

MAP2K1 (MEK1) Mutations Define a Distinct Subset of Lung Adenocarcinoma Associated with Smoking

Maria E. Arcila¹, Alexander Drilon², Brooke E. Sylvester³, Christine M. Lovly⁴, Laetitia Borsu¹, Boris Reva⁵, Mark G. Kris², David B. Solit³, and Marc Ladanyi^{1,3}

Abstract

Purpose: Genetic alterations affecting the MAPK/ERK pathway are common in lung adenocarcinoma (LAD). Early steps of the signaling pathway are most often affected with *EGFR*, *KRAS*, and *BRAF* mutations encompassing more than 70% of all alterations. Somatic mutations in *MEK1*, located downstream of *BRAF*, are rare and remain poorly defined as a distinct molecular subset.

Experimental Design: Tumors harboring *MEK1* mutations were identified through targeted screening of a large LAD cohort concurrently interrogated for recurrent mutations in *MEK1*, *EGFR*, *KRAS*, *BRAF*, *ERBB2/HER2*, *NRAS*, *PIK3CA*, and *AKT*. Additional cases were identified through a search of publically available cancer genomic datasets. Mutations were correlated with patient characteristics and treatment outcomes. Overall survival was compared with stage-matched patients with *KRAS*- and *EGFR*-mutant LADs.

Results: We identified 36 *MEK1*-mutated cases among 6,024 LAD (0.6%; 95% confidence interval, 0.42–0.85). The majority of patients were smokers (97%, $n = 35/36$). There was no association with age, sex, race, or stage. The most common mutations were K57N (64%, 23/36) followed by Q56P (19%, 7/36), all mutually exclusive with other driver mutations in the targeted panel. Transversions G:C>T:A were predominant (89%, 31/35), in keeping with smoking-associated DNA damage. Additional less common somatic mutations were identified in the kinase domain, all of which are predicted to converge into a single interaction area based on *in silico* 3D modeling.

Conclusions: *MEK1* mutations define a distinct subset of lung cancers (~1%) with potential sensitivity to MEK inhibitors. Mutations are predominantly transversions, in keeping with a strong association with smoking. *Clin Cancer Res*; 21(8):1935–43. ©2014 AACR.

Introduction

The MAPK/ERK pathway (RAS–RAF–MEK–ERK pathway) is one of the most extensively studied signal transduction pathways and regulates fundamental cell activities, including proliferation, transcriptional regulation, differentiation, and survival. Consistent with this critical role, defects in this pathway are implicated in the pathogenesis of several human malignancies, including lung

cancer. Genetic alterations affecting the MAPK/ERK pathway are common in non–small cell lung carcinoma (NSCLC), particularly those affecting the upstream components of the signaling cascade. Mutations in *EGFR* and *KRAS* (1, 2), for instance, constitute more than 70% of all reported driver mutations in this cancer, whereas those involving *BRAF* are less common but well described (3). Alterations in *MAP2K1* (*MEK1*), a primary downstream effector of RAF kinases, are uncommon and consequently tumors harboring these mutations have remained poorly defined as a distinct molecular subset in lung cancer (4).

MEK1, also known as the mitogen-activated protein kinase kinase 1 (MAP2K1), is a dual specificity kinase of the MAP2K/STE7 kinase family. This kinase family also includes five additional members but only *MEK1* and *MEK2* are known to participate in the MAPK/ERK cascade with pivotal roles in signal integration (5). These two kinases share a high degree of sequence homology, and although they seem to perform similar functions, they remain distinct in the ways they contribute to regulated ERK activity (6). The functional activation of the MAPK/ERK pathway begins with the extracellular binding of ligand to a receptor tyrosine kinase, such as *EGFR*, and is followed by sequential activation of RAS, RAF, and MEK. Once activated by RAF, *MEK1/2* catalyzes the concomitant phosphorylation of threonine and tyrosine residues on ERK (MAPK) within a specific Thr–Glu–Tyr sequence at codons 202 to 204. This locks the kinase domain of MAPK into a catalytically competent conformation, ultimately leading to nuclear signals that regulate cellular growth and

¹Department of Pathology, Memorial Sloan Kettering Cancer Center, New York, New York. ²Department of Medicine, Thoracic Oncology Service, Memorial Sloan Kettering Cancer Center, and Weill Cornell Medical College, New York, New York. ³Human Oncology and Pathogenesis Program, Memorial Sloan Kettering Cancer Center, New York, New York. ⁴Department of Medicine, Division of Hematology–Oncology, Vanderbilt University School of Medicine, Vanderbilt Ingram Cancer Center, Nashville, Tennessee. ⁵Computational Biology Center, Memorial Sloan Kettering Cancer Center, New York, New York.

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Current address for B. Reva: Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York, New York.

Corresponding Author: Maria E. Arcila, Memorial Sloan Kettering Cancer Center, Department of Pathology, Diagnostic Molecular Pathology Service, 1275 York Avenue, New York, NY 10065. Phone: 212-639-7879; Fax: 212-717-3515; E-mail: arcilam@mskcc.org

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Translational Relevance

Although genetic alterations activating the MAPK signaling pathway are common in lung adenocarcinoma (LAD), somatic mutations in *MEK1*, located downstream of *BRAF*, are relatively rare and have remained poorly defined as a distinct molecular subset. The present study of 36 patients with *MEK1* mutation-positive lung cancer represents the first systematic analysis of this molecular subset of LAD. We show that mutations in *MEK1* define a distinct subset of lung cancer that is strongly associated with smoking and that the mutations are recurrent, clustered, activating, and potentially "drugable." Though rare, based on estimated LAD incidence, mutations in *MEK1* may account for more than 600 patients with lung cancer a year in the United States alone who could benefit from specific targeted therapies directed at this driver oncogene.

proliferation. To date, ERK (MAPK) remains the only known MEK catalytic substrate. This tight selectivity, along with the unique dual specificity kinase function, confers a strategic role in the integration of signals through the pathway to the MEK1/2 proteins.

Initial insight into the critical role of MEK in cell proliferation and tumorigenesis has come from engineered mutations used in *in vitro* models. Deletions and mutations in the activation segment of *MEK1* have been shown to constitutively activate the protein. Activated *MEK1* mutants enhance cell proliferation, differentiation, and promote transformation (7–9). Conversely, dominant negative *MEK1* mutants can prevent cellular proliferation in fibroblasts and transformed cell lines. Naturally occurring mutations in *MEK1* and *MEK2* have been described in association with cardio-facio-cutaneous (CFC) syndrome as a germline event (10). In addition, somatic mutations have also been identified in small subsets of lung cancer (*MEK1* only; refs. 4, 11) and colorectal cancer (*MEK1* and *MEK2*; refs. 12, 13). More recently, *MEK1* mutations have also been reported in melanomas in the setting of resistance to BRAF-targeted therapy (14). Because MEK is a convergence point for multiple upstream kinases and intracellular signaling intermediaries, it constitutes a particularly attractive target for drug development and specific inhibitors are already available and are moving through various stages of clinical trials. Recently, trametinib, a selective inhibitor of MEK1 and MEK2, was approved by the FDA for use in patients with BRAF-mutant melanoma.

Despite the growing awareness of *MEK1* mutations and their potential as drug targets, to date, no comprehensive report has focused on the characteristics of patients with lung carcinoma harboring these mutations. In this study, we report the largest collection of lung cancer tumors screened for recurrent mutations in this gene. We describe the clinical and pathologic characteristics of these tumors and make comparisons with other molecularly defined subsets in lung carcinoma.

Materials and Methods

Patient data

Clinical cases of lung adenocarcinoma (LAD) received for routine molecular profiling at two institutions [Memorial

Sloan Kettering Cancer Center (MSKCC) and Vanderbilt-Ingram Cancer Center (VICC)] between January 2009 and June 2014 were identified for review. Clinical characteristics, including smoking history, clinical stage, age, gender, race (as reported by patient), and treatment, were recorded under Institutional Review Board (IRB)-approved waivers for specific subsets.

Tissue procurement and mutational profiling

Tumor specimens were obtained as standard of care for clinical management or with patients' consent under IRB-approved protocols. Genomic DNA was extracted from tumor samples using standard procedures. Clinical testing for the detection of mutations in *EGFR* (exon 19 deletions and L858R) and *KRAS* (exon 2) was carried out by a combination of methods, including fragment analysis, Sanger sequencing, mass spectrometry genotyping (Sequenom), or SNaPShot assays as previously described (15–17). Extended mutation analysis for other recurrent point mutations in *EGFR*, *KRAS*, *BRAF*, *ERBB2/HER2*, *NRAS*, *AKT*, *PIK3CA*, and *MEK1* (*MAP2K1*) was performed by mass spectrometry or SNaPShot assays. Specific *MEK1* mutations interrogated in the extended panels included Q56P, K57N and D67N. To further assess other variants, mutation data for *MEK1* were also extracted from publicly available sources, including TCGA (The Cancer Genome Atlas), cBio Portal, and the COSMIC database.

In vitro studies

Functional assays. Human embryonic kidney 293H cells were cultured in DME-HG, 10% FCS, 2 mmol/L glutamine, and 50 U/mL each of penicillin and streptomycin. Specific *MAP2K1* mutations were generated from the *MEK1*-GFP plasmid (Addgene, 14746) using the QuickChange Site-Directed Mutagenesis Kit (Stratagene). 293H cells were seeded for 70% to 90% confluency at the time of transfection, then transiently transfected with 10 µg of wild-type or mutant *MAP2K1* DNA using the Lipofectamine 2000 Transfection Reagent. At 24 hours, cells were lysed in 1% NP-40 lysis buffer and processed for immunoblotting as previously described (18). Rabbit polyclonal antibodies recognizing phosphorylated ERK1/2 (Thr202/Tyr204), ERK1/2, and phosphorylated p90RSK (Thr359/Ser363) were obtained from Cell Signaling Technology. Rabbit monoclonal antibodies recognizing RSK1/2/3 and GFP were obtained from Cell Signaling Technology. After incubation with horseradish peroxidase-conjugated secondary antibodies, proteins were detected by chemiluminescence (SuperSignal West Dura Chemiluminescent Substrate; Thermo Fisher Scientific) and visualized using a Fuji LAS-4000 (GE Life Sciences).

Drug treatments. Transfected 293H cells were grown under selection with Geneticin (Gibco). Cells were plated overnight, then treated with DMSO (control) or 1 µmol/L of AZD6244 for 0.5, 1, 2, or 6 hours.

Plasmid construction. To generate the MSCV-puro-MEK1 plasmid, *MEK1* cDNA was excised from the pEGFP-N1-MEK1-GFP plasmid using *XhoI/AgeI* digestion, then ligated into the *XhoI/AgeI*-digested MSCV-puro vector (Gateway). *MAP2K1* mutations were generated (specified above) from the MSCV-puro-MEK1 plasmid. All plasmids were verified by sequencing.

Viral production and infection of target cells. Retrovirus was generated by cotransfection of MSCV-puro vector, MSCV-puro-MEK1, or mutant MSCV-puro-MEK1 with psi-eco mouse packaging vector in 293T cells. NIH-3T3 cells were infected with virus-containing medium and 40 µg of polybrene (Santa Cruz Biotechnology) for 48 hours. Cells were then cultured in fresh medium and selected with 2 µg/mL of puromycin.

Soft agar colony formation assay. Log-phase NIH-3T3, NIH-3T3-MSCV-puro, NIH-3T3-MSCV-puro-Mek1, and mutant NIH-3T3-MSCV-puro-Mek1 cells (1×10^4) were mixed with agar (0.33%) and plated over a bottom layer of 0.5% agar on 6-well plates. Treated cells were mixed with agar and 1 µmol/L AZD6244, then plated. Cells were incubated at 37°C for 4 weeks. Wells were stained with crystal violet (Sigma-Aldrich) for 1 hour at 37°C. Colonies greater than 150 µm were imaged and counted using GelCount (Oxford Optronix). Two independent experiments were performed with each condition in duplicate wells per experiment.

Statistical analysis

Fisher exact tests were applied to test associations among MEK1 mutations, smoking history, and race. Overall survival (OS) and progression-free survival (PFS) were determined using the Kaplan-Meier method. For all MEK-mutated patients (stage I-IV), OS was calculated from the time of pathologic diagnosis of disease using Kaplan-Meier estimates. Comparisons of OS between MEK-mutant lung cancers and KRAS-mutant, EGFR-mutant, BRAF-mutant, ALK-rearranged, ROS1-rearranged, and RET-rearranged lung cancers were calculated from the time of diagnosis of metastatic disease.

Results

Mutation screening

Among 5,330 patients with LAD tested at two institutions (MSKCC and VICC), we identified 26 patients NSCLC harboring MEK1 mutations (MSKCC, 18 and VICC, 8). The distribution of driver mutations in this cohort is as follows: EGFR 20%, ERBB2 1%, KRAS 32%, BRAF 2%, PIK3CA 3%, MEK1 0.6%, and NRAS 0.25%. Of note, 211 cases of squamous cell carcinoma (SCC) were also tested at MSKCC with no MEK1 mutations identified.

To produce a more comprehensive view of LAD-associated MEK1 alterations, we combined the above datasets with next-generation sequencing data publicly available through the cBio Portal (TCGA and Broad; refs. 19, 20). Through this search, we identified 9 additional MEK1-mutated cases among 421 LAD samples. One additional (nonoverlapping) MEK1-positive case was identified in the COSMIC database (1/273). In total, this combined dataset included 36 MEK1-mutated cases among 6,024 LAD (0.6%)

Spectrum of MEK1 mutations

Among the 26 cases of MEK1-mutated lung cancer identified at the two institutions, K57N was the most common mutation detected, encompassing 77% (20/26) of all variants whereas all remaining cases harbored the Q56P (6/26). No D67N mutations were identified. Both the K57N and the Q56P constitute transversions, G>T and A>C, respectively. In all cases, MEK1 mutations were mutually exclusive with all other alterations tested for in the

panel, including known activating mutations in EGFR, ERBB2, KRAS, NRAS, and BRAF.

Among the 9 MEK1-mutated cases identified through publicly available datasets, there were three novel mutations: M146I (1/9), G301X (1/9), and S331R (1/9). Because the G301X mutation would be presumed to be inactivating and because the M146I and S331R mutations were reported in patients with concurrent KRAS mutations (G13C and G12V, respectively), these three MEK1 alterations were deemed as unlikely to be driver mutations. The six other cases harbored previously reported mutations in lung or other cancers, including F53L (1/9), Q56P (1/9), K57N (2/9), E102_1103del (1/9), and C121S (1/9; refs. 14, 19-21). No concurrent recurrent mutations were identified in these cases in EGFR, KRAS, BRAF, ERBB2/HER2, NRAS, AKT, or PIK3CA genes. Finally, the single case identified in the COSMIC database harbored a K57N mutation.

Among all 36 patients with MEK1 mutations, 92% (33/36) were G:C>T:A transversions. Most cases (86%, 31/36) were in exon 2.

The distribution of mutations is depicted on Fig. 1. A detailed summary of positive cases is presented in Table 1.

Characteristics of patients with MEK1 mutations

The clinical characteristics of 36 patients with MEK1 mutations are summarized in Table 2. The majority of patients were Caucasian (92%, 33/36), former or current smokers (97%, 35/36) with no gender predilection. Median pack-year history of smoking was 48. Patients most commonly presented with stage IV disease (39%, 14/36). The only never smoker in this series harbored a K57N mutation and was also the only patient of Asian ancestry.

Comparisons of MEK1 mutants versus EGFR- and KRAS-mutated subsets showed significant differences in the smoking history of MEK1- and EGFR-mutated cases. Compared with both EGFR and KRAS, MEK1 mutations were more common in males, although this difference only reached statistical significance in comparison with EGFR (Table 3).

Clinical outcomes and survival analysis

Early-stage disease (stage IA-III A). Of the 20 patients with stage IA-III A MEK1-mutated lung cancers, data on therapy were available for 15 patients. Fourteen of 15 patients underwent surgical resection of their lung cancers with curative intent. None of these patients ($n = 0/14$) developed disease recurrence (median recurrence-free survival 17 months, range 1-66 months). At the time of last follow-up, 13 of 14 remained alive and disease free, and 1 of 14 died of a nonmalignant cause. Recurrent disease was only noted in the remaining patient who was not a surgical candidate. This patient was treated with sequential chemotherapy and radiation, developed distant recurrence 3.2 years after completion of therapy, and passed away secondary to cancer.

Advanced/metastatic disease (stage IIIB-IV). Thirteen patients presented with stage IV disease at diagnosis. The most common site of metastatic disease at diagnosis was bone (73% of patients with available data, $n = 8/11$). Data on systemic therapy were available for 8 of 13 patients. The median number of lines of systemic therapy was 2 (range 1-4). Duration of disease control with systemic therapy was short, with a median PFS of 2.0 months [8 patients, 17 individual lines of therapy (n

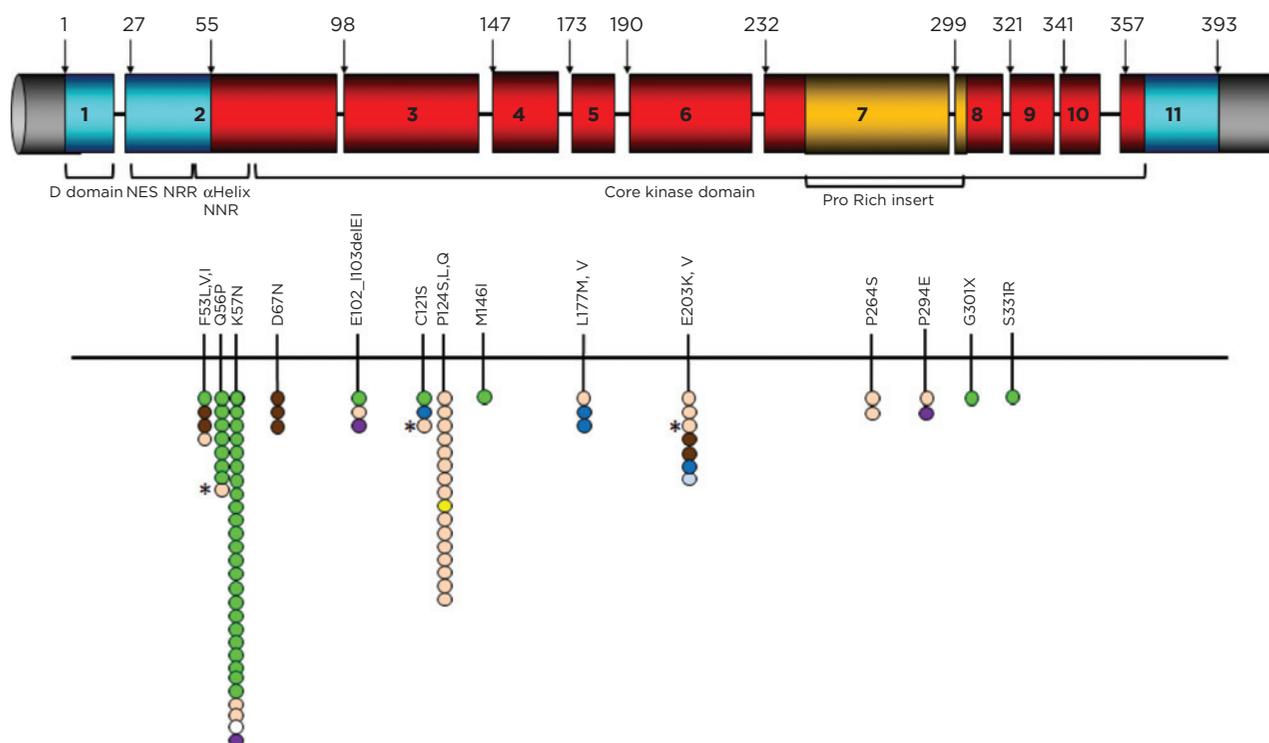


Figure 1. Top, linear model of the *MEK1* gene. Exons are represented by the numbered boxes. Red boxes, regions coding for the core kinase domain (residues 55–369). Bottom, mapping of all mutations identified in this study and publically available data (cBio portal and Cosmic database). Each circle, a single patient–LAD (green circle). Reported mutations in melanoma (light brown circle), colon carcinoma (brown circle), bladder (yellow), gastric carcinoma (blue), breast (light blue), head and neck (purple) are also mapped for comparison. D-Domain is the ERK docking site, Pro rich insert 262–307.

= 5/17 platinum doublet or triplet, $n = 3/17$ nonplatinum doublet, $n = 5/17$ single-agent cytotoxic, $n = 4/17$ targeted therapy with erlotinib, nintedanib, erlotinib+MET inhibitor, PI3K/mTOR+MEK inhibitor]. The patient who received targeted therapy with a combination of an MEK inhibitor and a PI3K/mTOR inhibitor on a phase I clinical trial developed progression of disease at first radiographic evaluation.

Two patients presented with locally advanced (stage IIIB) disease at diagnosis. Data on therapy were available for one of these patients who underwent chemoradiation with carboplatin and paclitaxel. During treatment, he was found to have metastatic disease to the adrenals. The median OS from date of pathologic diagnosis for all patients with stage IIIB/IV disease where survival data were available ($n = 12$) was 1.2 years (15 months). All deaths ($n = 8/11$ with known disease status at the time of last follow-up or death) were cancer related.

Table 1. *MAP2K1 (MEK1)*-mutated cases

AA mutation	CDS	Frequency	Type of change
p.F53L	c.157T>C	1 (3%)	Transition
p.Q56P	c.167A>C	7 (19%)	Transversion
p.K57N	c.171G>T	23 (64%)	Transversion
E102_I103del	c. 304_306delGAGATC	1 (3%)	—
p.C121S	c.361T>A	1 (3%)	Transversion
p.M146I	c.438G>C	1 (3%)	Transversion
p.G301X	c. 901G>T	1 (3%)	Transversion
p.S331R	c.991A>C	1 (3%)	Transversion

Survival comparison with other oncogene-driven lung cancers

Median OS of all patients with *MEK1*-mutant lung cancers (stage IA–IV, $n = 27$ with available data on survival) was 48.9 months (4 years). Median OS from the time of diagnosis of metastatic disease ($n = 11$ patients with *MEK*-mutant lung cancers who developed or were diagnosed with metastatic disease, and

Table 2. Clinical characteristics of patients with *MAP2K1 (MEK1)* mutations

Smoking history	
Never	3% (1/36)
Former <10 pack years	3% (1/36)
Former >10 pack years	94% (34/36)
Pack years	Median 48 pack years (range, 0–160)
Age at diagnosis	Median 68 (range, 48–89)
Sex	
M	50% (18/36)
F	50% (18/36)
Stage at presentation	
IA	19% (7/36)
IB	11% (4/36)
IIA	11% (4/36)
IIB	11% (4/36)
IIIA	3% (1/36)
IIIB	6% (2/36)
IV	39% (14/36)
Race	
Caucasian	91% (33/36)
Asian	3% (1/36)
Unknown	6% (2/36)

Table 3. Comparison of clinical characteristics of *MAP2K1* (*MEK1*)-mutant patients versus other molecularly defined subsets

	MAP2K1 (n = 36)	KRAS (n = 1,254) ^a	EGFR (n = 769) ^a
Gender (female/male)	18/18	788/466 (P = 0.07)	546/223 (P = 0.006)
Median age (range)	68 (48-89)	68 (37-90)	67 (30-92)
Smoking status (former or current/never smoker)	35/1	1136/118 (P = 0.36)	347/422 (<0.00001)

NOTE: P values in parentheses are based on comparison with *MEK1*.^aKRAS and EGFR groups are based on the MSKCC cohort.

with available survival data) was compared with other groups of oncogene-driven lung cancers ($n = 117$ *KRAS*-mutant, $n = 102$ *EGFR*-mutant, $n = 45$ *ALK*-rearranged, $n = 18$ *RET*-rearranged, $n = 10$ *ROS1*-rearranged, $n = 10$ *BRAF*-mutant).

No statistically significant differences in median OS from diagnosis of metastatic disease were found between *MEK1*-mutant and *KRAS*-mutant (14.9 vs. 13.7 months; HR, 0.89; $P = 0.75$), or *BRAF*-mutant lung cancers (14.9 vs. 20.9 months; HR, 1.64; $P = 0.30$). Median OS of *MEK1*-mutant lung cancers was inferior in comparison with *EGFR*-mutant (14.9 vs. 29.7 months; HR, 2.16; $P = 0.04$), *ALK*-rearranged (14.9 vs. 35.9 months; HR, 2.64; $P = 0.01$), *ROS1*-rearranged (14.9 vs. 76 months; HR, 4.00; $P = 0.02$), and *RET*-rearranged lung cancers (14.9 months vs. not reached; HR, 3.25; $P = 0.03$; Fig. 2).

Functional characterization of *MEK1* mutants

To determine how the two most common mutations (Q56P and K57N) identified in our cohort affect MAPK signaling, we assayed the ability of the mutants to induce ERK phosphorylation and its downstream effector ribosomal S6 kinase (p90RSK) in 293H cells. These were compared with other less common mutations, including F53L, D67N, E1102del, C121S, and E203K.

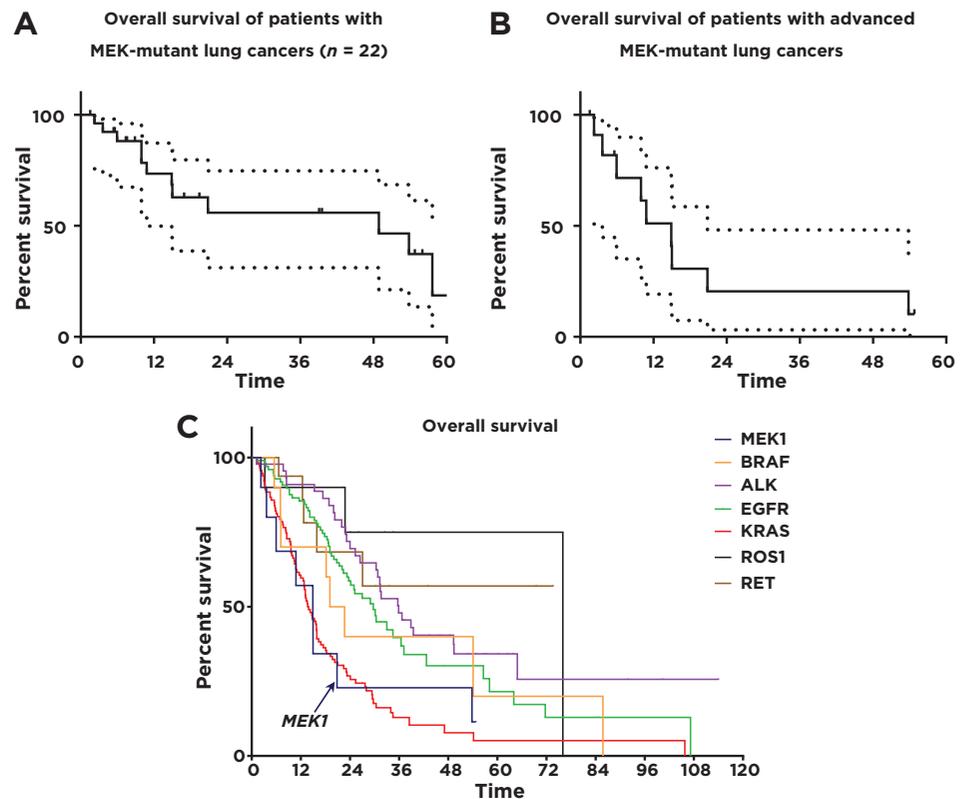
Western blot analysis demonstrated increased kinase activity in all mutants, except for the D67N. ERK phosphorylation levels in the D67N mutant did not vary significantly from the wild-type (Fig. 3A and B).

Anchorage-independent growth was also assessed for mutated cell lines harboring F53L, Q56P, K57N, and D67N mutations. Colony formation after 4 week culture in soft agar showed colonies of greater than 150 μm in the four mutant cell lines tested but not in the control or wild-type *MEK1* cells. Treatment with 1 $\mu\text{mol/L}$ selumetinib inhibited colony formation in all the mutated cell lines (Fig. 3C). Further assessment of ERK following treatment with Selumetinib at 0.5, 1, 2, or 6 hours showed significant ERK inhibition in all mutants after 30 minutes of treatment (Supplementary Fig. S1).

Discussion

Somatic mutations in *MEK1* were first described in NSCLC at MSKCC by Marks and colleagues (4) in 2008 and this led to the early inclusion of the known, recurrent *MEK1* point mutations in our multiplex genotyping panel for lung cancer. Since then, only rare reports have been published describing 1 or 2 mutation

Figure 2. Kaplan-Meier curves for OS in patients with *MEK1* mutations (time plotted in months). A, OS for MSKCC and VICC patients. B, OS for patients with advanced disease. C, survival comparison with other oncogene-driven lung cancers—the OS of the *MEK1*-mutant cohort was similar to the *KRAS*- and *BRAF*-mutant lung and significantly inferior to those patients with *EGFR* mutations and with rearrangements involving *ALK*, *RET*, and *ROS1*.



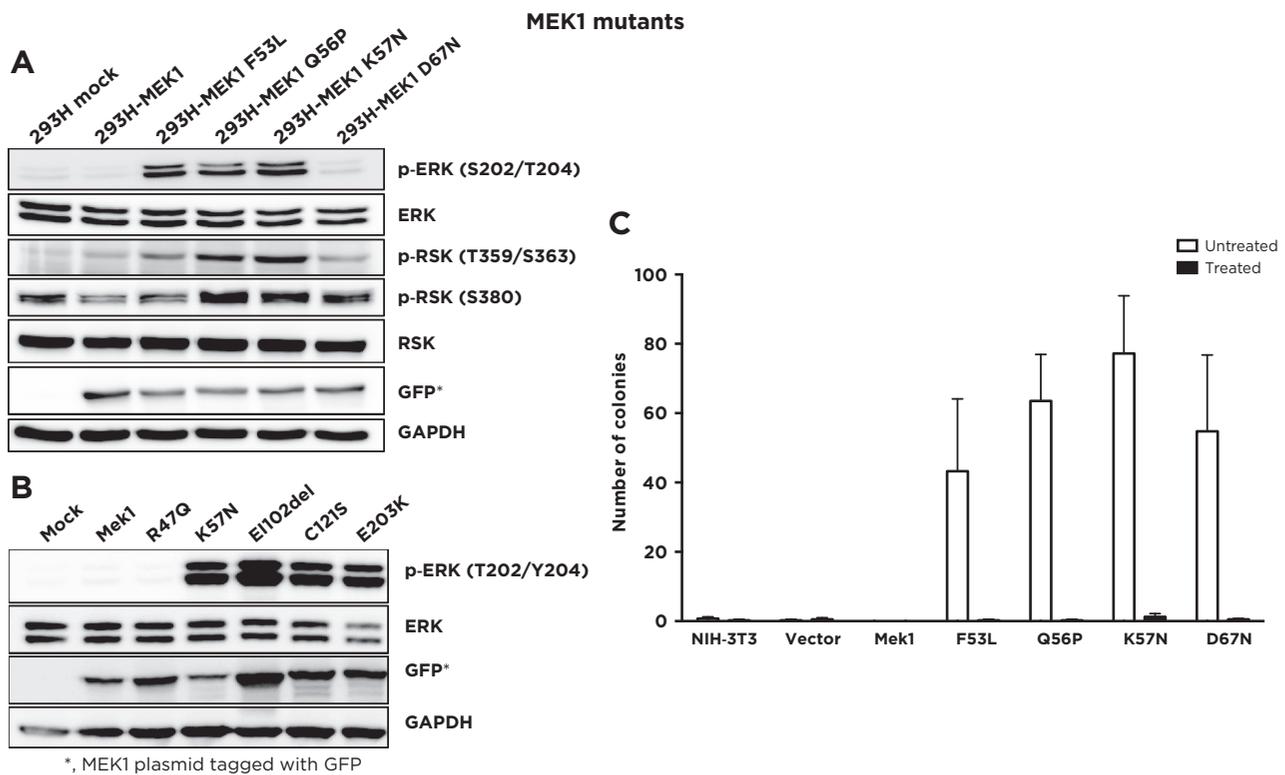


Figure 3.

Functional characterization of MEK1 mutants. 293H cells were transfected with a wild-type or mutant GFP-tagged Mek1 plasmid. Cell lysates were collected after 24 hours and analyzed by Western blotting. A, the F53L, Q56P, and K57N mutants that display hyperactive downstream MAPK pathway signaling based upon phosphorylation of ERK and its downstream effector ribosomal S6 kinase (RSK). In contrast, the D67N-mutant kinase activity is significantly lower than the other hyperactive mutants and not significantly different from the wild-type. B, the E1102del, C121S, and E203K mutants and display hyperactive downstream MAPK pathway signaling that was comparable with the K57N. C, NIH-3T3, NIH-3T3-vector, NIH-3T3-MEK1, and NIH-3T3-MEK1 mutant cells were cultured for 4 weeks in soft agar to assess anchorage-independent growth. Colonies greater than 150 μm in size formed in all four mutant cell lines but not in control and wild-type Mek1 cells. Treatment of mutant cell lines with 1 $\mu\text{mol/L}$ of selumetinib (AZD6244) prevented colony formation in all the mutant cell lines.

positive patients (11, 22), making it difficult to define the molecular, clinical, and pathologic characteristics of patients with these tumors. This study represents the largest series of *MEK1*-mutant lung cancer to date. Here, we describe the clinicopathologic characteristics associated with 36 patients with *MEK1* mutation-positive lung cancer identified among 6,024 tested (0.6%), and we further explore the molecular pathology of these tumors. We demonstrate that mutations in *MEK1* define a unique subset of lung cancer that is strongly associated with smoking and display a clinical behavior that most closely resembles *KRAS*-mutated lung cancer. Although the frequency of *MEK1* mutations in LAD is low, LAD is a common disease with at least 100,000 new cases in the United States each year. On the basis of our estimated prevalence of 0.6% of LAD, this would represent more than 600 patients with lung cancer a year in the United States alone who could potentially benefit from specific targeted therapies directed at this driver oncogene.

Mutations in *MEK1* were initially described in the germline setting in association with CFC syndrome (10). This observation was particularly noteworthy, as it was the first description of naturally occurring mutations in this gene and offered additional insight into the biochemistry of *MEK1* and *MEK2* that could not be obtained from *in vitro* models. Since then, additional mutations have been reported in CFC, and somatic variants have been

found in a small proportion of solid tumors and in melanomas in the setting of resistance to BRAF inhibitors. More recently, *MEK1* mutations have also been reported in a specific subset of hairy cell leukemias (23). Somatic mutations in *MEK1* cluster in the same functional regions as the previously identified germline alterations. The first cluster lies in and around the *MEK* NRR (negative regulatory region), whereas the second cluster localizes to the region flanked between codons 121 and 130 at the center portion of the kinase domain. In somatic cancer mutations, there seems to be a third preferentially mutated region surrounding codon 203. It is important to note, that although these clusters are separated at the level of the amino acid sequence, when mapped into a 3D model, all preferential regions are brought together forming a single mutational hot-spot that is proximal to the NRR and the ATP-binding site and thus well positioned to influence catalytic activity (Fig. 4; ref. 6).

In this study, *MEK1* testing was initially focused on point mutations in exon 2, namely Q56P, K57N, and D67N, based on the variants identified in initial reports. To further characterize the spectrum of mutations affecting this gene, we integrated publicly available comprehensive mutation analysis results from LAD studies (19, 20, 24) and nonoverlapping data from the COSMIC database. In this combined dataset, 86% of all mutations were identified in exon 2. Although this is influenced

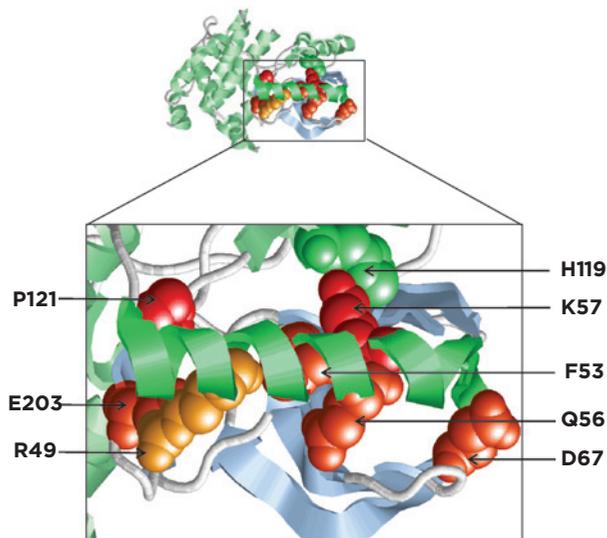


Figure 4. A 3D model of MEK1 showing the most common mutations identified in this study and selected mutations also identified in other solid tumors. Labeled residues identify the region of the mutation. Alpha helix—bright green (residues 43–67). Although in a linear model these mutations are separated into clusters in exons 2, 3, and 6, when mapped into a 3D model, mutations form a single hot spot.

by the original screening design, comprehensive unbiased datasets also identify this as the preferentially mutated region in lung cancer.

At the time of its initial description in lung cancer, it was unclear how the reported mutations would affect *MEK1* structure as the crystal structure of some regions had not been defined. Since then, the crystal structure has been solved for most of the protein, providing a structural rationale for the role of several residues and the impact of their alterations. In the study by Fischmann and colleagues (25), the boundaries of the *MEK1* consensus kinase domain were established to encompass the region of residues 55 to 369. Residues 43 to 61 fold into the alpha helix of the protein with significant packing interactions all along this segment. Residues F53, Q56, and K57 are located in the C-terminal end of the alpha helix, the area that is most involved with contacts to the N-terminal lobe of the protein and likely maintains helical integrity. Mutations in this region are likely to cause significant alterations in the helical structure that would deregulate kinase function due to their critical interface with the rest of the kinase domain. Of note, residue D67, mutated in colon cancer, lies outside the interface between the alpha helix and the rest of the N-terminal lobe. Its role in *MEK1* constitutive activation is unclear based on the kinase structure. Interestingly, in our functional characterization, we find that the D67N-mutant kinase activity is significantly lower than all other mutants tested (including F53L, Q56P, K57N, E102_I103del, C121S, and E203K) and not significantly different from the wild-type in our hands (Fig. 3). A previous study, however, shows that the D67N substitution does lead to constitutive activation of the MAPK pathway. In this study by Estep and colleagues (26), the level of ERK phosphorylation of the D67N *MEK1* was described to be less than that of *MEK1* Y130C mutant used for comparison. Additional studies are required to better understand the role of this

mutation in *MEK1* constitutive activation and how the mutation compares with other hyperactive mutations.

Additional mutations identified in our search of public databases include three novel mutations: M146I, G301X, S331R and two previously described mutations, E102_I103del and C121S (14, 27). Our assessment of the later two shows these to be functional with hyperactive downstream MAPK pathway signaling that was comparable with the K57N (Fig. 3B). The C121S mutation has also been associated with the development of resistance to both RAF and MEK inhibition *in vitro* (14). Of interest, the M146 residue is an important component within the binding pocket of the highly selective MEK inhibitor U0126 (23). The same binding pocket is shared by the MEK1 inhibitor PD-325901, which is currently in clinical trials for treatment of NSCLC and breast carcinoma (clinicaltrials.gov; ref. 23). The potential implication of these mutations in the binding of the drug remains to be explored.

Morphologically, *MEK1*-mutated tumors in our study were all adenocarcinomas, although one case also had a squamous cell component. No mutations were identified among 211 morphologic and immunophenotypically defined SCCs. The clinical characteristics of patients with *MEK1* mutations were also examined. Although we found no particular sex predilection, there was a strong association with smoking. Remarkably, all but 1 patient (35/36; 97%) were current or former smokers with a median smoking history of 48 pack years. In further support of the smoking association, we note that the vast majority of the identified mutations (89%) were G:C>T:A transversions, a pattern known to occur in association with direct exposure to tobacco carcinogens. A similar relationship has also been observed for *KRAS* (28–30) and *TP53* mutations in lung cancers from smokers (31). In contrast, *MEK1* mutations in melanoma, reported in up to 6% cases (32), predominantly involve C>T (majority) and G>A transitions, changes frequently associated with exposure to UV radiation (32, 33). Similar to melanoma, although based on a fewer number of reported cases, mutations in colon carcinomas are also transition predominant. Of note, and also in contrast with the data available in melanoma, in which *MEK1* mutations are often found to co-exist with *BRAF* or *NRAS* mutations (32–34), in lung cancers, we find most *MEK1* mutations to be mutually exclusive with all other established driver mutations, including *EGFR*, *KRAS*, *NRAS*, *BRAF*, *HER2*, *PIK3CA*, and fusions involving *ALK*. Only two novel, nonrecurrent *MEK1* mutations had concurrent *KRAS* alterations (both G>T Transversions). These two cases could represent passenger mutations in *MEK1* in smoking-associated lung cancers with a high mutation load.

In this series, median OS from the diagnosis of metastatic disease was not significantly different between patients harboring *MEK1*-mutant lung cancers and other lung cancers that have been associated with smoking, including those harboring *KRAS* and *BRAF* mutations. In contrast, the survival of patients with *MEK1*-mutant lung cancers was significantly inferior to that of patients whose lung cancers have been associated with a never or former light smoking history (*EGFR*-mutant, *ALK*-rearranged, *ROS1*-rearranged, and *RET*-rearranged lung cancers).

Overall, our data suggest that mutations in *MEK1*, although uncommon, define a molecular subset of LAD that is distinct and potentially "druggable." Although MEK inhibitors are currently in clinical testing and are approved either as a single agent or in combination with a second targeted agent in *BRAF*-

mutant melanomas, to the best of our knowledge, no ongoing or completed phase I or phase II trial of an MEK-selective inhibitor has yet enriched for MEK-mutant patients with non-small cell lung cancer and trials such as this remains an unmet need. In the past, testing for these rare mutations has been a bottleneck in this setting but more comprehensive tumor genotyping approaches should increasingly make it feasible to stratify patients on the basis of *MEK1* tumor genotype and this would have secondary benefits in furthering the understanding of the biology of these tumors and the clinical development of MEK1 inhibitors.

Disclosure of Potential Conflicts of Interest

C.M. Lovly reports receiving a commercial research grant from Astra Zeneca and Novartis, and speakers bureau honoraria from DAVA Oncology. M.G. Kris is a consultant/advisory board member for Astrazeneca. D.B. Solit reports receiving speakers bureau honoraria from Pfizer. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: M.E. Arcila, A. Drilon, C.M. Lovly, M.G. Kris, D.B. Solit, M. Ladanyi

Development of methodology: M.E. Arcila, A. Drilon, D.B. Solit, M. Ladanyi

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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): M.E. Arcila, A. Drilon, B.E. Sylvester, C.M. Lovly, L. Borsu, M.G. Kris, D.B. Solit

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): M.E. Arcila, A. Drilon, B. Reva, M.G. Kris, D.B. Solit, M. Ladanyi

Writing, review, and/or revision of the manuscript: M.E. Arcila, A. Drilon, C.M. Lovly, M.G. Kris, D.B. Solit, M. Ladanyi

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): M.E. Arcila, M.G. Kris, M. Ladanyi

Study supervision: M.E. Arcila, M. Ladanyi

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