Molecular Pathways: The Basis for Rational Combination Using MEK Inhibitors in KRAS-Mutant Cancers

Shunsuke Okumura¹ and Pasi A. Jänne¹,²,³

Abstract

Mutations in RAS oncogenes are frequently observed in human cancers, and the mutations result in activation of the RAS–RAF–MEK–ERK pathway, leading to cell proliferation and survival. The pathway is, therefore, a potent therapeutic target in the RAS-mutant cancers. MEK inhibitors can specifically block the pathway and are one of the key types of drugs for the treatment of the RAS-mutant cancers. As RAS proteins activate other downstream signaling proteins in addition to the RAS–RAF–MEK–ERK pathway, combination therapeutic approaches with MEK inhibitors are also being evaluated. Moreover, MEK inhibitors can arrest cancer cells in G₁ phase and repress prosurvival Bcl2 family proteins such as MCL1 and BCL2/BCLXL, and increase expression of Bim, a proapoptotic BH3-only family protein. This mechanism may explain the efficacy of the combination of MEK inhibitors with cytotoxic agents or other targeted inhibitors. A better understanding of the pathway will help us with development of rational combinations for the treatment of the RAS-mutant cancers. Clin Cancer Res; 20(16): 1–7. ©2014 AACR.

Background

Recent advances in the development of targeted therapies have improved survival in patients with a variety of cancers. For instance, in lung cancer, some of the most compelling clinical examples include the treatment of epidermal growth factor receptor (EGFR)–mutant or anaplastic lymphoma kinase (ALK) rearranged non–small-cell lung cancers (NSCLC) with either EGFR or ALK kinase inhibitors, respectively (1–5). Oncogenic mutations in RAS gene, most commonly in KRAS, are detected in approximately 30% of human cancers (6). Despite RAS mutations having been identified more than 30 years ago, successful therapies directly targeting the mutant RAS protein have not been developed. Mutant RAS activates several downstream signaling pathways that are involved in cell proliferation, differentiation, and survival (Fig. 1A; ref. 7). Among them, one of the most studied and characterized pathways is the RAS–RAF–MEK–ERK (RAS–ERK pathway). Efforts to directly inhibit mutant RAS, or its membrane association using farnesyl transferase inhibitors, failed to show clinical benefit in RAS-mutant cancers (8), with the result that the focus of recent efforts has shifted to the development of inhibitors of downstream kinases, including MEK inhibitors.

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The RAS genes are located on the short arm of chromosome 12 and encode 21-kDa membrane-localized small GTPases, which function as switches between the active GTP-bound RAS and the inactive GDP-bound one (Fig. 1A). There are four highly homologous RAS GTPases: HRAS, NRAS, and KRAS (KRAS4A and KRAS4B). Oncogenic KRAS mutations occur primarily in lung, colorectal, and pancreatic cancers, whereas HRAS mutations are detected in thyroid, kidney, and bladder cancers, and NRAS in hematologic malignancies (7). In normal epithelial cells, activation of RAS signaling is triggered in response to cell-membrane receptors, including tyrosine kinases (RTK), cytokine receptors, and G protein–coupled receptors. The activation of RAS is regulated by two proteins: guanine nucleotide exchange factors (GEF) stimulate GDP for GTP exchange for RAS, whereas GTPase-activating proteins (GAP) accelerate GTP hydrolysis (9). Upon the activation of the cell-membrane receptors, an adaptor protein Grb2 recruits GEFs to cell membrane where RAS proteins exist through prenylation, leading to increasing activated GTP–RAS proteins. Subsequently, the activated RAS interacts with RAF (A-RAF, B-RAF, and C-RAF/RAF-1), a serine/threonine kinase, leading to RAF activation through dimerization. Subsequently, RAF proteins directly phosphorylate and activate MEK1 and MEK2, tyrosine and serine/threonine dual-specificity kinases, which in turn activate ERK1 and ERK2 proteins that trigger activation of nuclear and cytoplasmic targets associated with transcription, cell proliferation, differentiation, and metabolism (Fig. 1A; ref. 9).

KRAS mutations in human cancer

Mutations in KRAS lead to constitutive activation of the RAS–ERK pathway. As intrinsic GTPase activity of RAS is...
**A**

RTKs → TKIs

- PI3K/AKT/mTOR inhibitors
  - PI3K
  - AKT
  - TORC1/2
  - RHEB
  - mTOR
- Survival, protein synthesis, cell-cycle progression, cell migration

- PI3K
  - Grb2
  - SOS
  - SH2
  - SH3
- RAS → GAP
  - GDP
  - RAS
  - GTP
- PI3K/AKT/mTOR inhibitors
  - RAF
  - MEK
  - MEK inhibitors
- Survival, proliferation, cell-cycle progression, differentiation, metabolism

**B**

- MEK inhibitors
- MEK
- PUMA
- BAD
- tBID
- BIM
- ERK
- MCL1
- BCL2
- BCLXL
- NOXA
- ABT-263 (navitoclax)
- Apoptosis
- BAX, BAK
- BAD
- p90RSK
- BAD
- Calcium signaling
- Vesicle trafficking
- Calcium signaling
- RAS
- RALGDS
- PLCε
- PKC
- Calcium signaling
- Ras
- Grb2
- SOS
- SH2
- SH3
- PI3K
- AKT
- TORC1/2
- RHEB
- mTOR
- Survival, protein synthesis, cell-cycle progression, cell migration
- Survival, proliferation, cell-cycle progression, differentiation, metabolism
- Calcium signaling
- Vesicle trafficking

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low, GTP hydrolysis of RAS, i.e., inactivation of RAS, relies heavily on the GAP function. KRAS mutations are frequently found in sites associated with binding of GAPs to RAS: codon 12 and 13 of exon 1, and codon 61 of exon 2. Amino acids in codon 12 and 13 are involved in van der Waals bonds between the RAS and GAPs, and mutations in codon 12 and 13 prevent formation of the bonds (9). Similarly, mutations in codon 61 also impair GTP hydrolysis, which in turn induces permanent activation of RAS proteins.

In NSCLC, KRAS is the most frequently mutated oncogene. KRAS mutations account for more than 90% of RAS mutations and are found mainly in the lung adenocarcinoma subtype (~30%), but rarely in squamous cell carcinoma of the lung or in small-cell lung cancer (SCLC; refs. 10, 11). Among the amino acid substitutions resulting from KRAS mutations, Gly12Cys (G12C) is the most frequent, followed by Gly12Val (G12V) and Gly12Asp (G12D; refs. 12, 13). Unlike NSCLC, in colorectal cancer, G12V is the most frequent, followed by G12D and G13D (14). In NSCLC, types of KRAS mutations seem to be related to smoking, and G12C KRAS mutations are found most commonly in former or current smokers, whereas G12D mutations are the dominant KRAS mutation in people who have never smoked (15). The phenotypic properties of mutant RAS proteins might differ between the different mutant amino acid substitutions. For example, a murine study demonstrated that mice with Kras-G12V tumors had a more aggressive phenotype compared with those with Kras-G12D tumors (16). Moreover, the KRAS-G12V protein interacts with Raf-1 and signals through the RAS–ERK pathway, whereas the KRAS-G12D protein does not activate the RAS–ERK pathway and instead transduces signals through the PI3K–AKT, JNK, p38, and FAK pathways (16). Ihle and colleagues also reported that in NSCLC cell lines, KRAS-G12C and KRAS-G12V activated the RAL signaling pathway downstream of RAS and decreased growth factor–dependent AKT activation. In contrast, KRAS-G12D resulted in AKT but not RAL activation (17).

Mechanisms of MEK inhibitor–induced apoptosis

The constitutive activation of the RAS–ERK pathway as a result of KRAS mutations has led to the development of MEK inhibitors as a potential therapeutic strategy for KRAS-mutant cancers. In vitro studies demonstrated a tendency toward sensitivity to MEK inhibitors in cell lines harboring KRAS mutations compared with those without KRAS mutations (18, 19), and in part inspired the development of clinical trials of MEK inhibitors for patients with KRAS-mutant cancers. Several MEK inhibitors are in clinical development, and one, trametinib, is currently approved by FDA, albeit for BRAF-mutant melanoma. As MEK1/2 does not have known targets other than ERK1/2, inhibition of MEK should lead to inhibition of all ERK1/2 target proteins. Moreover, most MEK inhibitors have the advantage of being noncompetitive inhibitors of ATP binding to MEK, thus conferring high specificity (20).

Unlike MEK proteins, ERK1/2 proteins have several nuclear and cytoplasmic targets. For instance, ERK protein plays a critical role in the cell cycle. ERK1/2 can promote phosphorylation of the Retinoblastoma protein (Rb) through activation of cyclin D1 and repression of p27KIP1 (21, 22). Because phosphorylation of Rb results in G1–S cell-cycle progression, deregulation of the RAS–ERK pathway leads to sustained cell-cycle progression. MEK inhibitors have been shown to reduce cyclin D1 levels and induce the p27KIP1 expression as well as cause dephosphorylation of Rb, arresting human cancer cells in the G1 phase (23, 24).

A second mechanism of antitumor activity of MEK inhibitors is a result of altered expression of the Bcl2 family proteins, consisting of the prosurvival proteins (Bcl2, MCL1, and BclXL), the proapoptotic Bcl-2 homology 3-only (BH3-only) proteins, and the proapoptotic Bax/Bak subgroup. The balance between the prosurvival and proapoptotic proteins is crucial to apoptosis caused by anticancer drugs, and when the proapoptotic members are dominant, downstream Bax and Bak proteins can stimulate release of apoptosis-inducing factor and cytochrome c from mitochondria, resulting in apoptosis (Fig. 1B). As ERK1/2 represses the proapoptotic proteins and induces expression of the prosurvival members, MEK inhibitors can tilt the balance toward apoptosis. In human lung cancer cells, exposure to the MEK inhibitor selumetinib (AZD6244) resulted in an increase of the proapoptotic proteins, Bim, PUMA, and NOXA (25), and in pancreatic cancer cells, the MEK inhibitor PD98059 caused a downregulation of the expression levels of Bcl2, BclXL, and MCL1 (24). However, despite these effects, the efficacy of MEK inhibitors alone might be insufficient as treatment for KRAS-mutant cancers (26, 27). Indeed, an in vitro study demonstrated the limited efficacy of MEK inhibitor alone in KRAS-mutant NSCLC (28), suggesting that combination therapies are likely to yield more robust clinical benefits.

Clinical–Translational Advances

KRAS mutations as prognostic factors

KRAS mutations have both prognostic and predictive roles in human cancer. In colorectal cancer, KRAS mutations are associated with a worse prognosis (29), and also with a lack of clinical benefit from treatment with anti-EGFR monoclonal antibody therapies (30). A recent analysis also
demonstrated that the amount of KRAS mutations detected using a sensitive PCR-based assay may correlate with the efficacy of anti-EGFR therapy in patients with human colorectal cancer (31). Similarly, in pancreatic cancer, KRAS mutations (codon 12 or 13) are associated with worse prognosis (32), and in NSCLC, patients with advanced KRAS-mutant cancer have a shorter survival compared with those with EGFR-mutant or EGFR/KRAS wild-type (WT) cancers (33, 34). In addition, KRAS mutations predict for resistance to treatment with EGFR tyrosine kinase inhibitors (17, 35, 36). Ilie and colleagues reported that patients with NSCLC whose tumors had either KRAS-G12C or KRAS-G12V mutations had worse progression-free survival (PFS) compared with patients whose tumors had other KRAS-mutant tumors or lacked KRAS mutations (17).

**MEK inhibitors in patients with KRAS-mutant cancer**

MEK inhibitors have been evaluated as both single agents and in combination with chemotherapy in KRAS-mutant cancers. A number of reports have demonstrated the preclinical efficacy both *in vitro* and *in vivo* of MEK inhibitors in KRAS-mutant tumors, but efficacy data obtained from clinical studies are, currently, limited to lung cancer. In colorectal cancer, a study suggested that inhibition of the MAPK pathway using MEK inhibitors exhibits favorable efficacy in patients with KRAS- or BRAF-mutant tumors (26), but this has not yet been demonstrated in a clinical study. There is limited clinical evidence of the efficacy of MEK inhibitors in patients with KRAS-mutant pancreatic cancer.

In lung cancer, a randomized phase II study evaluated selumetinib or pemetrexed in previously treated NSCLC, and demonstrated that the PFS in the selumetinib group was equivalent to that in the pemetrexed group, although this was a study of unselected patients with NSCLC (37). In contrast, a small study also examined the efficacy of selumetinib specifically in KRAS-mutant NSCLC, but demonstrated no responses (38). Selumetinib has also been evaluated in KRAS-mutant lung cancer. In a phase II study, patients with KRAS-mutant NSCLC were randomly assigned to either trametinib (GSK1120212) or docetaxel as second-line chemotherapy. The primary endpoint was PFS. Although there was no statistically significant difference in PFS between the treatment groups, patients treated with trametinib had a response rate of 12% (39).

In a randomized clinical phase II study comparing selumetinib and docetaxel versus docetaxel alone as second-line therapy demonstrated encouraging efficacy of the combination in patients with KRAS-mutant NSCLC (40). In this study, median PFS was prolonged in the combination group compared with the placebo group (5.3 vs. 2.1 months), and the combination had a significantly higher response rate (37% vs. 0%) than docetaxel alone. Although the median overall survival was not statistically significant, the preferable tendency toward survival in the combination has shed light on the treatment for KRAS-mutant NSCLC. On the basis of these results, a phase III study of docetaxel plus selumetinib or placebo in patients with KRAS-mutant NSCLC (SELECT-1) has been initiated (NCT 01933932). A single-arm phase I/II study evaluating the efficacy of trametinib and docetaxel in patients with KRAS-mutant and WT NSCLC has also been performed (41). In the preliminary analysis, the response rate was 28% in patients with KRAS-mutant NSCLC, and there appeared to be more antitumor activity in KRAS-G12C tumors compared with non-G12C tumors (41, 42). One challenge with the docetaxel/MEK inhibitor combinations is toxicity. The most dramatic of these is hematologic toxicity. Febrile neutropenia was significantly increased in the docetaxel/selumetinib combination compared with docetaxel alone (18% vs. 0%). Future studies will incorporate the use of primary prophylaxis with granulocyte colony-stimulating factor (G-CSF) in an effort to reduce this side effect.

Although the mechanism(s) of efficacy of the combination with docetaxel and a MEK inhibitor remains unclear, results from preclinical studies provide some intriguing observations (43, 44). In a murine study of *Kras* G12D NSCLC, the combination of docetaxel/selumetinib produced response rates of 92% compared with 30% in docetaxel (tumor volume difference was 43.7%, *P* = 0.03273), and the combination led to a prolonged PFS compared with docetaxel alone (12 weeks vs. 6 weeks, *P* = 0.0003) in the murine study (43). However, the efficacy of the combination was significantly blunted in mice whose tumors harbored both a *Kras* mutation and loss of *Lkb1* (43). *Lkb1* is a tumor-suppressor gene and encodes a serine/threonine protein kinase (45). Germline mutations of *Lkb1* cause Peutz-Jeghers syndrome, leading to an increased risk of cancer development (46). *Lkb1* mutations are frequently accompanied by KRAS mutations and observed in smokers with NSCLC (47, 48). It will be important to determine in future clinical trials whether KRAS-mutant NSCLC patients whose tumors also harbor a concurrent loss of *Lkb1* similarly do not derive benefit from the combination of docetaxel and MEK inhibitors. Other *in vitro* studies have further demonstrated a mechanistic explanation for the clinical efficacy of docetaxel and MEK inhibitors. Microtubule inhibitors, such as taxanes or vinorelbine, can lead to activation of the RAS–ERK pathway, and combining MEK inhibitors with these agents suppressed RAS-mediated signaling and increased the efficacy of the drugs in treatment of cancer (49, 50). Furthermore, Kawabata and colleagues have suggested, in a preclinical study, an interesting possibility that efficacy of the combination with microtubule inhibitors and MEK inhibitors is largely schedule dependent (44). The study demonstrated that when the microtubule inhibitor was administered first followed by a MEK inhibitor, cell death was observed, but not when the sequence was reversed (44). This observation may be related to the different phases of the cell cycle in which MEK inhibitors and chemotherapy act. MEK inhibitors can arrest most cells in the G1 phase, whereas microtubule inhibitors like docetaxel can affect cells in the M phase. However, treatment with microtubule inhibitors can sometimes cause mitotic slippage, in which the cells enter the following G1 phase without undergoing cell division (51). Thus,
sequential treatment with MEK inhibitors could also lead to clinical efficacy. Analogously, combining gemcitabine or pemetrexed, which affect cells mainly in S phase and inhibit DNA synthesis, with MEK inhibitors might also be schedule dependent. For instance, both concurrent combination with a MEK inhibitor AZD6244 and gemcitabine and sequential combination with AZD6244 followed by gemcitabine had limited efficacy, whereas gemcitabine followed by AZD6244 had efficacy in biliary cancer cells (52). These results may be, in part, due to G1 arrest caused by AZD6244, reducing gemcitabine-induced inhibition of DNA synthesis in the S phase. This observation may also be true for pemetrexed. Authors from an in vitro study reported that pretreatment with a MEK inhibitor before pemetrexed reduced S-phase arrest and apoptosis induced by pemetrexed in A549 KRAS-mutant lung cancer cells (53). Given these findings, the schedule of administration of chemotherapy and MEK inhibitors should be further evaluated in future clinical trials, and such studies may help with development of rational drug combinations with MEK inhibitors in other histologic cancers with KRAS mutations.

In lung cancer, a number of other clinical trials of combinations with MEK inhibitors and other targeted agents are ongoing. Among them, the well-studied targets are proteins in the PI3K–AKT–mTOR signaling cascade (Fig. 1A). Data from preclinical studies in a KRAS-mutant murine lung cancer model demonstrated that activation of the PI3K–AKT–mTOR pathway contributes resistance to AZD6244, and dual inhibition of the PI3K–AKT–mTOR and RAS–ERK pathway had synergistic effects (54–56). Several clinical trials have been initiated that combine MEK inhibitors with agents targeting one or more components of the PI3K signaling pathway. Preliminary data from one such phase I/II study of the combination with a MEK inhibitor GDC-0973 and a PI3K inhibitor GDC-0941 demonstrated that in advanced solid tumors, among 30 patients enrolled, 6 of 15 patients had a FG5-PET partial metabolic response and 5 patients had tumor reduction in RECIST-measurable targets (57). A major challenge has been toxicity of the treatment combination. It is possible and even likely that inhibition of both PI3K and MEK signaling sufficiently to fully inhibit these signaling pathways, albeit effective in preclinical models, is not tolerable in humans, and alternative schedules and/or treatment approaches will need to be developed.

The other intriguing targets for the combination are ant apoptotic Bcl2 family proteins (Fig. 1B). A preclinical experiment using a shRNA–drug screen library has indicated that a BclXL inhibitor ABT-263 (navitoclax) in combination with a MEK inhibitor led to dramatic apoptosis in KRAS-mutant xenografts and cell lines (58). In another preclinical study, Tan and colleagues reported the efficacy of the combination with ABT-263 and a MEK inhibitor, and further demonstrated that addition of a PI3K inhibitor GDC-0941 to the combination led to increased apoptosis in KRAS-mutant cancer cells (59). So far, although navitoclax has been studied mainly in SCLCs (60, 61), as MEK inhibitors can activate the proapoptotic proteins and suppress the prosurvival proteins, inhibition of Bcl2 prosurvi-

val family proteins in combination with a MEK inhibitor may be expected to be synergistic and thus could represent a potent therapeutic strategy in KRAS-mutant cancers.

Future Studies of MEK Inhibitors in NSCLC

To date, the most compelling clinical data exist for MEK inhibitors combined with docetaxel. Although this combination is now in phase III clinical development for KRAS-mutant NSCLC, several important questions and challenges remain. As highlighted above, hematologic toxicity is a potential limitation of the current chemotherapy combinations and likely to be associated as well with any other future chemotherapy combinations. Thus, alternative scheduling of MEK inhibitors and chemotherapy may need to be considered. This toxicity may also affect the choice of MEK inhibitors to combine with chemotherapy as MEK inhibitors with a short half-life may be more amenable to combine with chemotherapy compared with ones with a long half-life.

The development of chemotherapy/MEK inhibitors has so far focused on KRAS-mutant NSCLC, but it is not clear that this combination is specific for KRAS-mutant cancers. It is possible that MEK inhibitors merely enhance the efficacy of chemotherapy, independent of KRAS mutations, and hence would also be effective in KRAS WT cancers. In fact, the phase I trial of docetaxel/trametinib demonstrated a response RR of 21% in KRAS WT patients (41). Further studies will be necessary to determine the benefits of chemotherapy/MEK inhibitors specifically in KRAS WT tumors and whether the magnitude of benefit is similar or smaller than in KRAS-mutant cancers.

Until now, all KRAS-mutant tumors have been lumped together. However, it is more than likely that not all KRAS-mutant tumors will derive the same degree of benefit from MEK inhibitors or MEK inhibitor combinations. Data are already emerging on the outcome differences based on different subtypes of KRAS mutations. In addition, the genomic context of KRAS mutations is likely to affect therapeutic efficacy of MEK inhibitor combinations. Moving forward, it will be critical to comprehensively characterize KRAS-mutant cancers and correlate these with clinical efficacy.

Disclosure of Potential Conflicts of Interest

P.A. Jänne is a consultant/advisory board member for AstraZeneca, Boehringer Ingelheim, Chugai Pharmaceuticals, Clovis Oncology, Merck-Mack Pharmaceuticals, Pfizer, and Sanofi and reports receiving royalties from Dana-Farber Cancer Institute-licensed intellectual property on EGFR mutations licensed to LabCorp. No potential conflicts of interest were disclosed by the other author.

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