoforms—KRas and NRas are prenylated by GGTase I (33)—previously thought to be identical to that of HRas. Although HRas mutations are infrequent compared with KRas or NRas, FTase inhibitors would target HRas because HRas does not undergo alternative prenylation by GGTase I (33). Such findings could be exploited in a clinical trial of FTase inhibitors for patients with HRas-driven urothelial cancers.

In addition, any observed decreases in tumor size due to FTase inhibition do not correlate with KRas mutation status or other mutations in the Ras signaling pathway (34). This suggests alternative mechanisms of resistance such as Ras gene amplification, which has been observed as a resistance mechanism to MET tyrosine kinase inhibitors (35) or off-target effects, which could be mediated by the large number (>55) of prenylated substrates (e.g., other small GTPases; ref. 36). This may explain why there is not an adequate single biomarker, such as decreased Ras prenylation, for monitoring FTase inhibitor effects.

Nevertheless, many current clinical trials are investigating combination therapies of FTase inhibitors with other cytotoxic and targeted therapies (37). The failure of FT inhibition as a general strategy for targeting KRas has spurred preclinical studies of the other enzymes in the Ras processing pathway: Rce1, Icmt, and Pdeδ.

**RCE1, ICMT, PDEδ**

Disruption of the RCE1 gene slows mouse fibroblast cell growth (38); conversely, in another study, deficiency of RCE1 in a genetically engineered mouse model (GEMM) of KRas-driven myeloproliferative disease actually increased disease progression (39). These paradoxical results may reflect cell-specific differences in KRas signaling. Several inhibitors against yeast and human Rce1 have been developed (40); however, these bind with only low micromolar...
Inactivation of ICMT in the presence of activated KRas in fibroblasts leads to decreased cell growth and xenograft tumor formation (41). Concordantly, inactivation of ICMT in a GEMM of KRas-driven myeloproliferative disease also decreased lung tumor formation (42). Several small-molecule inhibitors of ICMT have been developed including cysmethynil, which reduces anchorage-independent cell growth in colon cancer cells (43). Of note, inhibition of ICMT has been shown to be an off-target effect of methotrexate, possibly through an increase in S-adenosylhomocysteine, a methyltransferase inhibitor (44).

Another recently discovered intracellular trafficking target is Pde6δ (phosphodiesterase delta), a subunit of the cyclic GMP phosphodiesterase complex. Pde6δ also functions as a chaperone protein that binds to farnesylated Ras and enhances the presence of fully processed KRas4b at the plasma membrane, and the presence of depalmitoylated HRas, NRas, and KRas4a at the Golgi (45; Fig. 3). Importantly, downmodulation of Pde6δ decreases oncogenic KRas signaling. A recent article reports benzimidazole small-molecule compounds that inhibit the mammalian Pde6δ–KRas interaction with nanomolar affinity, suppressing oncogenic signaling in vitro in KRAS-mutated pancreatic ductal adenocarcinoma cells and most tantalizingly in vivo in a pancreatic cancer mouse xenograft model (46). These findings likely will herald phase I trials of these novel inhibitors.

Direct Inhibition of the Ras Protein

The affinity of GTP for Ras is extremely potent, in the picomolar range (47); thus, attempts to inhibit Ras competitively would be difficult. Several previous reports have been given of Ras small-molecule inhibition—nucleotide exchange inhibitors (48) and Ras–Raf inhibitors (49)—but in the absence of clear structural data, it is difficult to know if
these effects are direct or indirect. This has led to a search for allosteric inhibitors of Ras.

On the basis of the crystal structure of SOS (a Ras GEF) bound to Ras, Patgiri and colleagues designed a cell-permeable peptide to inhibit Ras activation (50). As shown by nuclear magnetic resonance (NMR) spectroscopy, the peptide binds Ras with micromolar affinity at the same location as the analogous region of SOS, but does so about twice as avidly. This results in inhibition of downstream MAP kinase signaling.

Two recent groups (51, 52) have used NMR fragment-based lead discovery and structure-based drug design to find novel small-molecule allosteric inhibitors of KRas. Both groups’ inhibitors bind at a hydrophobic pocket between the switch 2 and core β sheet region of the protein, with micromolar affinity. The binding site is distinct from but partially overlapping with the SOS binding site such that SOS is unable to activate KRas.

Several FDA-approved tyrosine kinase inhibitors (e.g., afatinib, ibrutinib) take advantage of irreversible binding to a cysteine amino acid residue close to the active site. This approach inspired the development of irreversible inhibitors of KRas G12C, a specific mutant in which the glycine at the twelfth position of the KRas protein is mutated to cysteine (53). With submicromolar affinity, these inhibitors block SOS-mediated nucleotide exchange, favoring the binding of GDP instead of GTP, and rendering the KRas protein in an “off” state. When bound to KRas G12C, the compounds create a new binding surface mostly involving the switch 2 region. Importantly, they decreased viability and activated apoptosis in a KRas G12C–specific lung cancer cell line.

Although these approaches are appealing, more potent drugs that bind with nanomolar affinity would be needed for a viable drug. Nevertheless, these studies provide novel lead compounds for future optimization.

Inhibiting Downstream Ras Signaling

Many classes of inhibitors exist against components of the canonical Ras signaling pathway, including Raf, mTOR, PI3K, PI3K/mTOR, Axl, and MEK. Clinical trials with these agents are ongoing, but to date, these drugs have not been shown to be effective against Ras-driven cancers as single agents. This has led to the identification of new targets, combination approaches of multiple targeted therapies, and combination approaches of targeted therapies with conventional cytotoxic chemotherapy.

Identification of synthetic lethal targets

Synthetic lethality is a phenomenon through which perturbation in either one of two different genes does not cause cell death, but perturbation of both leads to cell death (54). In cancer, this initial perturbation could be a mutated oncogene or tumor suppressor, making this an attractive strategy for targeting cancer cells because normal cells would not have the mutation of interest and would theoretically be unaffected by a targeted treatment. This is also a strategy to develop ways to target oncogenes that are not readily “druggable” (e.g., Ras, transcription factors) or tumor suppressors in which protein target levels are decreased. The most successful example of this approach in oncology is the use of mTOR and VEGF inhibitors that indirectly target HIF1α in von Hippel-Lindau–mutated clear cell renal cell carcinoma (55).

Although the Ras signaling pathway is complex, it is thought that there may be critical nodes in the pathway that could be exploited. In KRas-mutated NSCLC, several small-molecule synthetic lethality screens have yielded lead compounds including lanperisone, which induces oxidative stress in KRas-mutated cells (56), and oncosin, which is synthetically lethal between KRas and PKCθ (protein kinase C iota) and functions through inhibition of RNA polymerase II (57). Moreover, the rheumatoid arthritis drug aurothiomalate, which inhibits PKCθ signaling, is currently in phase I clinical trials in NSCLC (58). More recently, RNA silencing technologies have facilitated the identification of new targets that, when deleted, are synthetically lethal with KRas mutations; this can be exploited clinically if the newly identified target has a known inhibitor. For example, in KRas-driven colon cancer, previous RNAi studies have shown the importance of TAK1 (TGFβ activated kinase 1), a MAP kinase kinase kinase, and Polo-like kinase 1, which functions at the mitotic spindle (59). Here, we focus on novel targets in preclinical development elucidated through synthetic lethality studies of NSCLC (Table 1).

**TBK1**

Using an shRNA screen targeting genes encoding druggable proteins—kinases, phosphatases, and oncogenes—Barbie and colleagues identified TANK-binding kinase 1 (TBK1) as essential in KRas-driven cancers (60). These

<table>
<thead>
<tr>
<th>Gene</th>
<th>Function</th>
<th>Inhibitors</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBK1</td>
<td>Kinase</td>
<td>6-aminopyrazolopyrimidine derivatives</td>
<td>60, 61, 62</td>
</tr>
<tr>
<td>WT1</td>
<td>Transcription factor</td>
<td>None</td>
<td>64, 65</td>
</tr>
<tr>
<td>CDK4</td>
<td>Kinase</td>
<td>PD-0332991</td>
<td>66</td>
</tr>
<tr>
<td>GATA-2</td>
<td>Transcription factor</td>
<td>Bortezomib + fasudil</td>
<td>67, 68</td>
</tr>
<tr>
<td>BCL-XL</td>
<td>Antiapoptotic factor</td>
<td>Selumetinib + navitoclax (ABT-263)</td>
<td>69</td>
</tr>
</tbody>
</table>

*Table 1. Genes that are synthetically lethal with KRas in KRas-mutated lung adenocarcinoma cells, their functions, and inhibitor or inhibitor combinations*
authors validated initially identified candidate genes through a higher-stringency secondary screen in lung adenocarcinoma cells, with TBK1 knockdown leading to the greatest amount of cell death, after KRas itself. TBK1 is linked to KRas through Rap1, a small GTPase downstream of KRas that is part of the Ras signaling pathway, and is activated by Rap1 and Sec5, a component of the exocyst, an intracellular tethering complex (61). Activated TBK1 leads to increased antiapoptotic NFκB signaling through B-cell lymphoma-extra large (BCL-XL) and the c-Rel proto-oncogene. TBK1 inhibitors with nanomolar affinity have been developed (62), confirming pharmacologic tractability.

**WT1**

Targeting genes from a previously identified KRas transcriptional signature (63), as well as transcriptional regulators, Vicent and colleagues identified the Wilm's tumor 1 transcription factor (WT1) as critical for oncogenic KRas signaling (64). Mechanistically, loss of WT1 decreases proliferation and increases cell senescence in KRas-driven cancers and was confirmed in mouse cell lines, GEMMs, and in human cell lines. Moreover, the authors were able to correlate WT1 expression levels with prognosis in patients with KRas-driven lung cancer, strengthening this connection. WT1 is a well-known tumor suppressor in Wilm's tumors, but recent data have implicated it as a possible oncogene with overexpression in lung cancer (65). Although WT1 is currently not druggable, further examination of WT1's role in cell senescence may yield future therapies.

**CDK4**

By querying the role of individual cyclin-dependent kinases (CDK) in KRas-driven NSCLC, Puyol and colleagues found a synthetic lethal interaction by disrupting CDK4, but not other related CDKs, causing cellular senescence (66). This effect was recapitulated in mouse embryonic fibroblasts and in a KRas-driven GEMM. Interestingly, the necessity of CDK4 in KRas-driven cancer was observed only in the lung, and not in the colon or pancreas, pointing to possible tissue-specific dependencies in the Ras pathway. In addition, these authors showed that small-molecule inhibition of CDK4 (using a CDK4/CDK6 dual antagonist) resulted in tumor regression, with decreased CDK4-mediated phosphorylation; however, this inhibition did not cause the previously observed senescence, pointing to the need for more potent and specific CDK4 inhibitors as well as using them in combination therapies.

**GATA-2**

Screening KRas-driven human NSCLC cell lines, Kumar and colleagues (67) discovered the transcription factor GATA-2 as necessary for cell viability, in vitro and in vivo in a lung cancer xenograft model. Gene expression analysis revealed multiple upregulated pathways: the proteasome, IL1 signaling, and Rho/ROCK signaling. The authors showed that GATA-2 normally upregulates the proteasome through Nrf1, IL1 signaling through TRAF6, and Rho signaling through STAT5. Together, these pathways converge onto NFκB signaling, which contributes to KRas-driven oncogenesis.

Because transcriptional factors are notoriously difficult to target, the authors relied on approved drugs that target these newly delineated components of the GATA-2 pathway: bortezomib, which inhibits the proteasome and NFκB indirectly, and fasudil, which inhibits Rho/ROCK signaling. Together, this combination therapy resulted in tumor regression in a KRas-driven lung cancer GEMM. This non-oncogene addiction to GATA-2 was also confirmed independently by a separate group (68).

**BCL-XL**

With the goal of finding genes whose inhibition may cooperate with the MEK inhibitor selumetinib in KRas-mutated cells, Corcoran and colleagues (69) designed a pooled shRNA drug screen of "druggable" genes and identified BCL-XL, an antiapoptotic gene. Using navitoclax, a BCL-XL small-molecule inhibitor, in combination with selumetinib, they showed increased apoptosis in KRas-driven lung adenocarcinoma cell lines. Alone, selumetinib increases the amount of BIM protein, a proapoptotic factor, but also increases the amount of BIM bound to BCL-XL; BIM must be unbound to induce apoptosis, so its levels induced by selumetinib are insufficient for this effect.

However, the combination of selumetinib with navitoclax increases the total amount of BIM, and decreases the amount of BIM/BCL-XL complex. Some of this free BIM forms a complex with MCL-1, and this complex is able to drive apoptosis. Thus, the authors proposed that the combination therapy "freezes up" more BIM to sufficient levels to drive apoptosis. Demonstrating the robustness and generalizability of this combination therapy, they show increased apoptosis in a large percentage of KRas-driven colorectal, lung, and pancreatic cancer cell lines; in KRas-mutant xenografts; and in a KRas-driven lung cancer GEMM.

**Clinical Trials Targeting KRas-Driven NSCLC**

Patients with KRas-mutated NSCLC derive less benefit from clinical trials compared with their KRas-wild-type patient counterparts (70, 71). Several targeted therapy clinical trials specifically address patients with KRas-mutated NSCLC. These trials represent the translational extension of the many decades of basic science research on the Ras pathway (Table 2).

**BATTLE and BATTLE-2**

As we have seen, biomarkers (e.g., mutations, overexpression) do not always correlate with effects of a targeted therapy. Moreover, as patients receive multiple treatment modalities, these markers may change even though the therapy is often dictated by the pretreatment tumor genotype. The phase II BATTLE trial addressed this issue in a novel manner (16). Ninety-seven patients with chemoresistant NSCLC were randomized into 4 treatment groups reflecting different combinations of targeted therapies. These newly delineated components of the GATA-2 pathway: bortezomib, which inhibits the proteasome and NFκB indirectly, and fasudil, which inhibits Rho/ROCK signaling. Together, this combination therapy resulted in tumor regression in a KRas-driven lung cancer GEMM. This non-oncogene addiction to GATA-2 was also confirmed independently by a separate group (68).
Table 2. Past and present clinical trials targeting KRas-mutated NSCLC

<table>
<thead>
<tr>
<th>Drug combination</th>
<th>Target</th>
<th>KRas-mutant patient response (vs. KRas wild-type patients)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sorafenib</td>
<td>Raf, VEGFR</td>
<td>Increased DCR (61% vs. 32%, ( P = 0.11 ))</td>
<td>NCT00409968</td>
</tr>
<tr>
<td>Selumetinib + docetaxel</td>
<td>MEK 1/2, microtubules</td>
<td>Increased PFS (5.3 months vs. 2.1 months, 80% CI, 0.42–0.79; one-sided ( P = 0.014 ))</td>
<td>NCT00890825</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Increased OS (9.4 months vs. 5.2 months, 80% CI, 0.56–1.14; one-sided ( P = 0.21 ))</td>
<td></td>
</tr>
<tr>
<td>Selumetinib + MK2206</td>
<td>MEK 1/2, Akt</td>
<td>Ongoing</td>
<td>NCT01248247</td>
</tr>
<tr>
<td>Bortezomib</td>
<td>Proteasome</td>
<td>Ongoing</td>
<td>NCT01833143</td>
</tr>
<tr>
<td>Retaspimycin + everolimus</td>
<td>Hsp90, mTOR</td>
<td>Ongoing</td>
<td>NCT01427946</td>
</tr>
<tr>
<td>Selumetinib + erlotinib versus</td>
<td>MEK 1/2, EGFR</td>
<td>Ongoing</td>
<td>NCT01229150</td>
</tr>
<tr>
<td>selumetinib alone</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NOTE: Drug combinations, targets, patient responses, and references are tabulated. DCR (disease control rate) = complete response, partial response, or stabilized disease.

mutation/copy number, treated with erlotinib; VEGF/VEGFR2 expression, treated with vandetanib; and RXRs/Cyclin D1 expression and CCND1 copy number, treated with beaxotene and erlotinib.

On the basis of the cumulative real-time results of the initial 97 patients over 8 weeks of treatment, 158 additional patients were adaptively randomized to receive the most effective therapy for their particular biomarker profile. In other words, if a patient from the first group with a KRas mutation had an adequate clinical response to sorafenib, there would be a greater than 25% chance that another patient with a KRAs mutation would be placed into that treatment arm.

In total, 20% of the 255 patients randomized had KRAs mutations. The overall 8-week disease control rate (DCR) was 46%; patients in the sorafenib arm had the highest DCR at 58%. Post hoc biomarker analysis showed that relative to the other treatments, sorafenib had a significantly higher DCR in EGFR wild-type patients (64% vs. 33%, \( P < 0.001 \)), and higher but nonstatistically significant DCR in KRAs-mutant patients (61% vs. 32%, \( P = 0.11 \)). Of note, in the KRAs/BRAf group, sorafenib gave a higher DCR than erlotinib (79% vs. 14%, \( P = 0.016 \)), with the G12C/G12V group associated with decreased PFS compared with other KRAs mutants or KRAs wild-type (1.84 months vs. 3.55 months vs. 2.83 months, \( P = 0.026 \)). These findings are similar to findings from a post hoc biomarker analysis of the MISSION phase III trial, in which EGFR mutation, but not KRAs mutations, predicted increased overall survival with sorafenib as third- or fourth-line monotherapy (72).

Together, the findings from this trial show the feasibility of real-time biomarker analysis and adaptive randomization, and provides a rationalization for further targeted therapy clinical trials, especially combination regimens, for patients with KRAs mutations. Of note, the BATTLE-2 trial, currently ongoing, is testing four treatment arms—erlotinib, erlotinib + MK2206 (an Akt inhibitor), selumetinib + MK2206, and sorafenib—with multiple biomarkers including KRAs (73).

### Selumetinib/docetaxel combination therapy

A recent phase II clinical trial tested a combination of MEK inhibition with cytotoxic chemotherapy (74). Jänne and colleagues randomized 87 patients with pretreated KRAs-mutant NSCLC to receive either docetaxel alone or in combination with selumetinib (74); selumetinib alone did not increase overall survival in NSCLC (75). Combination therapy increased overall survival, however, without statistical significance [9.4 months vs. 5.2 months; HR, 0.80; 80% confidence interval (CI), 0.56–1.14; one-sided \( P = 0.21 \)]. Median PFS was significantly increased (5.3 months vs. 2.1 months; HR, 0.58; 80% CI, 0.42–0.79; one-sided \( P = 0.014 \)). Of note, 37% (\( n = 16 \)) of patients given the combination therapy had an objective response, as measured by a decrease in tumor burden; however, 82% (\( n = 36 \)) of the combination group had adverse events of grade 3 or 4, mostly neutropenia, febrile neutropenia, or asthenia.

Waterfall plots (graphs that depict the continuum of tumor growth, positive to negative for all patients in a study) of response to this therapy were widespread, with 5 patients in the combination group having a 20% increase in tumor size (74). It would be interesting to know if these patients also had mutations in the Lkb1 tumor suppressor, which has been shown to potentiate resistance to selumetinib/docetaxel combination therapy in a KRAs-mutant GEMM (76). It will also be important to understand how MEK signaling cooperates with the microtubule depolymerizing activity of docetaxel and if KRAs is necessary for this functional interaction.

The results from these two clinical trials represent an important step forward in targeting KRAs. They show that patients with KRAs mutations have a small response to targeted therapies. However, KRAs mutation status is likely not the only marker involved because therapeutic responses in KRAs-mutant patients were not sufficiently robust, and because KRAs wild-type patients also had a response. Also remaining to be seen are the effects of other gene mutations,
such as those induced by smoking, on KRas, and how this may influence therapy.

Other ongoing clinical trials aimed at targeting KRas-mutant NSCLC include a phase II trial of bortezomib (77), a phase Ib/II trial of retaspinycin (an Hsp90 inhibitor) in combination with everolimus (78), and a phase II trial of selumetinib + erlotinib versus selumetinib alone (79; Table 2).

New Directions

The difficulties of targeting KRas-mutated NSCLC remain daunting, but a renaissance of discoveries—novel modes of inhibition, Ras regulatory proteins, and Ras-dependent targets—is defining a new battery of drugs. In turn, these drugs are informing a new wave of clinical trials. Although this process may seem slow and unforgiving, we should be reminded that paradigm changes take time—the renaissance lasted almost three centuries—and that continued studies of the Ras pathway will likely reveal novel therapies for this subset of patients, providing personalized medicine against this most common oncogene.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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49. The GDI-like solubilizing factor PDE 


79. Randomized phase II study of AZD6244 (MEK inhibitor) with erlotinib in KRAS wild type advanced NSCLC and a randomized phase II study of AZD6244 with erlotinib in mutant KRAS advanced NSCLC - ClinicalTrials.gov [Internet]. [cited 2014 Jun 16]. Available from: http://www.clinicaltrials.gov/ct2/show/NCT01229150.
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