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ERK Inhibitor LY3214996 Targets ERK Pathway–Driven Cancers: A Therapeutic Approach Toward Precision Medicine

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ABSTRACT

The ERK pathway is critical in oncogenesis; aberrations in components of this pathway are common in approximately 30% of human cancers. ERK1/2 (ERK) regulates cell proliferation, differentiation, and survival and is the terminal node of the pathway. BRAF- and MEK-targeted therapies are effective in BRAF V600E/K metastatic melanoma and lung cancers; however, responses are short-lived due to emergence of resistance. Reactivation of ERK signaling is central to the mechanisms of acquired resistance. Therefore, ERK inhibition provides an opportunity to overcome resistance and leads to improved efficacy. In addition, *KRAS*-mutant cancers remain an unmet medical need in which ERK inhibitors may provide treatment options alone or in combination with other agents. Here, we report identification and activity of LY3214996, a potent, selective, ATP-competitive ERK inhibitor. LY3214996 treatment inhibited the pharmacodynamic biomarker, phospho-p90RSK1, in cells and tumors, and correlated with LY3214996 exposures and antitumor activities. In *in vitro* cell proliferation assays, sensitivity to LY3214996 correlated with ERK pathway aberrations. LY3214996 showed dose-dependent tumor growth inhibition and regression in xenograft models harboring ERK pathway alterations. Importantly, more than 50% target inhibition for up to 8 to 16 hours was sufficient for significant tumor growth inhibition as single agent in *BRAF*- and *KRAS*-mutant models. LY3214996 also exhibited synergistic combination benefit with a pan-RAF inhibitor in a *KRAS*-mutant colorectal cancer xenograft model. Furthermore, LY3214996 demonstrated antitumor activity in *BRAF*-mutant models with acquired resistance *in vitro* and *in vivo*. Based on these preclinical data, LY3214996 has advanced to an ongoing phase I clinical trial (NCT02857270).

Introduction

The ERK pathway plays a critical role in oncogenesis and in the regulation of critical cellular processes such as cell cycle progression, cell proliferation, migration, survival, differentiation, senescence, metabolism, protein synthesis, and angiogenesis (1). This pathway is complex and regulated by various extracellular growth factors and mitogen signaling, which result in the activation of RAS (KRAS, NRAS, and HRAS). ERK1/2 (ERK) controls the output of the ERK pathway by phosphorylating and activating several downstream cytoplasmic and nuclear targets, which promote cell-cycle regulation and negative feedback mechanisms (2). The ERK pathway is activated in approximately 30% of all human cancers via *RAS*-, *BRAF*-, or *MAP2K1* (*MEK1*)-activating mutations and thus has attracted significant interest as a therapeutic cancer target (3). In addition, alterations known to activate the ERK pathway are also common in acquired resistance to BRAF, MEK, ALK, CDK4/6, TRKA, and EGFR inhibitors (4–6). Therefore, targeting ERK1/2 in the ERK pathway is an attractive strategy for the treatment of a variety of tumor types harboring ERK pathway alterations.

The ERK pathway is a clinically validated pathway in cancer as evidenced by the success of BRAF and MEK inhibitors in the treatment of *BRAF*-mutant melanoma and non–small cell lung cancer (NSCLC; refs. 7–10). Despite the success of BRAF and MEK inhibitor combination therapies, responses are typically transient, and many patients eventually develop resistance after approximately 9 to 11 months (7, 11). Several mechanisms of acquired resistance have been identified after treatment with either single-agent or combination therapies which include *BRAF* amplification, *NRAS* or *MEK* mutations, receptor tyrosine kinase (RTK) activation, upregulation of bypass or compensatory pathways, etc. (12–17). ERK reactivation is common to these mechanisms of acquired resistance leading to rapid recovery of ERK pathway resulting in resistance to single-agent BRAF inhibitor or combination of BRAF/MEK inhibitor therapies (18, 19). Therefore, treatment with an ERK inhibitor may provide the opportunity to avoid, delay, or overcome resistance from upstream signaling in these patients. Hence, several ERK inhibitors have been discovered to explore the clinical benefit to patients with acquired resistance to BRAF/MEK inhibition as well as for combination therapy, to tackle hard-to-treat *RAS*-mutant cancers. ERK inhibitors have demonstrated efficacy in preclinical cancer models, including those resistant to BRAF or MEK inhibitors (20–23). More recently, other ERK inhibitors have demonstrated clinical activity in patients with *BRAF* V600–mutant melanoma who were either refractory or naïve to BRAF and MEK

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Note: Supplementary data for this article are available at Molecular Cancer Therapeutics Online (http://mct.aacrjournals.org/).

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inhibitor combination therapy (24, 25). However, the potential value of ERK inhibitors expands beyond melanoma and acquired resistance in melanoma to target all cancers with ERK pathway alterations. Here, we demonstrate the potent antitumor activity of LY3214996 in a variety of tumor models with ERK pathway alterations as well as BRAF inhibitor acquired resistance.

Materials and Methods

Compounds

LY3214996 (example 1) was synthesized as described in U.S. patent number 9469652 (26). LY3009120 was synthesized as per published method (27). Trametinib, cobimetinib, and vemurafenib were purchased or synthesized using published methods. For *in vitro* studies, compounds were prepared as 10 mmol/L stock solution in DMSO and stored frozen.

Cell culture

HCT116, Colo205, Calu6, A375, SK-MEL-30, MiaPaCa-2, SW48, and other cell lines as part of the 60-cell line panel were obtained from the American Type Culture Collection (ATCC) and were characterized by PCR and short tandem repeat analysis for contamination and authentication. All cell lines used for *in vitro* experiments and xenograft studies were cultured in ATCC-recommended culture medium. Vemurafenib- or dabrafenib-resistant A375 cell lines were generated and cultured as previously described (17).

Western blotting

Cells were treated with indicated concentrations of LY3214996 for indicated time points in 10 cm dishes, and whole-cell lysates were prepared in RIPA Lysis Buffer (Millipore) supplemented with PMSF and Halt Phosphatase and Protease Inhibitor Cocktail at a final concentration of 5% for each reagent in buffer. Protein concentrations were determined via BCA assay (Thermo Fisher Scientific) following the manufacturer's guidance and subjected to SDS-PAGE and Western blotting with primary antibodies pCRAF, CRAF, pMEK1/2, MEK1/2, pERK1/2, ERK1/2, EGR1, c-MYC, DUSP4 (Cell Signaling Technology), pRSK1 (Abcam), and SPRY4 and Beta-Actin (Sigma-Aldrich). Secondary antibodies used were Alexa Fluor 680 goat anti-rabbit (Invitrogen), and goat anti-mouse and donkey anti-rabbit (LI-COR). Blots were read using the LI-COR Odyssey Classic Infrared Imaging System at 700 and 800 nm. Images were processed and analyzed using Image Studio version 3.1.

Cell proliferation assay

Sixty human lung, colorectal, pancreatic, and skin cancer cell lines were obtained from the ATCC. The cells were maintained in RPMI 1640 or DMEM supplemented with 10% FBS, sodium pyruvate, nonessential amino acids, L-glutamine, and penicillin–streptomycin (Invitrogen). All cultures were maintained in a humidified incubator at 37°C under 5% CO_2/95% air free of mycoplasma and pathogenic murine viruses. The cells were used for experiments at passages <7 after recovery from frozen stocks. Cells (3,000/well) were plated in 96-well black plates and cultured in the RPMI 1640 or DMEM with 10% FBS for 24 hours. The cells were treated with DMSO or LY3214996 at nine final dilutions from a 10 mmol/L stock solution (0.001, 0.003, 0.01, 0.03, 0.1, 0.3, 1, 3, and 10 μmol/L) in medium with 0.1% DMSO and 5% FBS or 10% FBS (melanoma cells) for 120 hours. Cell viability was measured by CellTiter-Glo luminescent cell viability assay (Promega). Data were presented as absolute IC_{50} (Abs IC_{50}) and analyzed using XLfit (IDBS).

In vivo studies

All *in vivo* studies were performed in accordance with the American Association for Laboratory Animal Care institutional guidelines. All experimental protocols were approved by The Eli Lilly and Company Animal Care and Use Committee. Briefly, 5×10^6 (HCT116, Colo205, SK-MEL-30, Calu6, MiaPaCa-2) or 10×10^6 (SW48) tumor cells in a 1:1 matrigel mix (0.2 mL total volume) were injected s.c. in to the right hind flank of 8- to 12-week-old (22–25 g) female athymic nude mice (Envigo). For CTG-0652 patient-derived xenograft (PDX) studies, tumor fragments were used for implantation (Champions Oncology). After tumors reached 200 to 300 mm^3, animals were randomized into groups of 5 or 6. All drugs were administered orally (gavage) in 0.2 mL volume of vehicle. Tumor growth and body weight were monitored over time to evaluate efficacy and signs of toxicity (24). The combination study of LY3214996 and LY3009120 in the HCT116 colorectal cancer xenograft model was performed in female NIH nude rats (Taconic Biosciences). Statistical analyses are described in the Supplementary Methods.

For pharmacodynamic (PD) studies in HCT116 colorectal cancer xenograft model, animals were randomized into groups of 5 and dosed orally with LY3214996 in hydroxyethylcellulose 1% (w/v)/P80 0.25% (v/v)/antifoam 1510-US 0.05% (v/v), trametinib in 20% captisol in 25 mmol/L phosphate buffer, pH 2.0, or cobimetinib in 1% HEC/0.25% tween-80/0.05% antifoam. Tumors and blood samples were collected after dose at indicated time points. Tumors were flash frozen and stored at -80°C until tumor lysates were processed for pRSK1 levels by ELISA method as described in the Supplementary Methods. TEC_{50} and TED_{50} were calculated using Excel (Microsoft Corporation) and XLfit. Blood was collected in EDTA-coated tubes, spun down to isolate plasma, and frozen at −80°C in a 96-well plate. Drug concentrations were measured by LC/MS-MS.

Results

LY3214996 is a potent and selective ATP-competitive inhibitor of ERK

6,6-Dimethyl-2-{2-[(1-methyl-1H-pyrazol-5-yl)amino]pyrimidin-4-yl}-5-[2-(morpholin-4-yl)ethyl]-5,6-dihydro-4H-thieno[2,3-c]pyrrol-4-one (LY3214996) was discovered by lead optimization of hits identified using a medium-throughput screen with recombinant ERK2 enzyme (**Fig. 1A**). LY3214996 is a potent ERK inhibitor with IC_{50} values of 5 ± 1 nmol/L against ERK1 and ERK2 recombinant enzymes (**Fig. 1B**). In the DiscoverX assays, IC_{50} values for ERK1 and ERK2 are <0.001 μmol/L for both enzymes (Supplementary Table S1). We also determined Ki values using ERK1-2P and ERK2-2P enzymes, and the average Ki values were 0.650 and 0.064 nmol/L respectively (**Fig. 1B**). Enzyme kinetics revealed LY3214996 to be a reversible, competitive inhibitor of ATP with rapid equilibrium kinetics (**Fig. 1C** and **D**). LY3214996 demonstrated excellent selectivity based on biochemical screening against 512 kinases in the DiscoverX panel (Supplementary Table S1). LY3214996 demonstrated >40-fold selectivity against 512 kinases and >1,000-fold selectivity against 486 kinases. LY3214996 was also tested for cellular selectivity in HCT116 cells using the ActivX platform (Supplementary Table S1). In the ActivX assay, cell IC_{50} for both ERK1 and ERK2 was 0.009 μmol/L. LY3214996 showed >110-fold selectivity against 245 kinases measured in HCT116 cell lysates.

To determine the ERK inhibition in tumor cells, we measured inhibition of pRSK1 (T359/S363) as a mechanistic readout in *KRAS*- or *BRAF*-mutant cell lines. LY3214996 potently inhibited pRSK1 in *KRAS*-mutant HCT116 (colorectal cancer) and Calu6 (NSCLC) cells with average IC_{50} values in the range of 0.200 to 0.223 μmol/L (**Fig. 1E**). Also, LY3214996 potently inhibited pRSK1 in *BRAF*-mutant

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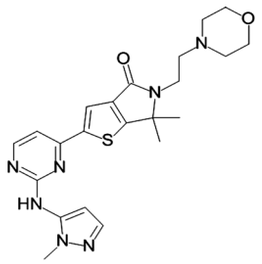
A

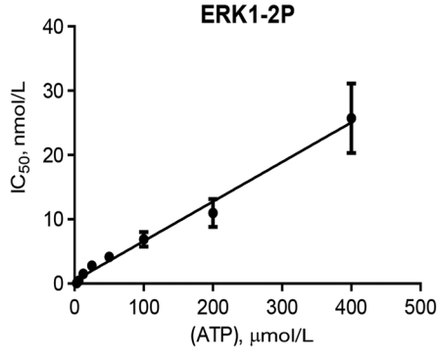


B

Kinase	Abs IC_{50}, nmol/L (Mean ± SD)	ATP Conc (μmol/L)	Avg Ki (nmol/L)
ERK1	5.0 ± 1.0	10	ND
ERK2	5.0 ± 1.0	10	ND
ERK1-2P	ND	NA	0.650
ERK2-2P	ND	NA	0.064

Abbreviations: Abs, Absolute; Conc, Concentration; NA, not available; ND, not determined; SD, standard deviation

C



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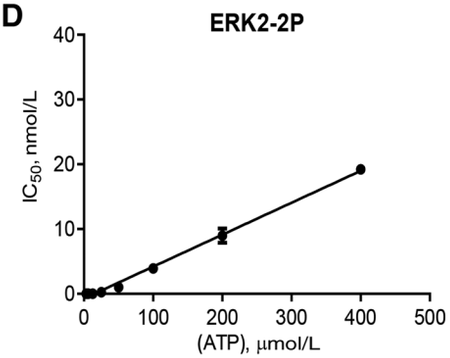

E

Cell line	Mutation	pRSK1 (T359/S363) cell IC_{50}, μmol/L (Sandwich ELISA)
HCT116	KRAS (G13D)	0.223 ± 0.110
Calu6	KRAS (Q61H)	0.200 ± 0.059
A375	BRAF (V600E)	0.183 ± 0.033
Colo205	BRAF (V600E)	0.054 ± 0.009

Figure 1.

Discovery, biochemical, and cell potency of LY3214996. **A,** Chemical structure of LY3214996. **B,** Biochemical potency of LY3214996 against ERK1 and ERK2 enzymes. **C** and **D,** Inhibitory potency of LY3214996 for ERK1-2P (**C**) and ERK2-2P (**D**) as a function of ATP concentration. **E,** Cellular potency of LY3214996 in *RAS*- and *RAF*-mutant cell lines.

A375 melanoma and Colo-205 colorectal cancer cells with average IC_{50} values in the range of 0.054 to 0.183 μmol/L, suggesting similar cell potency in *KRAS*- and *BRAF*-mutant cell lines tested. In summary, our data suggest that LY3214996 is a potent inhibitor of ERK signaling in *RAS*- or *RAF*-mutant cell lines *in vitro*.

LY3214996 induces G_1 arrest and apoptosis and shows potent inhibition of downstream signaling in cell lines with ERK alterations

To determine the effect of LY3214996 on cell-cycle regulation, *KRAS*-mutant HCT116 cells were treated with increasing concentrations of LY3214996 for 24, 48, and 72 hours and subjected to cell-cycle analysis by flow cytometry. LY3214996 arrested cells in the G_1 phase of the cell cycle and induced apoptosis in a dose- and time-dependent manner as demonstrated by an increasing percentage of cells in the sub-G_1 phase at 24, 48, and 72 hours after treatment (**Fig. 2A** and **B**; Supplementary Fig. S1). Furthermore, the percent apoptosis induced by LY3214996 was similar to apoptosis induced by a pan-RAF inhibitor LY3009120 or a MEK inhibitor trametinib at relevant concentrations, which was higher at 72 hours than 48 hours (Supplementary Fig. S1). More importantly, we confirmed the specificity of LY3214996 in inducing cell death by using ERK1 and ERK2 siRNA knockdown studies in HCT116 cells. As shown in **Fig. 2C**, >80% knockdown of both ERK1 and ERK2 in HCT116 cells resulted in an

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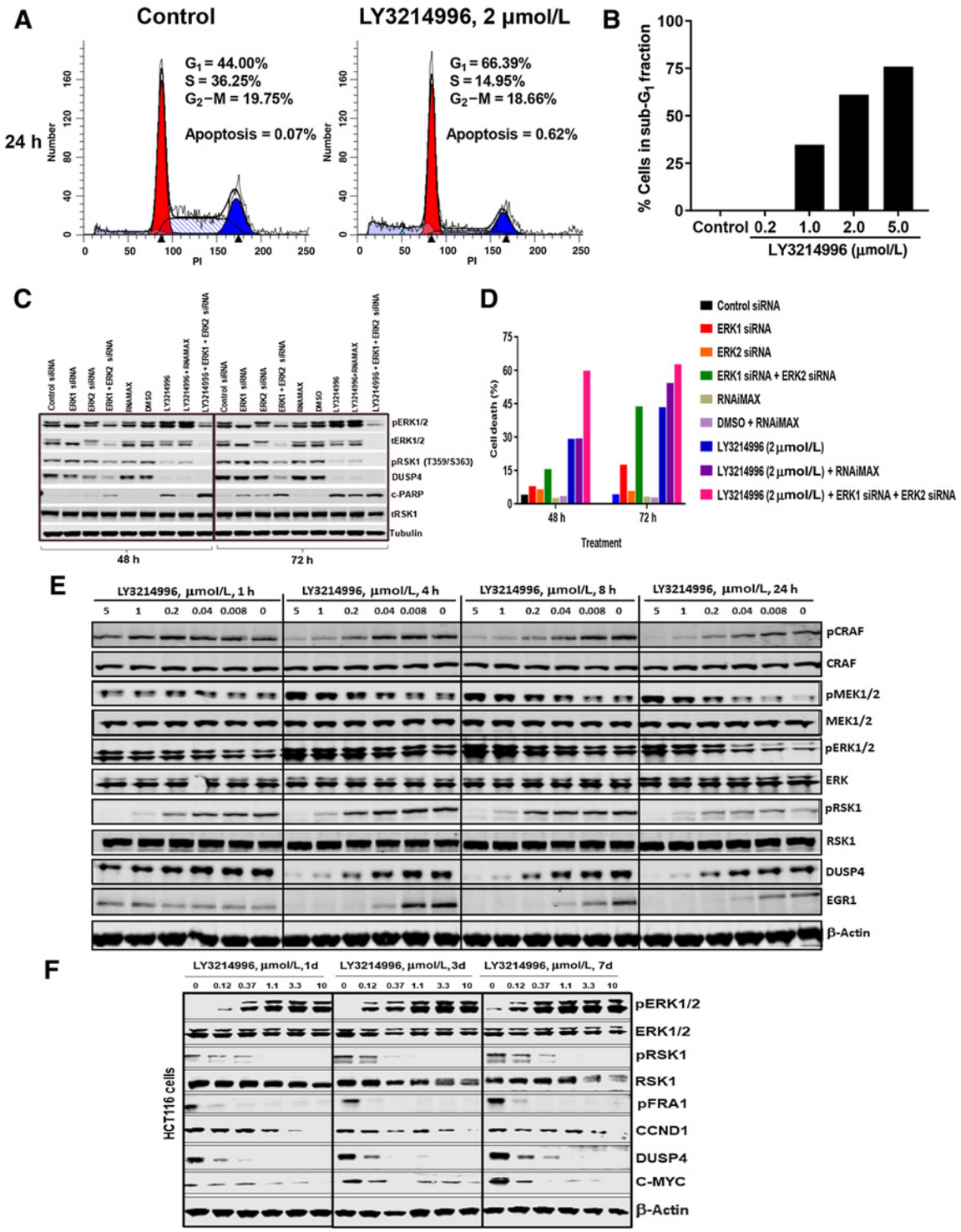

Figure 2.

LY3214996 induces G_1 arrest, apoptosis, and inhibition of downstream signaling in *KRAS*-mutant HCT116 colorectal cancer cells. **A** and **B,** Percentage of HCT116 cell population in G_1, S, and G_2–M phase of cell cycle after incubation for 24 hours with LY3214996 (2 μmol/L; **A**) and percentage of HCT116 cell population in sub-G_1 (indicator of apoptosis) phase of cell cycle after incubation for 48 hours with increasing concentrations of LY3214996 (**B**). **C,** Knockdown of ERK1 and ERK2 by siRNA and LY3214996 treatment and increased cleaved PARP. **D,** Cell death induced by ERK1 and ERK2 siRNA with and without LY3214996 treatment in HCT116 cells at 48 and 72 hours. **E,** Effect of increasing concentrations of LY3214996 treatment on negative feedback activation of pERK1/2 over time up to 24 hours. **F,** Potent inhibition of downstream signaling by LY3214996 up to 7 days with increasing concentrations of LY3214996 treatment in HCT116 cells.

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increase in apoptosis marker cleaved PARP (c-PARP) at 48 and 72 hours which is quite similar to the results with 2 μmol/L LY3214996 treatment which is expected to inhibit pRSK1 >90%. We have also measured cell death by FACS analysis followed by propidium iodide staining in HCT116 cells after both ERK1 and ERK2 knockdown for 48 and 72 hours, and treatment with or without 2 μmol/L LY3214996 as shown in **Fig. 2D**. The cell death induced by ERK1 and ERK2 siRNA is similar to 2 μmol/L LY3214996 treatment at 72 hours, and there was no additional significant increase in cell death by combination of 2 μmol/L LY3214996 with ERK1 and ERK2 siRNA compared with 2 μmol/L LY3214996 plus transfection control, suggesting that cell death induced by LY3214996 is specific to ERK1 and ERK2 inhibition and can take up to 2 to 3 days.

To further elucidate the mechanism of action of LY3214996, we measured ERK pathway inhibition in *KRAS*-mutant HCT116 colorectal cancer cells in a time-dependent manner. LY3214996 treatment resulted in sustained inhibition of pRSK1 in a dose- and time-dependent fashion despite increased pERK levels (**Fig. 2E**). ERK inhibitor–mediated reactivation of pERK is likely due to feedback activation through potent inhibition of pCRAF at the inhibitory ERK phosphorylation sites (S289/S296/S301; ref. 28), suggesting that the pERK increase is a result of CRAF activation. Consistently, we observed marked reduction in pCRAF (S289/S296/S301; **Fig. 2E**) after LY3214996 treatment in a dose- and time-dependent fashion. This mechanism was further supported by observed increases in pMEK1/2 (**Fig. 2E**). Treatment with a pan-RAF inhibitor LY3009120 blocked increase in pMEK1 and pERK suggesting pERK increase was due to inhibition of negative feedback on CRAF (Supplementary Fig. S2). To further characterize the mechanism of action and sustained effects on signaling elicited by LY3214996, we measured inhibition of phosphorylation or expression of various known downstream cytoplasmic and nuclear substrates of ERK by Western blotting. After treatment with multiple doses of LY3214996 for 1, 3, and 7 days, LY3214996 showed robust inhibition of pRSK1 (**Fig. 2F**). Inhibition of pRSK1 correlated with inhibition of pFRA1, CCND1, DUSP4, and c-MYC (**Fig. 2F**). Similar correlations of downstream pathway inhibition of pFRA1, CCND1, DUSP4, and EGR1 were observed in *BRAF*-mutant A375 melanoma and *MEK1*-mutant SW48 colorectal cancer cells treated with LY3214996 for 24 hours (Supplementary Fig. S3). These downstream targets have emerged as bonafide PD biomarkers for ERK inhibition in tumor models to establish pharmacokinetic (PK)/PD correlations. Taken together, LY3214996 induces G_1 cell-cycle arrest and cell death and potently inhibits ERK downstream signaling in cell lines with ERK pathway alterations. Furthermore, LY3214996 demonstrates sustained inhibition of ERK downstream signaling despite pERK feedback activation.

LY3214996 potently inhibits cell proliferation in most ERK pathway–altered cancer cell lines and melanoma cell lines resistant to BRAF inhibitors

Human cancer cell lines provide a vital model to study anticancer therapeutics and facilitate the development of predictive biomarkers. To link genetic alteration, lineage, and other cellular features of cancer cell lines to drug specificity and sensitivity, we tested LY3214996 in a panel of 60 cancer cell lines representing four major tumor types (pancreatic cancer, colorectal cancer, NSCLC, and melanoma) with high prevalence of ERK pathway alterations. Inhibition of cell proliferation was measured after treatment with a dose response of LY3214996 for 5 days, and data were plotted as absolute IC_{50} against each individual cell line (**Fig. 3A**). Cell lines showing IC_{50} value $\leq$ 2,000 nmol/L are defined as sensitive as it is expected to inhibit pRSK1 more than 80% in majority of cell lines. We focused on ERK pathway–activating alterations which included *BRAF* mutations (V600 and atypical), *BRAF* oncogenic deletion, *BRAF* copy gain, *RAS* mutations (*KRAS, NRAS, HRAS*), *MAP2K1* mutations, and *NF1* mutations or loss to predict the sensitivity. ERK pathway alterations present in various cell lines tested and the absolute IC_{50} value of LY3214996 in them are listed in Supplementary Table S2. There was only one cell line with *BRAF* copy gain which cooccurred with a *KRAS* mutation, and *NF1* mutations or deletions cooccurred with *RAS* or *RAF* mutations in several cell lines (Supplementary Table S2). There was no cell line with any *HRAS* mutations.

Among 11 pancreatic cancer cell lines tested, 10 were sensitive to LY3214996, and all of these sensitive cell lines had one or more ERK pathway alterations (11 *KRAS* mutations,1 *BRAF* oncogenic deletion, and 3 with *NF1* mutation or loss cooccurred with *KRAS* mutation or *BRAF* deletion; **Fig. 3A**). Among 17 colorectal cancer cell lines tested, 10 cell lines with one or more ERK pathway alterations were sensitive to LY3214996 (**Fig. 3A**). Among 27 NSCLC cell lines tested, 11 of them with one or more ERK pathway alterations were sensitive to LY3214996 treatment (**Fig. 3A**). All five melanoma cell lines (two with *BRAF* V600E mutations, three with *NRAS* mutations, *NF1* mutation or loss cooccurred with 2 *BRAF* V600E mutant, and 1 *NRAS*-mutant cell lines) were sensitive to LY3214996 (**Fig. 3A**). Overall, LY3214996 showed IC_{50} values less than 2,000 nmol/L in approximately 67%, 54%, 100%, and 60% of *RAS*-mutant or *BRAF*-mutant (or oncogenic deletion or copy-number gain) or *MAP2K1*-mutant or *NF1*-mutant (or loss) cancer cell lines respectively (**Fig. 3B**). Altogether, 57% of the cell lines with one or more ERK pathway alterations were sensitive to LY3214996, suggesting a potential patient selection strategy for clinical testing of LY3214996.

To test whether LY3214996 can overcome acquired resistance to BRAF inhibitors *in vitro*, we generated vemurafenib- and dabrafenib-resistant *BRAF* V600E–mutant A375 melanoma cells as previously described (17). A375-Dabrafenib-R cells had acquired *NRAS* Q61K mutation, and A375-vemurafenib-R cells showed elevated FGFR3/RAS signaling (17). As shown in **Fig. 3C**, both vemurafenib- and dabrafenib-resistant cell lines showed cross-resistance to vemurafenib and dabrafenib as compared with naïve parent A375 cells. However, LY3214996 demonstrated similar potency in naïve parent A375 cells and vemurafenib- or dabrafenib-resistant A375 cells (**Fig. 3C**). Our findings suggest that LY3214996 can overcome acquired resistance to BRAF inhibitors in metastatic melanoma.

LY3214996 demonstrates good PK/PD correlation in tumors that correspond to potent tumor growth inhibition

The kinetics of drug–target interactions can be quantified to predict *in vivo* PD and antitumor activity. A detailed PK/PD (pRSK1 inhibition) relationship of LY3214996 was generated in a *KRAS*-mutant HCT116 colorectal cancer xenograft model. After administration of a single dose of LY3214996 (6.25, 12.5, 25, 50, and 100 mpk) for dose–response study in nude mice bearing HCT116 xenografts, tumors were harvested at 4 hours after dosing, and pRSK1 was measured by sandwich ELISA (**Fig. 4A**).The PD effects correlated well with drug levels in the plasma (**Fig. 4A**). LY3214996 treatment showed dose-dependent increase in plasma drug exposure and inhibition of pRSK1 in tumors. LY3214996 was also evaluated at two different efficacious dose levels (50 and 100 mpk qd) for time-dependent plasma drug exposure and pRSK1 inhibition in the HCT116 colorectal cancer xenograft model. PD effects (pRSK1 inhibition) correlated well with PK (drug levels) in the plasma (**Fig. 4B** and **C**). After fitting to a four-parameter sigmoidal logistical model using XL fit, estimated TEC_{50}

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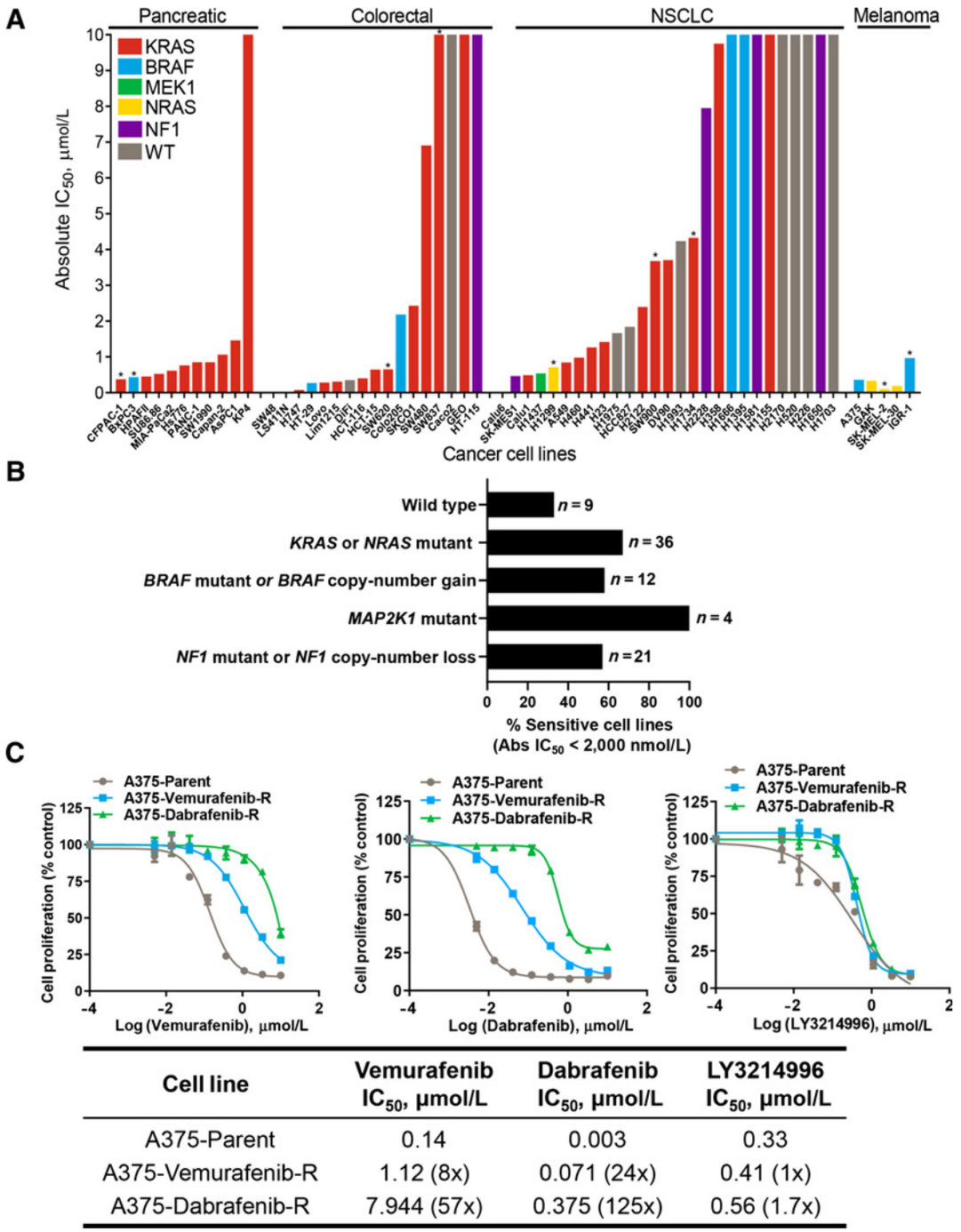

Cell line	Vemurafenib IC_{50}, μmol/L	Dabrafenib IC_{50}, μmol/L	LY3214996 IC_{50}, μmol/L
A375-Parent	0.14	0.003	0.33
A375-Vemurafenib-R	1.12 (8x)	0.071 (24x)	0.41 (1x)
A375-Dabrafenib-R	7.944 (57x)	0.375 (125x)	0.56 (1.7x)

Figure 3.

LY3214996 shows preferential antitumor activities in ERK pathway–altered cancer cell lines and can overcome acquired resistance to BRAF inhibitors in A375 melanoma models. **A,** Antiproliferative activity profile of LY3214996 in cancer cell lines. * denotes *NF1* mutations or loss co-occurred with *RAS, RAF,* or *MAP2K1*(*MEK1*) mutations. **B,** Summary of percentage of cell lines tested that is sensitive to LY3214996 based on one or more ERK pathway alterations, which included *KRAS* or *NRAS* mutations or *BRAF* mutations (V600 and atypical) or *BRAF* oncogenic deletion or *BRAF* copy gain or *MAP2K1* mutations or *NF1* mutations or loss. **C,** Antitumor activity and potency of LY3214996 in A375 parent, vemurafenib-resistant, and dabrafenib-resistant models. Assays performed at least twice, and representative data shown.

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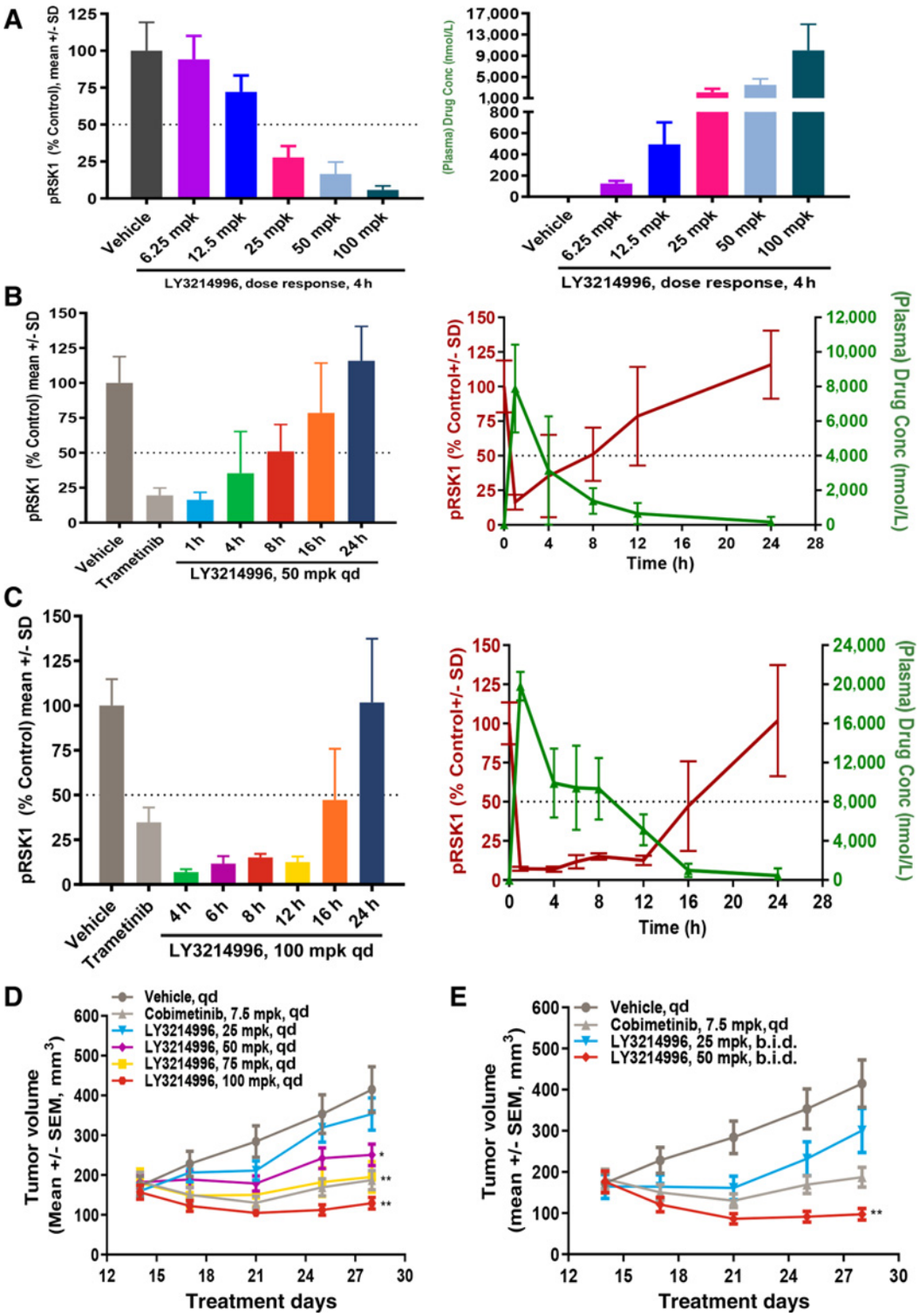

Figure 4.

LY3214996 demonstrates good PK, PD, and tumor growth inhibition in a *KRAS*-mutant HCT116 colorectal cancer model. **A,** Dose–response inhibition of pRSK1 in *KRAS*-mutant HCT116 colorectal cancer tumors and plasma exposure of LY3214996 in the plasma. **B** and **C,** Inhibition of pRSK1 PD biomarker in *KRAS*-mutant HCT116 colorectal cancer tumors at different time points (up to 24 hours) after single oral administration of LY3214996 at 50 mg/kg (**B**) or 100 mg/kg (**C**). Trametinib was dosed at 3 mg/kg as a reference MEK inhibitor. **D** and **E,** Multidose efficacy of LY3214996 in *KRAS*-mutant HCT116 after qd dosing (**D**), and *KRAS*-mutant HCT116 after b.i.d. dosing (**E**); *, $P < 0.05$; **, $P < 0.001$, vehicle control vs. LY3214996. b.i.d., twice a day; qd, daily.

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and TED_{50} (4 hours) values were 1,107 nmol/L and 16 mpk respectively. Our data suggest good PK and PD correlation of LY3214996 in a *KRAS*-mutant HCT116 colorectal cancer xenograft model.

In a subsequent efficacy study in in HCT116 *KRAS*-mutant colorectal cancer model dosed at 50 or 100 mpk qd, LY3214996 demonstrated 68% tumor growth inhibition and 25% tumor regression respectively as compared with vehicle control (**Fig. 4D**). Significant tumor growth inhibition by LY3214996 at 50 mpk qd and 100 mpk qd suggests strong PK/PD and efficacy correlation in *KRAS*-mutant HCT116 colorectal cancer xenograft model. All treatments were well tolerated, and efficacy was statistically significant in all treatment groups except 25 mpk qd and b.i.d. (twice daily) groups (Supplementary Fig. S4A and S4B). Robust efficacy of LY3214996 at 100 mpk qd or 50 mpk b.i.d. (**Fig. 4D** and **E**) suggests that ≥50% inhibition of pRSK1 for 8 to 16 hours in a given 24-hour period is sufficient for significant efficacy. More importantly, efficacy of LY3214996 at 50 mpk b.i.d. or 100 mpk qd was similar to efficacy of MEK inhibitor cobimetinib dosed at 7.5 mpk qd (approximate human equivalent dose in mice), which has been shown to cover the target inhibition >24 hours (29). In summary, analysis of our PK/PD/efficacy correlation data suggests ≥50% inhibition of the target for a minimum of 8 hours is sufficient for robust single-agent antitumor activity in *RAS*-mutant models without significant body weight loss.

LY3214996 demonstrates potent *in vivo* antitumor activity in *BRAF-, KRAS-, NRAS-,* and *MEK*-mutant models as a single agent

The *in vivo* efficacy of LY3214996 and MEK inhibitors was assessed in subcutaneous xenograft models derived from several colorectal cancer (*KRAS*-mutant HCT116, *BRAF*-mutant Colo205, and *MEK1*-mutant SW48), melanoma (*NRAS*-mutant SK-MEL-30), pancreatic cancer (*KRAS*-mutant MiaPaCa-2), and NSCLC (*KRAS*-mutant Calu6) models as representative examples of RAS/ERK pathway alterations. MEK inhibitors were dosed at predicted clinical efficacious doses in mice. LY3214996 treatment resulted in significant tumor regression of HCT116 (31%), Colo205 (76%), MiaPaCa-2 (66%), and Calu-6 (54%) xenograft tumors (**Fig. 5A**, **B**, **E**, and **F**; Supplementary Table S3). LY3214996 treatment also resulted in significant growth inhibition in SW48 colorectal cancer (%dT/C = 11) and SK-MEL-30 melanoma (%dT/C = 1) xenograft models (**Fig. 5C** and **D**; Supplementary Table S3). All single-agent treatments were well tolerated as represented in the HCT116 study (Supplementary Figs. S4 and S5). Taken together, our data suggest that LY3214996 has potent efficacy in xenograft models with various ERK pathway alterations including mutations of *BRAF, MEK1, NRAS,* or *KRAS* (Supplementary Table S3). Notably, the efficacy is similar compared with MEK inhibitor which reinforces the point that constant pRSK1 suppression (>50%) may not be required, especially in the most responsive tumor types. These genetic alterations are key biomarkers for patient selection and precision medicine of LY3214996 in clinical development (Supplementary Table S3).

LY3214996 shows durable response in an A375 melanoma parent model and potent antitumor activity in an A375 model resistant to vemurafenib or colorectal cancer PDX model with intrinsic resistance to vemurafenib

To further test if LY3214996 can overcome BRAF inhibitor resistance, we generated an *in vivo* acquired resistance model to vemurafenib using A375 melanoma cells. In the parental A375 xenograft model, LY3214996 (100 mpk qd) showed significant tumor regression resulting in four of six complete responses and complete cure as those animals were tumor free for 115 days after 21 days of treatment (**Fig. 6A**). We used the same model to generate an *in vivo* acquired resistance to vemurafenib model by administering vemurafenib (15 mpk b.i.d.) over time. Acquired resistance was first demonstrated after 45 days (Supplementary Fig. S6). Tumor fragments from those resistant tumors were implanted for the LY3214996 efficacy study shown in **Fig. 6B**. LY3214996 dosed at 50 mpk b.i.d. showed 95% tumor growth inhibition (%dT/C = 5), whereas the vehicle control grew in the presence of vemurafenib (15 mpk b.i.d.; **Fig. 6B**). These results suggest that LY3214996 can overcome acquired resistance to vemurafenib in *BRAF* V600E–mutant melanoma.

Efficacy of LY3214996 was also tested in PDX models that maintain morphologic similarities and recapitulate molecular profiling of the original tumors. In a *BRAF* V600E–mutant colorectal cancer PDX model CTG-0652, which is intrinsically resistant to vemurafenib, LY3214996 treatment showed 83% tumor growth inhibition (%dT/C = 17; **Fig. 6C**). Overall, our data suggest that LY3214996 has single-agent activity in BRAF inhibitor–resistant melanoma and colorectal cancer models.

LY3214996 demonstrates enhanced efficacy in combination with pan-RAF inhibitor LY3009120 in the HCT116 colorectal cancer xenograft model

Inhibition of multiple targets in the ERK pathway has been used to enhance therapeutic response in melanoma (30). Using this paradigm, we have explored combination of LY3214996 with pan-RAF inhibitor LY3009120 (31) in a *KRAS*-mutant HCT116 colorectal cancer xenograft model. LY3214996 alone, LY3009120 alone, and the combination of both resulted in 52%, 29%, and 94% tumor growth inhibition, respectively, suggesting synergistic effect for the combination ($P < 0.001$; **Fig. 6D** and **E**). All tested doses were well tolerated as indicated by body weight measurements in the study (Supplementary Fig. S7).

Discussion

Approximately 30% of all human cancers have an aberrantly activated ERK pathway, thus targeted therapeutics are vital to treating ERK pathway–activated human cancers. BRAF and MEK inhibitors can target this pathway, but often result in resistance. ERK, a key downstream signaling molecule in the ERK pathway, is therefore an attractive target for inhibition to overcome BRAF and MEK inhibitors' resistance and indirectly target *RAS*-mutant cancers. LY3214996, a potent and selective small-molecule ATP-competitive inhibitor of ERK, was designed to treat cancers with ERK pathway alterations. Our data suggest that ERK inhibition by LY3214996 is a potential therapeutic option for precision medicine of patients with ERK pathway–altered cancers including *BRAF, KRAS, NRAS, MEK1* (*MAP2K1*), or *NF1* mutations as well as patients whose cancers relapse on BRAF and/or MEK inhibitor therapies

LY3214996 has demonstrated potent target inhibition in *in vitro* and *in vivo* cancer models having ERK pathway mutations. *In vitro*, LY3214996 demonstrated potent inhibition of the pathway as demonstrated by inhibition of both cytoplasmic (e.g., pRSK1) and nuclear targets (e.g., DUSP4, EGR1) of ERK. Interestingly, treatment with LY3214996 increased phosphorylation of ERK in certain contexts, which is also reported for other ERK inhibitors (20, 23). The increase in pERK is likely caused by the loss of negative feedback activation through ERK-mediated inhibition of CRAF phosphorylation (28). Despite this increase in pERK, LY3214996 treatment maintained the blockade of downstream signaling as indicated by potent inhibition of pRSK1. These findings led to the successful use of pRSK1 or DUSP4

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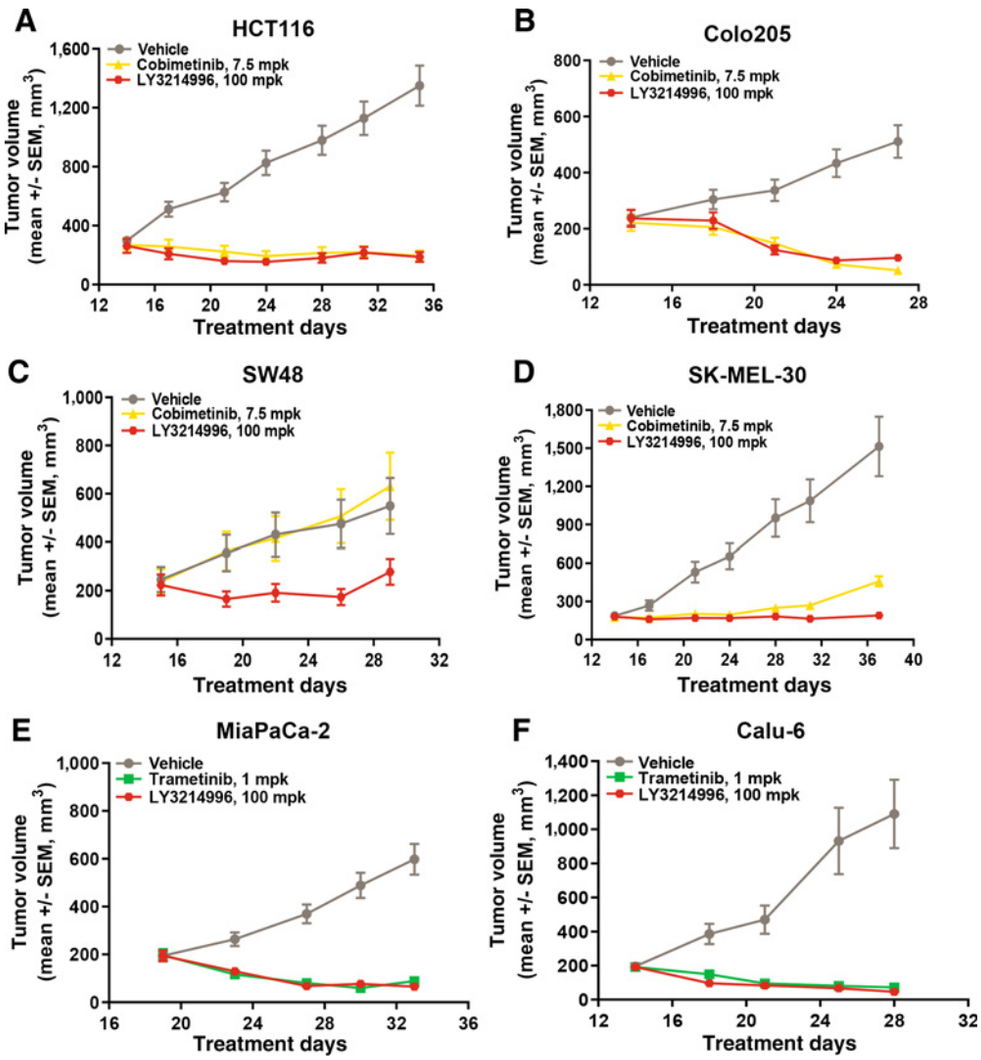

Figure 5.

Differential antitumor activity of LY3214996 as compared with known MEK inhibitors in cancer models with ERK pathway alterations. Single-agent efficacy of LY3214996 (100 mg/kg) and MEK inhibitors (cobimetinib, 7.5 mg/kg and trametinib, 1 mg/kg) in *KRAS*-mutant HCT116 colorectal cancer model (**A**), *BRAF*-mutant Colo205 colorectal cancer (**B**), *MAP2K1*-mutant SW48 colorectal cancer (**C**), *NRAS*-mutant SK-MEL-30 melanoma (**D**), *KRAS*-mutant MiaPaCa-2 pancreatic cancer (**E**), and *KRAS*-mutant Calu6 NSCLC (**F**) xenograft tumors.

levels in tumors as reliable PD biomarkers to measure LY3214996-mediated inhibition of ERK activity *in vitro* and *in vivo*, which translated into robust antitumor activity. Interestingly, continuous pRSK1 inhibition may not be required for efficacy, especially in the most responsive tumor populations. Our data provide guidance for testing LY3214996 in the clinic and selected patient population using an optimal biological dose and schedule for achieving efficacy. Theoretically, sustained drug exposure and target inhibition may appear optimal for efficacy as monotherapy. However, safety concerns observed during the clinical investigation of single-agent MEK inhibitors prompted us to ask about the minimal duration and magnitude of target inhibition (pRSK1) required for significant efficacy. Summary of the analysis of our PK/PD/efficacy correlation data suggests $\geq$50% inhibition of the target for a minimum of 8 hours is sufficient for robust antitumor activity in *RAS*- or *RAF*-mutant models without significant body weight loss.

In a panel of cell lines including melanoma, colorectal cancer, pancreatic cancer, and NSCLC, LY3214996 demonstrated preferential *in vitro* sensitivity to cell lines harboring ERK pathway alterations (*BRAF, KRAS, NRAS, MEK1,* or *NF1* mutations). Similar findings were observed in ERK pathway–altered xenograft models where LY3214996 potently inhibited *in vivo* tumor growth. In contrast to *MEK1*-mutant cell lines in which all cell lines tested were sensitive to LY3214996, only two thirds of *KRAS*- or one-half of *BRAF*-mutant cell lines showed sensitivity to LY3214996. This contrast was expected and somewhat recapitulated the observations made with other ERK inhibitors (20, 23). *BRAF* mutations themselves appear to be the major driver for many *BRAF*-mutated cancers, whereas *KRAS*-mutant cancers appear to be driven by not only ERK pathway, and in some cases by other mechanisms including non-RAS genetic alterations which affect the biology and contribute to resistance. ERK plays a critical role in *KRAS*-mutant cancers as signaling through the ERK pathway from RTK

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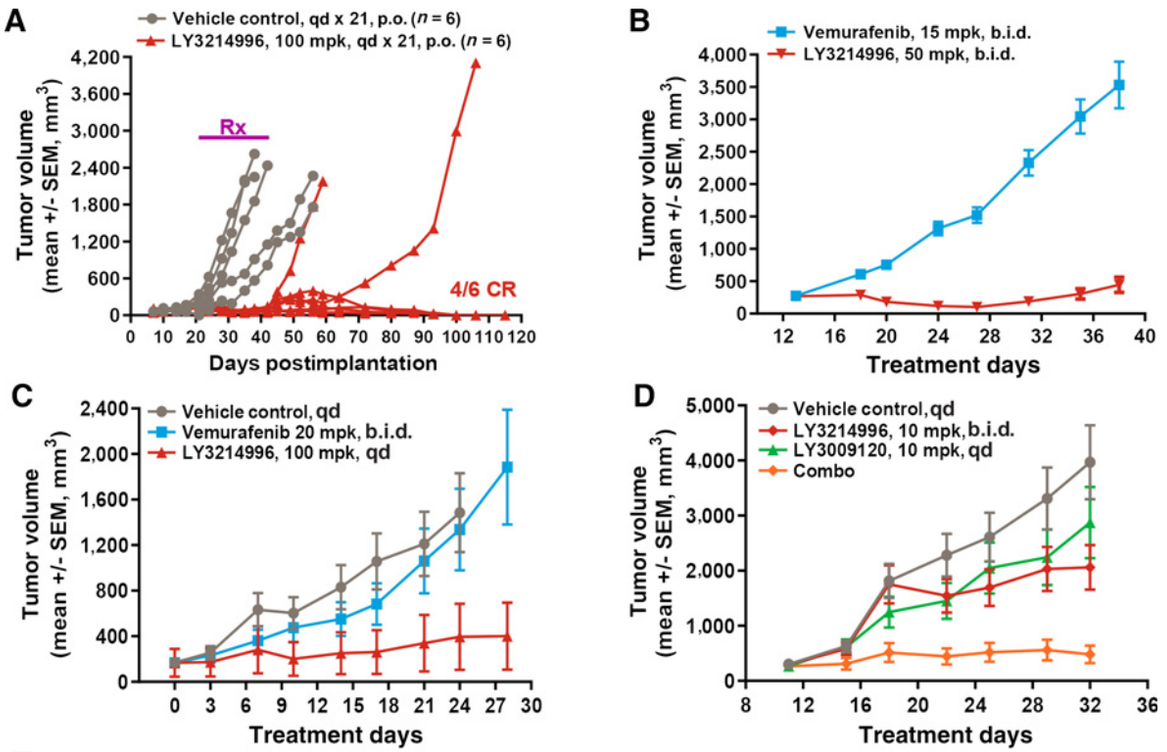

E

Treatment	%dT/C	*P* value	Treatment 1	Treatment 2	*P* value
LY3214996, 10 mpk, qd	48	0.038[b*]	Vehicle control	Combo	<0.001[b*]
LY3009120, 10 mpk, b.i.d.	71	0.303	LY3214996, 10 mpk, qd	Combo	<0.001[b*]
Combo[a] (LY3214996 + LY3009120)	6	<0.001[b*]	LY3009120, 10 mpk, b.i.d.	Combo	<0.001[b*]

Abbreviations: b.i.d., twice a day; qd, daily.
[a]Synergistic.
[b]Compares vehicle vs. treatment.
*Significance: $P < 0.05$.

Figure 6.

LY3214996 shows potent antitumor activity in a vemurafenib-resistant *BRAF* V600E–mutant A375 melanoma xenograft model and a *BRAF* V600E–mutant colorectal cancer PDX model and shows enhanced efficacy in combination with pan-RAF inhibitor LY3009120 in a *KRAS*-mutant HCT116 colorectal cancer xenograft model. **A–C,** Single-agent efficacy of LY3214996 in *BRAF* V600E–mutant A375 melanoma xenograft tumors (**A**), *BRAF* V600E–mutant A375 melanoma xenograft tumors with acquired resistance to vemurafenib (**B**), and *BRAF* V600E–mutant CTG-0652 colorectal cancer PDX model (**C**). **D,** Combination efficacy of LY3214996 with pan-RAF inhibitor LY3009120 in *KRAS*-mutant HCT116 colorectal cancer xenografts implanted in nude rats. **E,** Data for %dT/C and *P* value for single agent and combination treatments. b.i.d., twice a day; p.o., orally; qd, daily.

through RAS culminates in ERK. This is supported by the current data and a previous analysis which show inhibition of the ERK pathway by targeting ERK in *KRAS*-mutant cancers may be advantageous (32). Although we often view the ERK pathway in a simplified linear cascade, each level has its own distinct regulatory mechanisms which, upon inhibition, may contribute to and/or drive inhibitor resistance as evidenced by clinical experience with RAF and MEK inhibitor treatment in *BRAF*-mutant melanomas (33, 34). Moreover, in *RAS*-mutant cancers, RAF and MEK inhibitors are not very effective in blocking ERK reactivation by the loss of negative feedback regulation (35, 36). In addition, recent nonclinical reports demonstrated that only approximately 50% of *RAS*-mutant cancer cell lines are sensitive to ERK inhibitors such as SCH772984 and BVD-523, in spite of showing resistance to MEK inhibitors (20, 23), further supporting the idea that ERK inhibition may be preferable in ERK pathway–altered cancers (21).

Acquired resistance leading to disease progression is common even after initial successful responses with BRAF and MEK inhibitors in melanoma and other cancers (37). Resistance to RAF/MEK inhibition often involves reactivation of the ERK pathway by multiple mechanisms (33, 38). Although multiple distinct mechanisms to achieve this effect have been described, ERK remains the major point of

convergence. Thus, ERK represents an attractive target to overcome acquired resistance to BRAF/MEK inhibitors. LY3214996 has shown potent antitumor activity *in vitro* in BRAF inhibitor–resistant (vemurafenib- or dabrafenib-resistant) cell lines and *in vivo* in a vemurafenib-resistant A375 melanoma model. More importantly, the *MEK1*-mutant model (SW48) is sensitive to LY3214996 despite an insensitivity to MEK inhibitor cobimetinib. Our data suggest that LY3214996 is an effective agent to overcome acquired resistance resulting from ERK activation and that treatment with LY3214996 is a promising strategy to delay or revert resistance to BRAF and MEK inhibitor therapy.

Apart from BRAF-mutant cancers and cancers resistant to BRAF/MEK inhibitor therapy, ERK inhibitors are likely to show benefit in *RAS*-mutant cancers because ERK is central in this pathway. RAS mutations are prevalent in approximately 25% of all human cancers (39), and targeting ERK with LY3214996 is a rational treatment approach for these cancers. More than 95% of patients with pancreatic cancer have activated *KRAS* mutations, and half of pancreatic cancer cell lines with *KRAS* mutations are sensitive to long-term ERK inhibition, which has been shown to induce senescence, mediated in part by the degradation of MYC protein (40). The reactivation of ERK has limited the effectiveness of RAF and MEK inhibitors in *RAS*-mutant cancers; for example, in a phase I study, BVD-523 demonstrated partial response in patients with *NRAS*- and *BRAF*-mutated solid tumors (24) but not in *KRAS*-mutant cancers (41). This reinforces the complexity of *KRAS*-mutant cancers and supports the use of combinational approaches to effectively target tumors of patients with *KRAS* mutations. Although LY3214996 has demonstrated potent single-agent activity in *KRAS*-mutant xenograft models, one third of the *RAS*-mutant cancer cell lines were insensitive to single-agent LY3214996 treatment (42); thus, it is important to identify additional key players or pathways that could be targeted to optimize therapeutic response. Importantly, the combination of RAF1 suppression and MEK inhibition proved to be synthetically lethal using siRNA and shRNA screening of colorectal cancer cell lines (43), highlighting the delicate balance of the ERK pathway. Prolonged inhibition of both RAF and MEK leads to persistent ERK suppression and apoptosis induction (44). Therefore, we explored the combination of LY3214996 with a pan-RAF inhibitor LY3001920 in a *KRAS*-mutant colorectal cancer xenograft model. We found that dual inhibition of the ERK pathway against ERK and RAF1 using LY3214996 with LY3009120 resulted in a synergistic combinational benefit in a *KRAS*-mutant colorectal cancer xenograft model. Our data highlight the relevance of developing novel combinatorial regimens to target the ERK pathway in *KRAS*-mutant cancers. Based on potent single-agent activity and combination potential, we emphasize a number of areas of highly unmet medical need that could benefit from LY3214996-based combinations, including patients with relapsed/refractory colorectal cancer, pancreatic cancer, and NSCLC.

In summary, our data suggest that an ERK inhibitor, such as LY3214996, that demonstrates potent and selective ERK inhibition with >50% target inhibition for 8 to 16 hours is sufficient for significant tumor growth inhibition as a single agent in *KRAS*- and *BRAF*-mutant models. LY3214996 may hold promise of precision medicine for the treatment of patients with cancers harboring ERK pathway alternations such as mutations of *BRAF, MEK, KRAS, NRAS,* and *NF1*, including patients whose tumors have acquired resistance to other targeted therapies. More importantly, LY3214996 has the potential to safely be combined with other targeted agents to maximize therapeutic benefit in hard-to-treat *KRAS*-mutant cancers. A phase I study of LY3214996 administered alone or in combination with other agents in patients with advanced/metastatic cancer is currently ongoing (NCT02857270).

Disclosure of Potential Conflicts of Interest

S.V. Bhagwat is Sr. Research Advisor at and has an ownership interest (including patents) in Eli Lilly and Company. B. Zhao is Biologist at Eli Lilly and Company. W. Shen is Associate Scientist at and has an ownership interest (including patents) in Eli Lilly and Company. L. Kindler is Senior Tech at Eli Lilly and Company. R.S. Flack has an ownership interest (including patents) in Eli Lilly and Company. B. Anderson is Consultant at and has an ownership interest (including patents) in Eli Lilly and Company. M. Pogue is Senior Biologist at and has an ownership interest (including patents) in Eli Lilly and Company. R.D. Van Horn is Consultant Biologist at and has an ownership interest (including patents) in Eli Lilly and Company. X. Rao is Research Scientist at Eli Lilly and Company and has an ownership interest (including patents) in Eli Lilly and Company. D. McCann is Principal Research Scientist at Eli Lilly and Company. A.J. Dropsey and J. Manro are Senior Research Scientists at and have an ownership interest (including patents) in Eli Lilly and Company. J. Walgren is Advisor, Regulatory Affairs, at Eli Lilly and Company. E. Yuen is an employee at Eli Lilly and Company. M.J. Rodriguez is Principal Research Scientist at and has an ownership interest (including patents) in Eli Lilly and Company. G.D. Plowman is VP Oncology at Eli Lilly and Company. R.V. Tiu is Senior Medical Advisor/Medical Advisor and Senior Medical Director & Global Medical Lead at Astellas Pharma US and has an ownership interest (including patents) in Eli Lilly and Company. S. Joseph is a research fellow at Eli Lilly and Company. No potential conflicts of interest were disclosed by the other authors.

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References

1. Sebolt-Leopold JS, Herrera R. Targeting the mitogen-activated protein kinase cascade to treat cancer. Nat Rev Cancer 2004;4:937–47.
2. Roskoki R Jr. ERK1/2 MAP kinases: structure, function, and regulation. Pharmacol Res 2012;66:105–43.
3. Roberts PJ, Der CJ. Targeting the Raf-MEK-ERK mitogen-activated protein kinase cascade for the treatment of cancer. Oncogene 2007;26:3291–310.
4. Groenendijk FH, Bernards R. Drug resistance to targeted therapies: déjà vu all over again. Mol Oncol 2014;8:1067–83.
5. de Leeuw R, McNair C, Schiewer MJ, Neupane NP, Brand LJ, Augello MA, et al. MAPK reliance via acquired CDK4/6 inhibitor resistance in cancer. Clin Cancer Res 2018;24:4201–14.
6. Cocco E, Schram AM, Kulick A, Misale S, Won HH, Yaeger R, et al. Resistance to TRK inhibition mediated by convergent MAPK pathway activation. Nat Med 2019;25:1422–7.
7. Robert C, Karaszewska B, Schachter J, Rutkowski P, Mackiewicz A, Stroiakovski D, et al. Improved overall survival in melanoma with combined dabrafenib and trametinib. N Engl J Med 2015;372:30–9.
8. Planchard D, Besse B, Groen HJM, Souquet PJ, Quoix E, Baik CS, et al. Dabrafenib plus trametinib in patients with previously treated BRAF (V600E)-mutant metastatic non-small cell lung cancer: an open-label, multicentre phase 2 trial. Lancet Oncol 2016;17:984–93.
9. Larkin J, Ascierto PA, Dréno B, Atkinson V, Liszkay G, Maio M, et al. Combined vemurafenib and cobimetinib in BRAF-mutated melanoma. N Engl J Med 2014; 371:1867–76.
10. Dummer R, Ascierto PA, Gogas H, Arance A, Mandala M, Liszkay G, et al. Results of COLUMBUS Part 2: a phase 3 trial of encorafenib (ENCO) plus binimetinib (BINI) versus ENCO in BRAF-mutant melanoma [abstract]. Ann Oncol 2017;28 Suppl 5:429.
11. Long GV, Stroyakovskiy D, Gogas H, Levchenko E, de Braud F, Larkin J, et al. Dabrafenib and trametinib versus dabrafenib and placebo for Val600 BRAF-mutant melanoma; a multicentre, double-blind, phase 3 randomised controlled trial. Lancet 2015;386:444–51.
12. Corcoran RB, Dias-Santagata D, Bergethon K, Iafrate AJ, Settleman J, Engelman JA. BRAF gene amplification can promote acquired resistance to MEK inhibitors in cancer cells harboring the BRAF V600E mutations. Sci Signal 2010;3:ra84.
13. Wagle N, Van Allen EM, Treacy DJ, Frederick DT, Cooper ZA, Taylor-Weiner A, et al. MAP kinase pathway alterations in BRAF-mutant melanoma patients with acquired resistance to combined RAF/MEK inhibition. Cancer Discov 2014;4:61–8.
14. Shi H, Hugo W, Kong X, Hong A, Koya RC, Moriceau G, et al. Acquired resistance and clonal evolution in melanoma during BRAF inhibitor therapy. Cancer Discov 2014;4:80–93.
15. Nazarian R, Shi H, Wang Q, Kong X, Koya RC, Lee H, et al. Melanomas acquire resistance to B-RAF(V600E) inhibition by RTK or N-RAS upregulation. Nature 2010;468:973–7.
16. Villanueva J, Vultur A, Herlyn M. Resistance to BRAF inhibitors: unraveling mechanisms and future treatment options. Cancer Res 2011;71:7137–40.
17. Yadav V, Zhang X, Liu J, Estrem S, Li S, Gong XQ, et al. Reactivation of mitogen-activated protein kinase (MAPK) pathway by FGF receptor 3 (FGFR3)/Ras mediates resistance to vemurafenib in human B-RAF V600E mutant melanoma. J Biol Chem 2012;287:28087–98.
18. Paraiso KHT, Fedorenko IV, Cantini LP, Munko AC, Hall M, Sondak VK, et al. Recovery of phospho-ERK activity allows melanoma cells to escape from BRAF inhibitor therapy. Br J Cancer 2010;102:1724–30.
19. Long GV, Fung C, Menzies AM, Pupo GM, Carlino MS, Hyman J, et al. Increased MAPK reactivation in early resistance to dabrafenib/trametinib combination therapy of BRAF-mutant metastatic melanoma. Nat Commun 2014;5:5694.
20. Morris EJ, Jha S, Restaino CR, Dayananth P, Zhu H, Cooper A, et al. Discovery of a novel ERK inhibitor with activity in models of acquired resistance to BRAF and MEK inhibitors. Cancer Discov 2013;3:742–50.
21. Hatzivassiliou G, Liu B, O'Brien C, Spoerke JM, Hoeflich KP, Haverty PM, et al. ERK inhibition overcomes acquired resistance to MEK inhibitors. Mol Cancer Ther 2012;11:1143–54.
22. Blake JF, Burkard M, Chan J, Chen H, Chou KJ, Diaz D, et al. Discovery of (S)-1-(1-(4-Chloro-3-fluorophenyl)-2-hydroxyethyl)-4-(2-((1-methyl-1H-pyrazol-5-yl)amino)pyrimidin-4-yl)pyridin-2(1H)-one (GDC-0994), an extracellular signal-regulated kinase 1/2 (ERK1/2) inhibitor in early clinical development. J Med Chem 2016;59:5650–60.
23. Germann UA, Furey BF, Markland W, Hoover RR, Aronov AM, Roix JJ, et al. Targeting the MAPK signaling pathway in cancer: promising preclinical activity with the novel selective ERK1/2 inhibitor BVD-523 (ulixertinib). Mol Cancer Ther 2017;16:2351–63.
24. Sullivan RJ, Infante JR, Janku F, Wong DJL, Sosman JA, Keedy V, et al. First-in-class ERK1/2 inhibitor ulixertinib (BVD-523) in patients with MAPK mutant advanced solid tumors: results of a phase I dose-escalation and expansion study. Cancer Discov 2018;8:184–95.
25. Moschos SJ, Sullivan RJ, Hwu WJ, Ramanathan RK, Adjei AA, Fong PC, et al. Development of MK-8353, an orally administered ERK1/2 inhibitor, in patients with advanced solid tumors. JCI Insight 2018;3:e92352.
26. Cortez GS, Joseph S, McLean JA, McMillen WT, Rodriguez MJ, Zhao G. ERK inhibitors. United States patent US9469652. 2016.
27. Henry JR, Kaufman MD, Peng S-B, Ahn YM, Caldwell TM, Vogeti L, et al. Discovery of 1-(3,3-Dimethylbutyl)-3-(2-fluoro-4-methyl-5-(7-methyl-2-(methylamino)pyrido[2,3-*d*]pyrimidin-6-yl)phenyl)urea (LY3009120) as a pan-RAF inhibitor with minimal paradoxical activation and activity against BRAF or RAS mutant tumor cells. J Med Chem 2015;58:4165–79.
28. Dougherty MK, Muller J, Ritt DA, Zhou M, Zhou XZ, Copeland TD, et al. Regulation of Raf-1 by direct feedback phosphorylation. Mol Cell 2005;17:215–24.
29. Wong H, Vernillet L, Peterson A, Ware JA, Lee L, Martini JF, et al. Bridging the gap between preclinical and clinical studies using pharmacokinetic-pharmacodynamic modeling: an analysis of GDC-0973, a MEK inhibitor. Clin Cancer Res 2012;18:3090–9.
30. King AJ, Arnone MR, Bleam MR, Moss KG, Yang J, Fedorowicz KE, et al. Dabrafenib; preclinical characterization, increased efficacy when combined with trametinib, while BRAF/MEK tool combination reduced skin lesions. PLoS One 2013;8:e67583.
31. Peng SB, Henry JR, Kaufman MD, Lu WP, Smith BD, Vogeti S, et al. Inhibtion of RAF isoforms and active dimers by LY3009120 leads to anti-tumor activities in RAS or BRAF mutant cancers. Cancer Cell 2015;28:384–98.
32. Merchant M, Moffat J, Schaefer G, Chan J, Wang X, Orr C, et al. Combined MEK and ERK inhibition overcomes therapy-mediated pathway reactivation in RAS mutant tumors. PLoS One 2017;12:e0185862.
33. Welsch SJ, Rizos H, Scolyer RA, Long GV. Resistance to combination BRAF and MEK inhibition in metastatic melanoma: where to next? Eur J Cancer 2016;62: 76–85.
34. Corcoran RB, Settleman J, Engelman JA. Potential therapeutic strategies to overcome acquired resistance to BRAF and MEK inhibitor in BRAF mutant cancers. Oncotarget 2011;2:336–46.
35. Ryan MB, Der CJ, Wang-Gillam A, Cox AD. Targeting RAS-mutant cancers: is ERK the key? Trends Cancer 2015;1:183–98.
36. Lake D, Corrêa SA, Müller J. Negative feedback regulation of the ERK1/2 MAPK pathway. Cell Mol Life Sci 2016;73:4397–413.
37. Hazar-Rethinam M, Kleyman M, Han GC, Liu D, Ahronian LG, Shahzade HA, et al. Convergent therapeutic strategies to overcome the heterogeneity of acquired resistance in $BRAF^{V600E}$ colorectal cancer. Cancer Discov 2018;8:417–27.
38. Griffin M, Scotto D, Josephs DH, Mele S, Crescioli S, Bax HJ, et al. BRAF inhibitors: resistance and the promise of combination treatments for melanoma. Oncotarget 2017;8:78174–92.
39. Hobbs GA, Der CJ, Rossman KL. RAS isoforms and mutations in cancer at a glance. J Cell Sci 2016;129:1287–92.
40. Hayes TK, Neel NF, Hu C, Gautam P, Chenard M, Long B, et al. Long-term ERK inhibition in KRAS-mutant pancreatic cancer is associated with MYC degradation and senescence-like growth suppression. Cancer Cell 2016;29:75–89.
41. Sullivan RJ, Janku F, Li BT, Wong D, Sosman J, Keedy V, et al. Activity of ERK1/2 inhibitor ulixertinib (BVD-523) in patients with BRAF and NRAS mutant melanoma. Cancer Res 2017;77 Suppl 13. Abstract nr CT003.
42. Bhagwat SV, McMillen WT, Cai S, Zhao B, Whitesell M, Kindler L, et al. Discovery of LY3214996, a selective and novel ERK1/2 inhibitor with potent antitumor activities in cancer models with MAPK pathway alterations [abstract]. Cancer Res 2017;77 Suppl 13. Abstract nr 4973.
43. Whittaker SR, Cowley GS, Wagner S, Luo F, Root DE, Garraway LA. Combined pan-RAF and MEK inhibition overcomes multiple resistance mechanisms to selective RAF inhibitors. Mol Cancer Ther 2015;14:2700–11.
44. Lamba S, Russo M, Sun C, Lazzari L, Cancelliere C, Grernrum W, et al. RAF suppression synergizes with MEK inhibition in KRAS mutant cancer cells. Cell Rep 2014;8:1475–83.

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