

Preexisting MEK1^{P124} Mutations Diminish Response to BRAF Inhibitors in Metastatic Melanoma Patients

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Abstract

Background: MEK1 mutations in melanoma can confer resistance to BRAF inhibitors, although preexisting MEK1^{P124} mutations do not preclude clinical responses. We sought to determine whether recurrent, preexisting MEK1^{P124} mutations affected clinical outcome in BRAF inhibitor-treated patients with melanoma.

Methods: Data from four published datasets were analyzed to determine whether preexisting MEK1^{P124} mutations affect radiologic response or progression-free survival (PFS) in patients with BRAF^{V600}-mutant metastatic melanoma treated with vemurafenib or dabrafenib. The effects of MEK1^{P124} mutations on MAPK pathway activity and response to BRAF inhibition were also investigated in a series of cell models.

Results: In a pooled analysis of 123 patients, the presence of a pretreatment MEK1^{P124} mutation ($N = 12$, 10%) was associated with a poorer RECIST response (33% vs. 72% in MEK1^{P124Q/S} vs. MEK1^{P124} wild-type, $P = 0.018$), and a shorter PFS (median 3.1 vs.

4.8 months, $P = 0.004$). Furthermore, MEK1^{P124Q/S} mutations were shown to have independent kinase activity and introduction of these mutations into a BRAF-mutant melanoma cell line diminished inhibition of ERK phosphorylation by dabrafenib and enhanced clonogenic survival in the presence of dabrafenib compared with cells ectopically expressing wild-type MEK1. Consistent with these data, two BRAF-mutant cell lines with endogenous MEK1^{P124} mutations showed intermediate sensitivity to dabrafenib, but were highly sensitive to downstream inhibition of MEK or ERK.

Conclusion: Taken together, our data indicate that preexisting MEK1^{P124} mutations are associated with a reduced response to BRAF inhibitor therapy and identify a subset of patients with BRAF-mutant melanoma likely to benefit from combination therapies involving MEK or ERK inhibitors. *Clin Cancer Res*; 21(1); 98–105. ©2014 AACR.

Introduction

In patients with BRAF^{V600}-mutant metastatic melanoma, the type I BRAF inhibitors vemurafenib and dabrafenib elicit response rates of 40% to 50% and improve overall survival, when compared with standard chemotherapy (1–4). Despite this activity, 50% of patients treated with BRAF inhibitors develop disease progression within 7 months (1, 3).

Mechanisms of acquired resistance to BRAF inhibitor therapy have been described, and these predominantly reactivate MAPK signaling. Key mechanisms include elevated expression of the kinases CRAF, COT1, or mutant BRAF (5–7), aberrant splicing of BRAF (8), activating mutations in N-RAS (9) and RAC1 (10), loss of NF1 (11), persistent activation of receptor tyrosine kinases (RTK; refs. 9, 12, 13), and amplification of the downstream effector MITF (14). Activation of the PI3K pathway has also been implicated in BRAF inhibitor resistance and acquired mutations activating the AKT (15) and PIK3CA (14) kinases have been detected in BRAF inhibitor-resistant melanomas. Additional somatic variants of unknown significance (PIK3CG, PHLLP1, PIK3R1, and HOXD8) have also been identified in progressing melanomas (14, 16).

Genetic alterations identified in pretherapy BRAF^{V600}-mutant tumors can also modulate initial responses to BRAF inhibitors. For instance, loss of the PTEN, deletions encompassing the p16^{INK4a} cyclin-dependent kinase inhibitor gene (CDKN2A) and *cyclin D1* gene amplification are each associated with shorter progression-free survival (PFS) in patients with BRAF^{V600}-mutant melanoma treated with BRAF inhibitors (17). These genetic markers do not necessarily confer intrinsic resistance but appear to function as predictive markers of duration of response. Consequently, PTEN-deficient and p16^{INK4a}-null BRAF^{V600}-mutant melanoma cells can remain exquisitely sensitive to BRAF inhibition (18) and PTEN-null, BRAF^{V600}-mutant tumors can show dramatic responses to BRAF inhibitors (19).

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Translational Relevance

BRAF inhibitors improve overall survival in patients with BRAF^{V600}-mutant melanoma. Patient responses are variable, however, and up to 25% of patients will progress within 3 months. Few predictors of BRAF inhibitor response have been identified and these may prove useful in customizing patient therapy. Our data indicate that the presence of a MEK1^{P124} mutation at baseline is associated with a poorer response and shorter progression-free survival in BRAF inhibitor-treated patients. MEK1^{P124} mutations show elevated kinase activity and cause a significant, albeit slight, decrease in BRAF inhibitor sensitivity in BRAF-mutant melanoma cell lines. Significantly, carriage of a MEK1^{P124} mutation does not diminish melanoma cell sensitivity to downstream inhibition at the MEK and ERK nodes. This study identifies patients with BRAF^{V600}- and MEK1^{P124}-mutant melanomas as a subgroup that is likely to have a poor response to single-agent BRAF inhibition, but may benefit from combination therapies involving MEK or ERK inhibitors.

In this report, we explored the potential role of recurrent mutations affecting the *MEK1* gene (*MAP2K1*) for predicting clinical responses. Mutations affecting MEK1 and MEK2 can promote resistance to BRAF and MEK inhibitors (14, 20–22), but preexisting MEK1 mutations, which occur in 5% of melanomas (23), do not preclude objective responses to BRAF inhibition in patients with BRAF^{V600}-mutant melanoma (19, 24). This is despite the fact that MEK1 alterations affecting Proline at codon 124, are recurrent melanoma-associated mutations, have been identified in melanomas progressing on the MEK inhibitor AZD6244 (20), and were selected in a random mutagenesis screen for resistance to AZD6244 (20). MEK1^{P124} mutations display weakly elevated kinase activity in some studies (20, 24, 25) and have been described in patients with cardiofaciocutaneous syndrome, a genetic disorder caused by germline mutations in *MAPK* (26). We analyzed the clinical impact of MEK1^{P124} mutations on responses to BRAF inhibition in patients with BRAF^{V600}-mutant melanoma. We identified that pretherapy MEK1^{P124} mutations are associated with poorer RECIST response and shorter PFS in patients with BRAF^{V600}-mutant melanoma treated with BRAF inhibitors. In accordance with these clinical data, we also showed that MEK1^{P124S} and MEK1^{P124Q} have slightly elevated kinase activity and cause a significant, albeit slight decrease in dabrafenib sensitivity. Furthermore, two BRAF-mutant cell lines with endogenous MEK1^{P124} mutations showed intermediate sensitivity to dabrafenib, but were highly sensitive to downstream inhibition of MEK or ERK.

Materials and Methods

Patients

Treatment and outcome data from four published cohorts of patients with BRAF^{V600}-mutant metastatic melanoma treated with single-agent BRAF inhibitor (dabrafenib or vemurafenib) were pooled for analyses (14, 19, 22, 24). Cohort 1 includes patients treated on the phase II trial of vemurafenib (27), and the subset of 28 patients with available *MEK1* exon 3 mutation data were included in our analysis (19). Cohort 2 includes 30 patients treated with dabrafenib ($n = 22$) or vemurafenib ($n = 8$) at our institutions and the complete *MEK1*-coding sequence was examined from melanoma tissue taken from each patient (22). Cohort 3 also examined patients treated with dabrafenib ($n = 8$) or vemurafenib ($n = 23$), and *MEK1* exon 3 tumor-derived mutation data were available for all patients (24). Cohort 4 includes 39 patients treated with dabrafenib ($n = 11$) or vemurafenib ($n = 28$) with *MEK1* next-generation exome sequence data (14). Five patients common to cohorts 2 and 3 were excluded from cohort 3.

Our analysis included 123 patients with available clinical response, PFS and MEK1 mutation data (Table 1). The best objective response and PFS were determined using RECIST for patients in cohorts 1, 3, and 4 and those on clinical trials from cohort 2. For patients in cohort 2, not on a clinical trial or patients without measurable disease at treatment commencement ($n = 4$), the treating physician determined disease progression and categorized the best objective response as "response" ($\geq 30\%$ reduction in tumor burden) or "no response" ($< 30\%$ reduction; ref. 22).

Statistical analysis

Fisher exact tests were used to compare categorical variables. PFS was calculated from the date of initiation of BRAF inhibitor therapy until progression of disease or last clinical follow-up. Univariate Kaplan–Meier analysis of MEK mutation status compared median survival between groups using the log-rank test. Experimental data are presented as the mean \pm SE from at least two independent experiments. Paired t tests were used to compare mean values. A two-tailed P value of < 0.05 was considered statistically significant. Statistical analysis was performed using Stata version 13.1 (Stata Corp.) or Prism 6 (GraphPad Software Inc.).

Cell culture and cell screening

SKMel28 and D28 melanoma cells were obtained from Professors P. Hersey (Kolling Institute of Medical Research, University of Sydney, New South Wales, Australia) and N. Hayward (Oncogenomics Laboratory, QIMR Berghofer Medical Research Institute, Herston, Queensland, Australia). The patient from which the short-term culture SMU030P was established, consented to tumor biopsies and the creation of a short-term cultures as previously described (28). Cells were grown in DMEM with 10% FBS and glutamine (Gibco BRL) and cultured in a 37°C

Table 1. Cohort profiles and mutation testing methods

Cohort	<i>n</i>	MEK1 mutations	MEK1 screening	Ref*
1	28	P124L(4), P124S(2)	Targeted sequencing	19
2	30	P124S(1), I111S(1), G176S(1)	cDNA sequencing	22
3	26	P124S(3)	Targeted sequencing	24
4	39	P124L(1), P124S(1), F52Y(1), G276W(1)	Exome sequencing	14

NOTE: Number of patients with the indicated MEK1 mutations is shown in parentheses. Five overlapping patients identified in cohorts 2 and 3 have been excluded from cohort 3 *Reference.

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incubator with 5% CO₂. Clonogenic and pharmacologic growth inhibition assays were performed as previously described (22, 29). HEK293T cells were transfected using Lipofectamine 2000 (Life Technologies) according to the manufacturer's instructions. Media were changed 7 hours after transfection to DMEM/10% FBS or serum-free DMEM media. Cells were collected after an additional 24 or 48 hours in culture. Stocks of dabrafenib, trametinib, and VX-11e (supplied by Active Biochem) were made in DMSO. Cell authentication of SKMel28 and SMU030P cells was confirmed using the StemElite ID system from Promega, and the MEK1 mutation profile of the D28 cells was verified by sequencing the complete open reading frame of the *MAP2K1* cDNA. Lentiviruses were produced in HEK293T cells as described previously (30). Cells were infected using a multiplicity of infection of 10 to provide an efficiency of infection above 90%. A reverse transcription resistance screen was used to examine the expression of *BRAF* splice variants, the complete coding sequence of *MEK1*, *MEK2*, *N-RAS*, *PTEN*, and *p16^{INK4a}* and the 5' half of *AKT1* cDNA as previously described (22). Amplification and sequencing primers for *PTEN* and *p16^{INK4a}* were PTEN_F 5'-CATTTCATCCTGCAGAAGA-3', PTEN_R 5'-CTGA-

CACAATGTCCTATTG-3', *p16^{INK4a}_F* 5'-TGGCTGGTCACCAGAGGGT-3' and *p16^{INK4a}_R* 5'-AAACTACGAAAGCGGG-3'.

Western blotting

Total cellular proteins were extracted at 4°C using the RIPA lysis buffer containing protease inhibitors (Roche) and phosphatase inhibitors (Roche). Total proteins (30–40 µg) were resolved on a 10% to 12% SDS-PAGE and transferred to Immobilon-P membranes (Millipore). Western blots were probed with the following antibodies: total ERK (137F5; Cell Signaling), p-ERK^{Y204} (E4; Santa Cruz Biotechnology), FLAG (Sigma-Aldrich), MEK1/2 (L38C12; Cell Signaling Technology), β-actin (AC-74; Sigma-Aldrich), p-pRb^{S807/811} (Cell Signaling Technology), CCND1 (G124-326; Becton Dickinson), EGFR (D7A5; Cell Signaling Technology), p-rpS6^{S235/236} (2F9; Cell Signaling Technology), p27^{Kip1} (Becton Dickinson), DUSP6 (Abcam), p-AKT^{S473} (736E11; Cell Signaling Technology), p-AKT^{T308} (D25E6; Cell Signaling Technology), Bcl-2 (100; Santa Cruz Biotechnology), p-GSK-3β (Cell Signaling Technology), PRAS40 (C77D7; Cell Signaling Technology), p-BAD^{S136} (D25H8; Cell

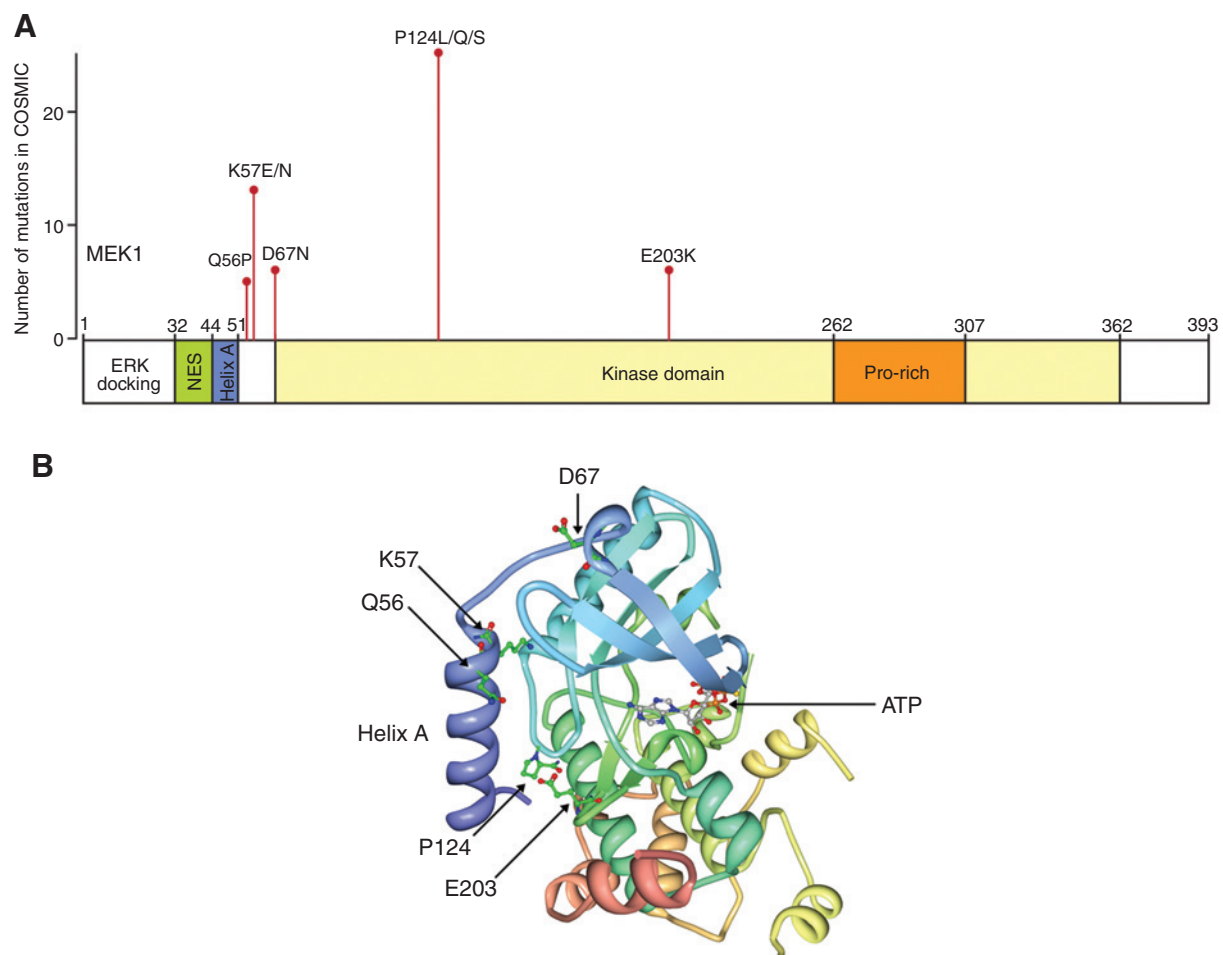


Figure 1.

A, recurrent MEK1 mutations in human cancer. Schematic diagram of domains and common (identified in at least five tumors) cancer-associated MEK1 mutations. B, the crystallographic model of MEK1 with mapped common mutations (PDB code: 3EQC).

Signaling Technology), IGF1R (Cell Signaling Technology), PDGFR β (C82A4; Cell Signaling Technology), BRAF (55C6; Cell Signaling Technology), PTEN (A281; Santa Cruz Biotechnology), and p-p90RSK^{S363} (Santa Cruz Biotechnology).

Constructs

Wild-type and mutant FLAG-tagged MEK1 constructs were each cloned into the *pCDH-CuO-MCS-EF1-CymR-PURO* lentiviral vector (System BioSciences).

Results

Positive selection of MEK1 Pro124 mutations during melanoma progression

The *MAP2K1* gene displays a significant mutation burden in melanoma, with a ratio of nonsynonymous to synonymous mutations of 6:2 (23). Alterations affecting Proline at codon 124 (P124L, P124Q, and P124S) are the most frequent MEK1 mutations (Fig. 1A) with a prevalence of 3.6% in melanomas unexposed to MAPK inhibitors (24/652 melanomas; COSMIC; ref. 31), suggestive of positive selection during melanoma progression. These alterations are caused by C>T (P124S and P124L) or C>A (P124Q) mutations at dipyrimidine sites and are typical of UV-induced mutations (32). In the crystal structure of wild-type MEK1, P124 is located in the interface between the negative regulatory helix A (residues 43–61) and the MEK1 kinase domain (Fig. 1B; ref. 33), and the functional consequences of these mutations are classified as deleterious by the missense substitution algorithms SIFT and Polyphen-2 (Supplementary Table S1).

Preexisting MEK1^{P124} mutations are associated with a reduced clinical response in patients treated with BRAF inhibitors

We assessed the impact of preexisting MEK1^{P124} alterations on clinical response of BRAF^{V600}-mutant metastatic melanoma to BRAF inhibitor therapy. Pretreatment MEK1^{P124} mutations were found in 12 of 123 (10%) tumor biopsies in four published cohorts (Table 1). Four tumors carried non-P124 MEK1 mutations (Table 1). The presence of a preexisting MEK1^{P124Q/S} mutation was associated with fewer RECIST responses (complete response + partial response) and a shorter PFS compared with patients who had MEK1^{P124}-wild-type tumors (response rate 33% vs. 72%, $P = 0.018$; median PFS 3.1 vs. 4.8 months, HR, 2.46 [95% confidence interval (CI), 1.32–4.56; $P = 0.004$, Fig. 2]. Both comparisons for response and PFS remained significant when patients with non-P124 MEK1 tumor mutations ($N = 4$) were excluded (i.e., P124-mutant compared with no detected MEK1 mutation; data not shown).

MEK1^{P124} status does not correlate with age, gender, M stage, or BRAF mutation genotype

Given the association between MEK1^{P124} mutations and clinical outcome, we also examined whether MEK1^{P124} mutation status correlated with additional clinical and genetic variables. On the basis of available data, we were able to examine patient age, gender, BRAF mutation status, and M stage at therapy initiation. As shown in Supplementary Table S2, MEK1^{P124} mutation status did not correlate with patient age or M stage at commencement of therapy (Supplementary Table S2), and although there was a trend

MEK1 Mutations in BRAF Inhibitor-Treated Melanoma

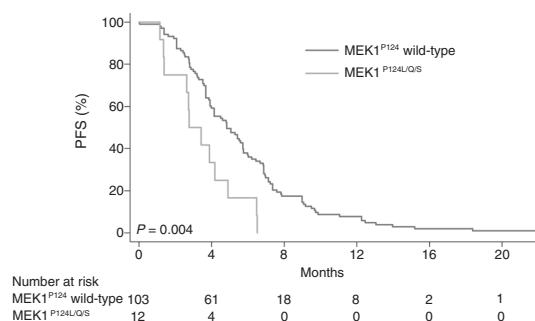


Figure 2. MEK1^{P124} mutation status and PFS of patients treated with BRAF inhibitor (dabrafenib or vemurafenib) therapy ($N = 123$).

toward an association between MEK1^{P124} mutations and gender and BRAF^{V600K/R} genotype, these did not reach statistical significance ($P = 0.07$ and $P = 0.09$, respectively; Supplementary Table S2). Importantly age, gender, and BRAF mutation genotype did not correlate with radiologic response or PFS (Supplementary Tables S3 and S4). In this combined cohort, there was a trend toward a longer PFS in those with M1a disease (Supplementary Table S4).

MEK1^{P124Q} and MEK1^{P124S} mutations display RAF-independent kinase activity and modulate dabrafenib sensitivity

To examine the kinase activity of P124 MEK1 mutations, the MEK1^{P124S} and MEK1^{P124Q} mutants were introduced into the BRAF-wild-type HEK293T cells, which show minimal phosphorylation of the MEK1 targets ERK1/2 (Supplementary Fig. S1). The overexpression of wild-type MEK1 in HEK293T cells for 24 and 48 hours did not induce ERK1/2 phosphorylation, whereas MEK1^{P124Q} and, to a lesser degree, MEK1^{P124S} consistently increased ERK1/2 phosphorylation. The diminished level of MEK1^{P124S}-mediated ERK phosphorylation at 48 hours (1.2-fold pERK/ERK increase compared with wild-type MEK1, Supplementary Fig. S1) appears to reflect the reduced accumulation of this mutant protein. In comparison, the K57E mutation, which disrupts the negative regulatory region of MEK1 (Fig. 1B; ref. 33) induced strong and persistent ERK1/2 activation (Supplementary Fig. S1). The MEK1 mutants also retained ERK kinase activity in the absence of serum, confirming RAF-independent kinase activity (Supplementary Fig. S1).

We also introduced these MEK1 variants into the BRAF^{V600E}-mutant SKMel28 melanoma cells, which are wild-type for MEK1 and MEK2 (Supplementary Table S5). As expected, dabrafenib induced a dose-dependent decrease in ERK activation in this cell model, and this inhibitory effect was overcome upon low-level expression of the MEK1^{K57E} variant (Fig. 3A). Significant levels of phosphorylated ERK were also retained in the presence of 10 nmol/L dabrafenib when cells expressed the MEK1^{P124Q} or MEK1^{P124S} variants. Importantly, ERK activation was inhibited when cells expressing wild-type MEK1, MEK1^{P124Q}, or MEK1^{P124S}, but not MEK1^{K57E}, were exposed to 100 nmol/L of dabrafenib (Fig. 3A). Consistent with these data, SKMel28 cells expressing MEK1^{K57E} were resistant to dabrafenib in clonogenic assays using 10 and 50 nmol/L dabrafenib, whereas cells expressing MEK1^{P124Q} or MEK1^{P124S} showed intermediate resistance (Fig.

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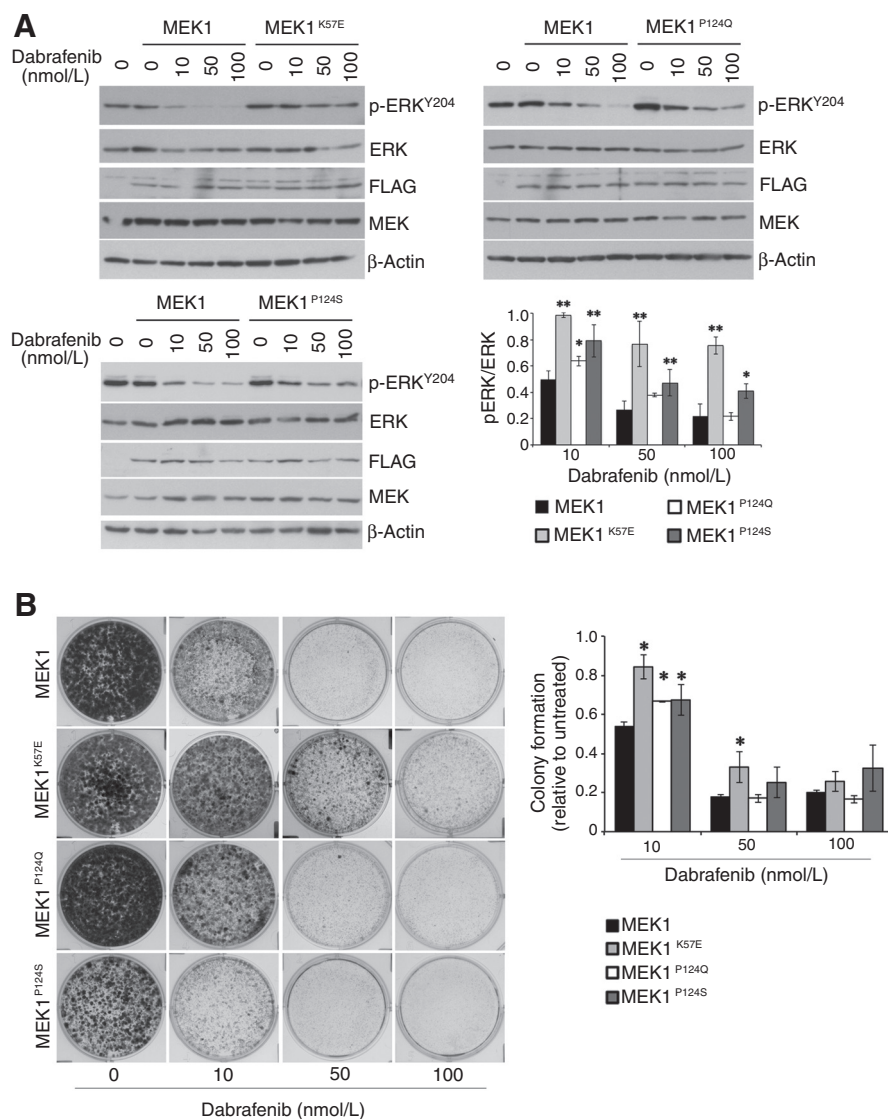


Figure 3. MEK1^{K57E} confer melanoma cell resistance to dabrafenib whereas MEK1^{P124Q} mutations decrease cell sensitivity to dabrafenib. A, SKMel28 melanoma cells were stably transduced with the indicated constructs. Cell lysates were analyzed for the indicated proteins 4 hours after incubation with dabrafenib at 0, 10, 50, and 100 nmol/L concentrations. Ectopically expressed MEK1 proteins were tagged with the FLAG epitope. Histogram, the ratio of p-ERK to ERK, relative to control-treated, transduced cells. Results are the average of at least two independent transduction experiments \pm SE. Significant differences between pERK/ERK ratios in cells expressing MEK1 variants versus wild-type MEK1 are shown; *, $P < 0.05$; **, $P < 0.01$ (Student two-tailed t test). B, transduced SKMel28 cells were seeded at low density and 24 hours after seeding were treated with the indicated concentrations of dabrafenib every 72 to 96 hours. Colonies were stained with crystal violet 12 days after transduction. Photographs, representative of at least two independent transduction experiments. Histogram, colony formation quantitated by crystal-violet staining relative to the matched transduced and untreated (0 nmol/L dabrafenib) cells. Results are the average of at least two independent experiments, each performed in triplicate \pm SE. Significant differences between colony-forming capacity of cells expressing MEK1 variants versus wild-type MEK1 are shown; *, $P < 0.05$. Colony formation was also increased in MEK1^{K57E} versus MEK1^{P124Q} and MEK1^{P124S} transduced cells treated with 10 nmol/L dabrafenib ($P < 0.05$).

3B). We also observed a slight increase in the viability of SKMel28 cells transduced with MEK1^{P124Q} compared with cells transduced with wild-type MEK1 (Supplementary Fig. S2).

To examine the effects of activated MEK1 on alternate signaling pathways, we examined the influence of MEK1^{P124S} and MEK1^{P124Q} on PI3K/AKT signaling and on the accumulation of key molecules involved in BRAF inhibitor resistance. As shown in Supplementary Fig. S3, MEK1 mutants caused a slight increase in the phosphorylation of AKT^{S473}, but not at AKT^{T308}, and this did not result in phosphorylation and activation of the AKT downstream targets pS6, p70S6K, GSK-3 β , or PRAS40. Furthermore, the MEK1 mutants did not alter the accumulation of bcl-2 (Supplementary Fig. S3) and phosphorylation of the proapoptotic protein BAD was not detected (data not shown). Similarly, we did not observe any changes in the expression of PTEN, p16^{INK4a}, or in the accumulation of the RTKs IGF1R, PDGFR β , or EGFR (Supplementary Fig. S3). As expected in the setting of a BRAF-mutant cell model, MAPK signaling, as determined by the phosphorylation of ERK and p90RSK, was

also indistinguishable in the MEK1-transduced BRAF-mutant cells (Supplementary Fig. S3).

We also examined two BRAF-mutant melanoma cell lines with endogenous MEK1^{P124Q} (SMU030P) and MEK1^{P124L} (D28; ref. 34) mutations. The SMU030P cell line was derived, before treatment, from a 27-year-old man with V600K-mutant disease who was treated with combination dabrafenib and trametinib (29). This patient had a best RECIST response of stable disease, with a decrease in the size of his target lesions of 14%. His PFS was 3.1 months, well below the median reported for combined dabrafenib and trametinib (35). Both the pretreatment cell line (SMU030P) and the cell line generated on progression (SMU030R) carried the MEK1^{P124Q} mutation (Supplementary Fig. S4). Intriguingly, when compared with a small panel of BRAF^{V600}/MEK1 wild-type melanoma cell lines, the SMU030P and D28 cell lines showed intermediate sensitivity to dabrafenib, but were exquisitely sensitive to the downstream MEK and ERK inhibitors, trametinib and VX-11e (Fig. 4A). Importantly,

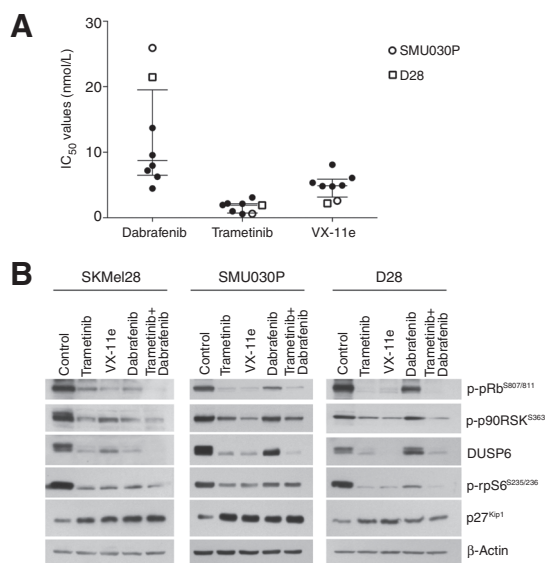


Figure 4. MEK1^{P124} mutations are associated with diminished BRAF inhibitor sensitivity. A, IC₅₀ values of dabrafenib, trametinib, and VX-11e in a panel of BRAF-mutant melanoma cell lines. The highlighted SMU030P and D28 cells are heterozygous for MEK1^{P124Q} and MEK1^{P124L}, respectively. Other melanoma cells include the BRAF^{V600}/MEK1 wild-type NM176, MM200, M238, SKMel28, and NM182. The 0.01 × IC₅₀ values shown for VX-11e. The median values and interquartile ranges are shown. B, SKMel28, SMU030P, and D28 cells were treated with DMSO (control), trametinib, VX-11e, dabrafenib, or combined trametinib and dabrafenib for 24 hours. Western blots of lysates showing protein markers of MAPK signaling.

these cell lines were screened for established mechanisms of resistance, and all were wild-type for *N-RAS*, *MEK2*, and *AKT*, did not overexpress the EGFR, IGF1R, or PDGFRβ RTKs, did not display alternate BRAF splicing and showed variable CRAF expression and PTEN and p16^{INK4a} status that did not correlate with MAPK inhibitor sensitivity (Supplementary Fig. S5 and Supplementary Table S5).

These data suggest that SMU030P and D28 were dependent on MAPK activity but that MAPK pathway activity was not solely driven by mutant BRAF. Accordingly, exposure of SMU030P and D28 to trametinib or VX-11e produced substantially diminished levels of the ERK transcription target DUSP6 and diminished phosphorylation of the ERK target p90RSK, whereas in contrast, dabrafenib caused only minor reduction in DUSP6 levels, and minor changes in p-p90RSK accumulation (Fig. 4B). In comparison, the BRAF^{V600}/MEK1 wild-type SKMel28 control cells showed a consistent reduction in MAPK activation markers in response to dabrafenib and trametinib or VX-11e (Fig. 4B).

Discussion

Treatment with BRAF inhibitors alone or in combination with MEK inhibitors has variable activity in BRAF-mutant melanoma, and a proportion of patients have only minor transient responses, with up to 25% of patients progressing by 12 weeks when treated with single-agent BRAF inhibitors (27, 36). A number of clinical factors are correlated with response to BRAF inhibition. For instance, early heterogeneity of response, as measured by FDG-PET is associated with a shorter PFS (37). Loss of PTEN expression,

deletions involving the p16^{INK4a} gene and amplification of the *cyclin D1* gene have all been associated with a shorter PFS in patients treated with BRAF inhibitors (17, 19). Importantly, these genetic changes do not preclude clinical or preclinical responses to BRAF inhibition.

We now show that MEK1 mutations affecting Proline 124 are associated with fewer patient responses and a shorter PFS with BRAF inhibitor therapy compared with patients without P124 mutations. We and others have also confirmed that MEK1^{P124} mutations have slightly elevated kinase activity (24, 25) and that melanoma cells carrying these mutants show diminished sensitivity to BRAF inhibition (14). It is worth noting that the reported influence of MEK1^{P124} mutations on BRAF inhibitor sensitivity is not always consistent (24). Collectively, these observations suggest that the background tumor genotype may influence the impact of MEK1^{P124} mutations and that additional genetic changes may contribute and be required for BRAF inhibitor resistance.

We surmise that the elevated kinase activity of MEK1^{P124} mutants leads to a slightly diminished BRAF inhibitor response, and this facilitates a greater pool of surviving tumor cells and the acquisition of resistance mechanisms. This is supported by our previously reported analyses of the SMU030R cell line. This short-term cell model is derived from the same patient as the SMU030P pretreatment cell line, but was isolated from a lesion that had progressed on BRAF/MEK inhibitor therapy. Both the matched pretreatment SMU030P and progressing SMU030R cells carry the MEK1^{P124Q} mutation, but the resistant SMU030R cells have also acquired an amplification encompassing the *BRAF* gene, a further progressing lesion was found to carry an NRAS^{Q61K}, suggesting that the MEK1^{P124} mutation is insufficient to confer resistance (29).

We also showed that two BRAF^{V600}/MEK1^{P124} double-mutant melanoma cell lines were less sensitive to dabrafenib, but showed similar responses to downstream MEK and ERK inhibition, when compared with six BRAF^{V600}/MEK wild-type cell lines. Although the two MEK1^{P124}-mutant cell lines were BRAF^{V600K}, dabrafenib shows comparable activity against BRAF^{V600E} and BRAF^{V600K} at the enzyme and cellular levels (38–40). This finding suggests that patients carrying baseline MEK1^{P124} mutations may gain greater benefit from treatment combinations, which include an MEK or ERK inhibitor. Formal examination of this hypothesis is required in patients treated with combinations involving these inhibitors; particularly given that patient SMU030 had only a minor transient response to combined dabrafenib and trametinib. This disconnect between preclinical and clinical activity in the SMU030 tumor may be attributable to limitations in delivering effective MEK inhibitor concentrations *in vivo* and/or additional genetic alterations that further diminish response duration.

It is possible that mutations affecting MEK1 at Proline124 are prognostic rather than predictive of BRAF inhibitor response. Our data, however, showing that MEK1^{P124} mutations show RAF-independent kinase activity and that melanoma cells with these MEK1 mutations retain sensitivity to the downstream MEK and ERK inhibitors, while displaying intermediate sensitivity to BRAF inhibition, support our hypothesis that these MEK1 alterations are predictive of BRAF inhibitor response. We also show that MEK1^{P124} mutations do not correlate with several significant clinical variables, including M stage, patient age, and BRAF genotype. It is worth noting that our analysis of clinical correlates was limited to the variables available in previous studies

and further investigations are required to examine possible interactions between MEK1^{P124} mutations, clinical factors, and patient outcomes.

This work highlights the importance of defining baseline predictors of clinical response to targeted therapies. Multiple predictors of BRAF inhibitor response have now been described, including MEK1^{P124} mutations, PTEN expression, *CDKN2A* deletions, and *cyclin D1* amplification (17, 19), and analyses of larger cohorts with detailed genetic and clinical data will help determine the precise clinical significance of each genetic variant. Biomarkers help define patients that may respond poorly to current therapies and also help identify patients who may benefit from alternative first-line combination treatments.

Disclosure of Potential Conflicts of Interest

M.S. Carlino reports receiving speakers bureau honoraria from Glaxo-SmithKline and Novartis. R.F. Kefford reports receiving speakers' bureau honoraria from Merck and Bristol-Myers Squibb, is a consultant/advisory board member for Bristol-Myers Squibb, GlaxoSmithKline, Merck, Novartis and Roche, and other conflicts from Roche and Bristol-Myers Squibb. G.V. Long reports honoraria from speakers' bureau from GlaxoSmithKline and Roche, and is a consultant/advisory board member for GlaxoSmithKline and Roche. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: M.S. Carlino, H. Rizos

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