A Phase I Study of LY3009120, a Pan-RAF Inhibitor, in Patients with Advanced or Metastatic Cancer

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ABSTRACT

Mutations in ERK signaling drive a significant percentage of malignancies. LY3009120, a pan-RAF and dimer inhibitor, has preclinical activity in *RAS*- and *BRAF*-mutated cell lines including *BRAF*-mutant melanoma resistant to BRAF inhibitors. This multicenter, open-label, phase I clinical trial (NCT02014116) consisted of part A (dose escalation) and part B (dose confirmation) in patients with advanced/metastatic cancer. In part A, oral LY3009120 was dose escalated from 50 to 700 mg twice a day on a 28-day cycle. In part B, 300 mg LY3009120 was given twice a day. The primary objective was to identify a recommended phase II dose (RP2D). Secondary objectives were to evaluate safety, pharmacokinetics, and preliminary efficacy. Identification of pharmacodynamic biomarkers was exploratory. In parts A and B, 35 and 16 patients were treated, respectively ($N = 51$). In part A, 6 patients experienced eight dose-limiting toxicities. The RP2D was 300 mg twice a day. Common (>10%) any-grade drug-related treatment-emergent adverse events were fatigue ($n = 15$), nausea ($n = 12$), dermatitis acneiform ($n = 10$), decreased appetite ($n = 7$), and maculopapular rash ($n = 7$). The median duration of treatment was 4 weeks; 84% of patients completed one or two cycles of treatment. Exposures observed at 300 mg twice a day were above the preclinical concentration associated with tumor regression. Eight patients had a best overall response of stable disease; there were no complete or partial clinical responses. Despite adequate plasma exposure levels, predicted pharmacodynamic effects were not observed.

Introduction

The RAF serine/threonine protein kinase family comprises three isoforms (A-, B-, and C-RAF) that play a pivotal role in the MAPK pathway (RAS/RAF/MEK/ERK) in transducing extracellular signals to the nucleus (1, 2). The RAF protein kinases are involved in cell proliferation, survival, invasion, and angiogenesis (3). *BRAF* point mutations within the kinase domain of the protein are often single amino acid substitutions (e.g., *V600E*) that result in a significant increase in kinase activity and are known to occur in several human cancers, including melanoma, thyroid, colon, ovarian, and lung cancers (4, 5). In melanoma, *BRAF* mutations are found in approximately 50% of patients (over 90% *V600E*; refs. 5, 6), whereas *NRAS* mutations occur in 15% to 20% and are associated with poorer survival rates (7). Therefore, *BRAF* is an attractive therapeutic target for these patients.

Three BRAF-mutant–specific kinase inhibitors, dabrafenib (8–10), vemurafenib (11, 12), and encorafenib (13), are currently approved for treatment of patients with melanoma with the *BRAF V600E/K* mutation. Moreover, three MEK kinase inhibitors, trametinib (14–16), cobimetinib (17), and binimetinib (13), are currently approved for treatment of melanoma patients with *BRAF V600E/K* mutations who have not received previous treatment with a BRAF inhibitor. However, almost 20% of patients have tumors with intrinsic resistance to BRAF inhibitors, and those who do respond eventually develop acquired mechanisms of resistance to BRAF inhibition and disease relapse (5, 18).

In preclinical models, LY3009120 was demonstrated to be active against vemurafenib-resistant melanoma cells with MAPK pathway reactivation via *NRAS* mutation, *BRAF* splice variants, and FGFR3 activation; therefore, LY3009120 may have activity in patients failing established BRAF or MEK inhibitor treatment (19). In addition, in a preclinical model of cancer cells with microdeletions leading to BRAF homodimers, LY3009120 inhibited cell proliferation and induced tumor cell apoptosis, whereas vemurafenib had minimal activity (20).

LY3009120 is a pan-RAF inhibitor with minimal paradoxical activation and activity against *BRAF*- or *RAS*-mutant tumor cells *in vitro* and *in vivo* (19, 21, 22). The primary objective of this first-in-human phase I study (NCT02014116) was to determine the recommended phase II dose (RP2D) of LY3009120 that could be administered to patients with advanced cancer. Secondary objectives were to characterize the safety and toxicity profile of LY3009120, to

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estimate the pharmacokinetic parameters of LY3009120, and to document any antitumor activity observed with LY3009120. Exploratory objectives included investigation of pharmacodynamic biomarkers.

Materials and Methods

Study design and eligibility

This phase I study was a multicenter, nonrandomized, open-label clinical trial that consisted of part A: dose escalation in patients with advanced/metastatic cancer; and part B: dose confirmation in patients with advanced or metastatic melanoma carrying *BRAF* or *NRAS* mutations and non–small cell lung cancer (NSCLC) or colorectal cancer carrying *KRAS* or *BRAF* mutations. Key inclusion criteria for the study were as follows: patients $\geq$18 years of age with a performance status of 0 or 1 on the Eastern Cooperative Oncology Group (ECOG) scale and the presence of measurable and/or nonmeasurable disease, defined by the RECIST version 1.1; patients had failed or were ineligible for available therapies for their disease, or had a disease with no proven effective therapy; patients had discontinued all previous therapies for cancer (including chemotherapy, immunotherapy, corticosteroids, and radiotherapy); and patients had recovered from the acute effects of therapy for at least five half-lives or a minimum of 4 weeks before initiating study treatment.

Inclusion criteria for the three dose confirmation cohorts in part B were: (cohort A) patients with advanced unresectable/metastatic melanoma carrying a *BRAF V600X* (where X represents any amino acid) mutation that has relapsed after treatment with BRAF inhibitors, MEK inhibitors, or the combination of BRAF/MEK inhibitors; (cohort B) advanced unresectable/metastatic melanoma carrying a *NRAS Q61X* mutation; and (cohort C) advanced unresectable/metastatic NSCLC or colorectal carcinoma carrying a *KRAS* or *BRAF* mutation.

A total of eight dosing levels were planned, starting with 50 mg twice a day up to 700 mg twice a day of LY3009120 (Supplementary Fig. S1). Dose range was based on nonclinical toxicology and PK/PD modeling of nonclinical tumor growth and phospho-ERK (pERK) data, with twice a day dosing recommended due to predicted human elimination half-life of 6 hours and to maintain maximum pERK inhibition, given the observed direct relationship of pharmacokinetics with pERK inhibition in preclinical studies. LY3009120 was given orally as 50-mg or multiples of 100-mg capsules twice a day in a 28-day cycle. The planned duration of treatment was two cycles, but patients continued to receive study treatment if they were receiving clinical benefit [stable disease (SD), partial response (PR), or complete response (CR)] or until disease progression or unacceptable toxicity occurred. Estimation of the maximum tolerated dose (MTD) was conducted using the Bayesian model-based toxicity band method (23). This method utilized prior dose toxicity information and all available dose-limiting toxicity (DLT) data to estimate the MTD. The MTD was defined as a safe dose with the highest probability of DLT in the targeted toxicity interval (20%–35%).

This study protocol was approved by institutional review boards and ethics committees prior to initiation, and conducted in accordance with the Declaration of Helsinki. Patients provided written informed consent before entering the study.

Safety

Safety was assessed by monitoring adverse events (AE) including severity and possible relationship to study drug, dose adjustments, DLTs, laboratory values, vital signs, electrocardiogram readings, ophthalmologic assessments, and dermatologic evaluations. All AEs observed during the study were graded using the NCI Common Terminology Criteria for Adverse Events version 4.0. A DLT was defined as an AE during cycle 1 for a patient enrolled in part A (dose escalation) that was possibly related to the study drug and fulfilled any of the following criterion: grade $\geq$3 nonhematologic toxicity (exceptions could be made for nausea, vomiting, diarrhea, or constipation controlled with appropriate care and resolved within 2 days); grade 3 elevations of alanine aminotransferase and/or aspartate aminotransferase lasting fewer than eight days, without evidence of other hepatic injury; grade 3 rash that resolved or improved to grade $\leq$2 within 7 days; grade 4 hematologic toxicity that persisted for more than 5 days; grade 4 thrombocytopenia; grade 3 thrombocytopenia with bleeding; grade 3 febrile neutropenia; or any other significant toxicity deemed to be dose limiting. A DLT-equivalent toxicity was defined as an AE that met the criteria for DLT but occurred between day 1 and day 28 of any cycle (other than cycle 1) for a patient enrolled in part A, or in any cycle for a patient enrolled in part B.

Efficacy

Tumor response was assessed radiologically in alignment with RECIST version 1.1 criteria. Overall response rate, duration of response, and duration of SD were evaluated. Progression-free survival (PFS) was defined as the time from study entry to disease progression or death.

Pharmacokinetics and pharmacodynamics

Blood samples for measurement of LY3009120 concentration in plasma were obtained according to the schedule of events in the study protocol. Human plasma samples (drawn into ethylenediaminetetraacetic acid tubes) obtained during this study were analyzed at Squared Solutions BioSciences, LLC. Plasma concentration of LY3009120 was measured using a validated liquid chromatography and mass spectrometry method. Biomarker data were taken from pretreatment fresh biopsies at baseline or available archival samples (<6 months before enrollment with no intermittent therapies). The on-treatment samples were fresh biopsies taken after 4 weeks of treatment with LY3009120 (cycle 1 day 28 $\pm$ 14 days). The expression of antigen Ki-67 (Ki-67), cyclin-dependent kinase inhibitor 1B (p27), and pERK were investigated using IHC; changes in the expression of 11 nuclear targets of ERK were investigated via real-time PCR. Gene expression, and genetic mutations hypothesized to be related to safety, efficacy, drug disposition, or pathways associated with the mechanism of action of LY3009120 were also investigated.

Statistical procedures

Safety

All patients who received at least one dose of LY3009120 were evaluated for safety and toxicity. DLTs were summarized for all patients on therapy during cycle 1 in part A. In addition, after each cohort, the toxicity-band method was used to summarize the posterior distribution of probability of a DLT and to assist in the decisions related to dose escalation and determination of the MTD.

All study drug and protocol procedure-related AEs and safety data were summarized using descriptive statistics, as appropriate. Safety parameters, such as vital signs, electrocardiogram readings, and specific laboratory values, were also summarized with descriptive statistics.

Efficacy

The study was not designed to make an efficacy assessment; however, best overall response (BOR) data were reported according to study part and patient cohort. The percentage of patients with a confirmed response, defined as CR or PR, was calculated along with a 90% confidence interval (CI). Kaplan–Meier analysis estimated preliminary median PFS with a 95% CI.

Pharmacokinetics and pharmacodynamics

Pharmacokinetic analyses were conducted on patients receiving at least one dose of the study drug. Primary analytic parameters included: maximum plasma concentration, area under the concentration–time curve from zero to time t and from zero to infinity, terminal half-life ($t_{1/2}$), apparent clearance, and apparent volume of distribution, derived from the noncompartmental analyses. Descriptives of pre- and posttreatment difference in gene expression for selected biomarkers were also provided for available biopsy samples.

Results

Patients

A total of 68 patients were entered into the study with 17 screen failures, and 51 patients enrolled. All enrolled patients received at least one dose of LY3009120. In parts A and B combined, there were 28 females (55%) enrolled in the study; 96% of all patients were white, and most had an ECOG performance status of 1 (69%). The median age was 59 years (range: 24–82 years; **Table 1**).

In part A, 35 patients were treated in dose-escalation: 3 patients at 50 mg; 4 patients at 100 mg; 3 patients at 200 mg; 16 patients at 300 mg; 7 patients at 400 mg; and 2 patients at 500 mg. In part B, dose-confirmation, 16 patients were treated [1 melanoma (cohort A), 5 melanoma (cohort B), and 10 adenocarcinoma of the lung (cohort C); Supplementary Fig. S1]. Most patients had tumors harboring either a *BRAF* or *KRAS* mutation (**Table 1**).

The most common primary reason for treatment discontinuation was disease progression ($n = 40$; 78.4%). Two patients (3.9%) discontinued study treatment due to myalgia (500 and 300 mg dose levels). Additional reasons for treatment discontinuation included: patient decision ($n = 3$), physician decision ($n = 1$), and loss to follow-up ($n = 1$). One patient remained on study treatment at data cutoff (April 10, 2017). Three deaths were reported during the study treatment period, all of which were unrelated to the study drug (due to disease progression, intracranial hemorrhage, and cardiac arrest).

The majority of patients (84.3%) completed one or two cycles of treatment, with a median duration of treatment of 4.14 weeks (range: <1 week to 64.1 weeks). Sixteen (31.4%) patients had at least one dose adjustment, including 16 (31.4%) patients with dose omissions and 2 (3.9%) patients with dose reductions (**Fig. 1**).

Safety

DLTs

Eight DLTs were reported in 6 patients during the dose-escalation phase (part A); one DLT was reported in the dose-confirmation phase (part B, **Table 2**). No DLTs were reported for the following dose levels: 50 mg twice a day, 100 mg twice a day, 200 mg twice a day, and 400 mg twice a day. Both patients enrolled at 500 mg twice a day reported DLTs: 1 patient experienced grade 3 pain and grade 3 stomatitis; the other patient reported grade 3 arthralgia and grade 3 myalgia. The dose

Table 1. Baseline characteristics of study population.

Parameters	Part A ($n = 35$)	Part B ($n = 16$)
Median age, years (range)	59 (24–82)	63.5 (41–78)
Female, *n* (%)	22 (62.9)	6 (37.5)
Race		
White	33 (94.3)	16 (100.0)
Unknown	2 (5.7)	0 (0)
ECOG PS		
0	13 (37.1)	3 (18.8)
1	22 (62.9)	13 (81.3)
Number of prior regimens, *n* (%)		
1	4 (11)	5 (31)
≥2	31 (89)	11 (69)
Cancer subtypes, *n*		
CRC	9 (*KRAS* mutant: 8; *BRAF* mutant: 1)	0
NSCLC	9 (*KRAS* mutant: 6; *BRAF* mutant: 3)	10 (*KRAS* mutant: 5; *BRAF* mutant: 5)
PDAC	5 (*KRAS* mutant: 2; unknown: 3)	0
Others	12 (liver: 2; breast: 2; cholangiocarcinoma: 1; uterine: 1 appendiceal: 1; melanoma: 1; rectal neuroendocrine: 1; pilocytic astrocytoma: 1; nasal cavity: 1; mesothelioma: 1)	6 (*BRAF* mutant: 1 – melanoma; *NRAS* mutant: 5 – melanoma: 4 and breast: 1)
Genomic alterations, *n*		
KRAS mutant	18	5
BRAF mutant	7	6
Others	*BRAF* WT: 19 *KRAS* and *BRAF* WT: 5; *NRAS* mutant: 1; not available: 4	5 (*NRAS* mutant: 5)

Abbreviations: Amp, amplification; CRC, colorectal cancer; *n*, number in group; PDAC, pancreatic ductal adenocarcinoma; WT, wild type.

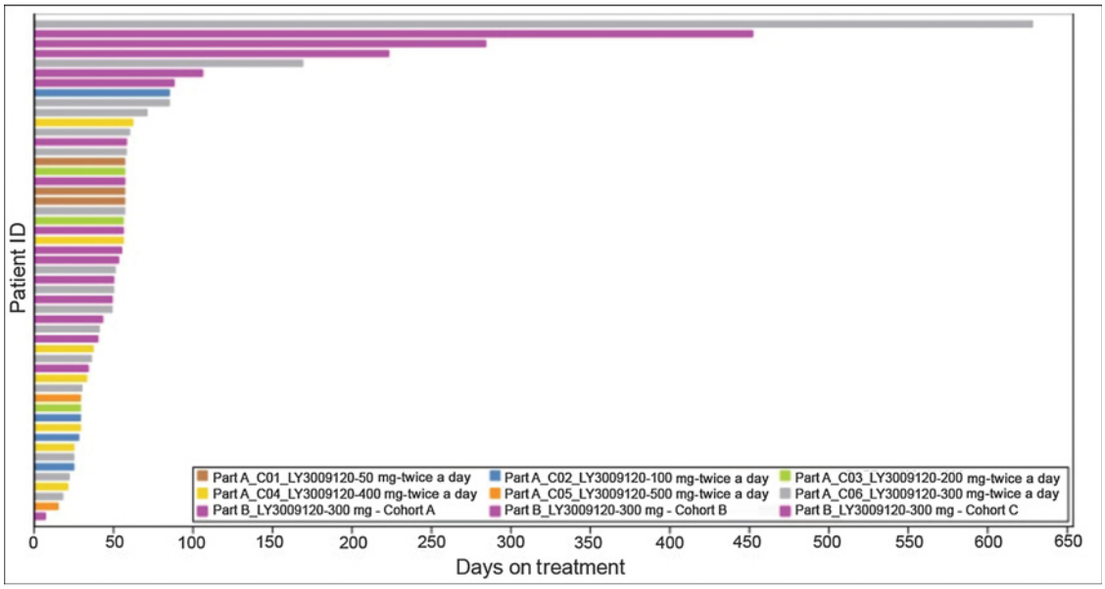

Figure 1.
Duration of treatment with LY3009120 in dose escalation (part A) and dose confirmation (part B). The Napoleon plot shows the number of days on treatment with LY3009120 for individual patients in the part A dose escalation and the part B dose confirmation portions of the study. Cohorts and dose levels are each shown according to the figure legend.

was deescalated to 400 mg twice a day. At 400 mg twice a day, 2 of the 3 additional patients enrolled after deescalation reported DLTs (grade 2/3 alanine aminotransferase increase). As a result, the dose was further deescalated to 300 mg twice a day. A total of 16 patients were enrolled at 300 mg twice a day either initially or through deescalation, with 2 patients reporting DLTs (grade 2 blurred vision and grade 3 dermatitis acneiform). Thus, the RP2D was identified as 300 mg twice a day. Dose levels of 600 and 700 mg twice a day were not attempted, given the dose de-escalations needed at 400 and 500 mg.

Treatment-emergent AE

Fifty patients (98%) experienced at least one treatment-emergent AE (TEAE). The most common (>20%) TEAEs regardless of causality were fatigue ($n = 23$; 45%), nausea ($n = 21$; 41%), constipation ($n = 19$; 37%), dyspnea ($n = 17$; 33%), decreased appetite ($n = 16$; 31%), anemia ($n = 15$; 29%), vomiting ($n = 15$; 29%), abdominal pain ($n = 11$; 22%), dermatitis acneiform ($n = 11$; 22%), and insomnia ($n = 11$; 22%). Forty patients (78%) experienced at least one TEAE considered related to study treatment. The majority (83%) of the related TEAEs were grade 1 or 2 in severity. The most common (>10%) any-grade TEAEs related to study treatment were fatigue ($n = 15$; 29%), nausea ($n = 12$; 24%), dermatitis acneiform ($n = 10$; 20%), decreased appetite ($n = 7$; 14%), and maculopapular rash ($n = 7$; 14%).

Thirty (59%) patients experienced at least one grade 3 TEAE or greater (grade 3, $n = 24$; grade 4, $n = 4$; grade 5, $n = 2$). Seven patients experienced grade $\geq$3 TEAEs possibly related to the study drug, and the maximum severity reported was grade 3. Treatment-related grade 3 TEAEs included: stomatitis, fatigue, pain, increased alanine aminotransferase, increased aspartate aminotransferase, increased bilirubin, arthralgia, myalgia, and dermatitis acneiform. The only study treatment-related grade 3 event reported by more than 1 patient was myalgia ($n = 2$).

Table 2. DLTs and dose-limiting equivalent toxicities.

Part A[a]				
Dose (twice a day)	**Age**	**Sex**	**Disease**	**Description of DLT**
400 mg	71	F	PDAC	Gr3 ALT increase
400 mg	39	F	NSCLC	Gr2 ALT increase
500 mg	54	M	PDAC	Gr3 pain; Gr3 stomatitis
500 mg	55	F	NSCLC	Gr3 arthralgia; Gr3 myalgia
300 mg	59	M	PDAC	Gr2 blurred vision
300 mg	67	M	CRC	Gr3 dermatitis acneiform
Part B				
Dose (twice a day)	**Age**	**Sex**	**Disease**	**Description of DLET**
300 mg	53	F	NRAS melanoma	Gr3 myalgia

Abbreviations: ALT, alanine aminotransferase; CRC, colorectal cancer; DLET, dose-limiting equivalent toxicity; F, female; Gr, Grade; M, male; NRAS, neuroblastoma RAS viral oncogene homolog; PDAC, pancreatic ductal adenocarcinoma.
[a]No DLTs were reported for the following dose levels in part A: 50 mg twice a day, 100 mg twice a day, 200 mg twice a day, and 400 mg twice a day.

There were 55 serious AEs (SAE) reported in 33 (65%) patients, of which nine events in three patients (6%) were assessed as related to study treatment. Treatment-related SAEs were: maculopapular rash (two incidences), stomatitis, pain, pyrexia, increased bilirubin, myalgia, dermatitis acneiform, and palmar–plantar erythrodysesthesia syndrome (one incident each).

Efficacy

There were no CRs or PRs reported in the study. A BOR of SD was observed in 8 patients. The median PFS for patients in part B was 1.8 months (95% CI, 1.3–7.2 months).

A total of 12 *BRAF*-mutant, 17 *KRAS*-mutant, and 5 *NRAS*-mutant patients with cancer had tumor response assessments in the study. Of the 8 patients who had a BOR of SD, 5 patients had *BRAF* mutations (median SD duration, 3.7 months), 2 had *KRAS* mutations (SD duration, 7.3 and 1.8 months), and 1 had a *NRAS* mutation (SD duration, 5.3 months). All patients received prior systemic therapy, 63% had prior radiotherapy, and 71% had prior surgery.

Of the 8 patients who had BOR of SD, 5 were patients with NSCLC enrolled in cohort C: 4 patients with *BRAF* mutations and 1 patient with a *KRAS* mutation (median SD duration, 7.2 months). The patient with NSCLC with the *KRAS* mutation was on treatment and progression-free at the time of data lock for this study report.

Other patients with BOR of SD were: 1 patient with nasal squamous cell adenocarcinoma (SD duration, 5.3 months), 1 patient with uterine carcinoma (SD duration, 1.8 months), and 1 patient with pilocytic astrocytoma (SD duration, 3.7 months).

Pharmacokinetics

Single-dose pharmacokinetics

Pharmacokinetic data for LY3009120 after a single oral administration were available from 32 patients, with doses ranging from 50 to 500 mg. In general, the increase in LY3009120 exposure was approximately dose-proportional within the studied dosage range, although the exposure of the 400-mg group was slightly lower than that of the 300-mg group. The time of maximum observed plasma concentration (T_{max}) ranged from 1 to 10 hours. The $t_{1/2}$ was similar across all cohorts and ranged from 3.1 to 9.9 hours (**Fig. 2**). The exposures observed at 300 mg twice a day were above the half-maximal effective concentration (EC_{50}) for the *BRAF*-mutant preclinical model and estimated to be above the *KRAS*-mutant preclinical model.

Multiple-dose pharmacokinetics

Similar to single-dose pharmacokinetic results, approximately dose-proportional exposure increase was observed between 50 and 300 mg on days 15 and 28. Exposures at 400 mg appeared to be slightly lower than those at 300 mg. Trough concentrations appeared to be similar on days 15, and 28, indicating steady state was achieved by day 15 (**Fig. 3**). In addition, the LY3009120 exposures on day 28 were comparable to those observed on day 15, suggesting no time-dependent change in the steady state pharmacokinetic properties. After multiple twice a day doses, minimum accumulation was observed, with accumulation index ranging from 1.05 to 1.44. The T_{max} and $t_{1/2}$ were similar to those obtained from single-dose data (**Fig. 3**).

Pharmacodynamics

Five patients with paired pre- and postbiopsy samples were available for pharmacodynamic evaluations of Ki-67, p27, and p-ERK expression (Supplementary Fig. S2), as well as for changes in the expression of eleven ERK pathway nuclear targets (Supplementary Fig. S3).

Two cases of patients treated at the RP2D are highlighted in Supplementary Fig. S2: a 56-year-old female patient with rectal neuroendocrine cancer who is *KRAS* and *BRAF* wild-type (Supplementary Fig. S2A), and a 26-year-old male patient with hepatocellular carcinoma with unknown mutational status (Supplementary Fig. S2B). No differences in staining for the three biomarkers were observed from baseline to day 28 (Supplementary Figs. S2A and S2B). Gene expression of eleven ERK nuclear targets was assessed for pharmacodynamic changes across 5 patients; no discernible PD effects were observed (Supplementary Fig. S3).

Although no objective responses were observed, minor decreases were observed. There were 4 patients with negative best tumor percentage change; two with BRAF mutations, and two with KRAS mutations. Specifically, 1 patient had the best tumor percentage change of $\leq$30%.

Discussion

LY3009120 is a pan-RAF, dimer inhibitor with preclinical activity against *RAF*- and *RAS*-mutant cancers. The primary objective of this study was to determine a RP2D of LY3009120 that could be safely administered to patients with advanced and/or metastatic cancer. Secondary objectives were to assess the safety, toxicity, pharmacokinetic parameters, and antitumor activity of the compound. Pharmacodynamic biomarkers were also explored.

The RP2D was 300 mg, administered twice a day. At this dose, drug exposure levels were above the preclinical concentration associated with treatment response in both *BRAF*- and *KRAS*-mutant models. However, during dose escalation and dose confirmation, there was limited clinical activity identified [no RECIST 1.1 defined responses, best response of SD in only 8 of 51 (15.7%) patients] and there was no evidence of target inhibition noted in the analysis of the limited available on-treatment biopsies.

These results follow an unexpected and rather unsettling trend in pan-RAF inhibitors in the MAPK pathway of mutated or activated tumors. The first of these agents, sorafenib, is a potent inhibitor of RAF and of wild-type and mutant BRAF, as well as a number of other serine–threonine kinases such as VEGFR1/2, PDGFR, and KIT (24). On the basis of preclinical data in *BRAF V600*-mutant melanoma (25), a development program for this drug was launched and included a number of phase I, II, and III studies (although not specifically in the *BRAF*-mutated population); studies have failed to show benefit of sorafenib as monotherapy in melanoma, including no benefit in a subgroup analysis of patients with *BRAF* mutations (26). The next pan-RAF inhibitor to be evaluated in the clinic, RAF-265, was also an inhibitor of VEGFR and exclusively studied in patients with melanoma in a phase I trial, based on strong preclinical data in *BRAF*-mutant and wild-type populations (27–29). RAF-265 was associated with a number of side effects common with small-molecule inhibitors such as fatigue, diarrhea, weight loss/anorexia/dysgeusia, and nausea, as well as hematologic (thrombocytopenia, neutropenia) and ophthalmologic (vitreous floaters) toxicities (29). Objective responses were uncommon (8/66 with evaluable disease; 12%) and were independent of *BRAF*-mutation status. Importantly, this trial preceded the approvals of vemurafenib and dabrafenib, and therefore represents perhaps the last targeted therapy trial of pan-RAF inhibitors that include patients with *BRAF V600*-mutant melanoma not previously treated with mutant-specific BRAF inhibitors.

RAF (particularly the B- and C-isoforms) remains a relevant target in cancer, as evidenced by the success of BRAF, MEK, and ERK inhibitors in patients with *BRAF*-mutant cancers (8, 11, 14, 30, 31). In

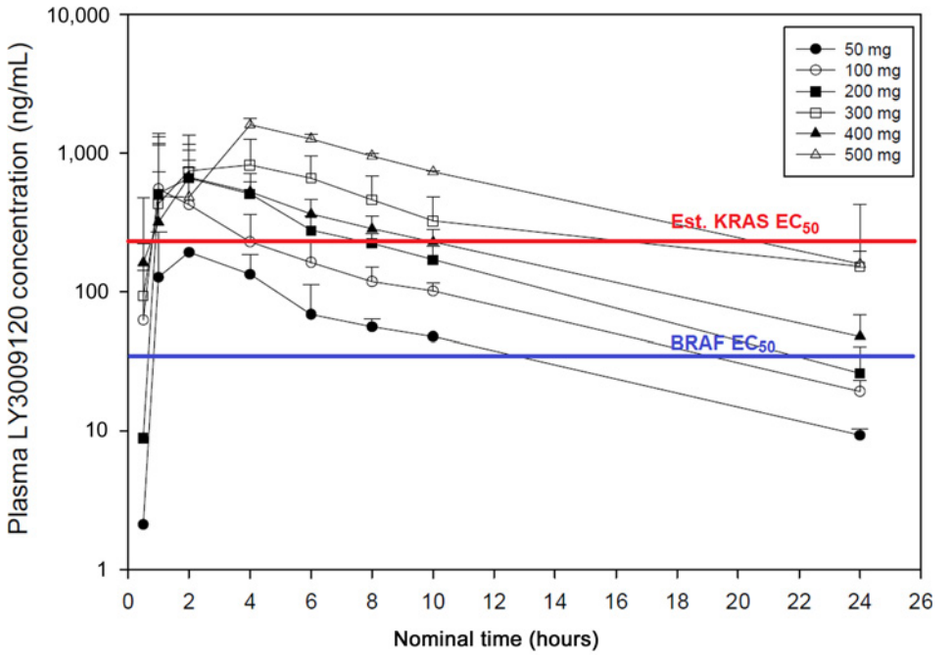

Figure 2.
Mean plasma LY3009120 concentration–time profiles following single dose administration during dose escalation part A (50–500 mg). The mean pharmacokinetic profile is shown for LY3009120 after a single oral administration at 50, 100, 200, 300, 400, and 500 mg. The EC_{50} determined from *BRAF* A375–mutant preclinical models (blue line) and the EC_{50} determined from *in vivo KRAS*-mutant preclinical models (red line) are shown.

fact, one of the more promising features of pan-RAF inhibitors is that they should be able to get around the paradoxical activation of the MAPK pathway in the setting of the more specific BRAF inhibitors (e.g., vemurafeib, dabrafenib; refs. 32, 33). LY3009120 qualifies as a "paradox breaking" RAF inhibitor in that it can inhibit BRAF without paradoxically activating cell lines with upstream mutations (e.g., *KRAS* or *NRAS*). Similarly, other agents in early-phase clinical trials, such as TAK 580 (NCT01425008; ref. 34), BGB-283 (NCT02610361; ref. 35), and PLX8394 (NCT02428712; ref. 36), also do not lead to paradoxical activation of MAPK signaling preclinically. To date, responses in *BRAF*-mutant patients not previously treated with BRAF inhibitors have been documented with BGB-283 (35) and TAK 580, but responses in patients with *NRAS*-mutant melanoma remain rare with TAK 580 (34), although a partial response with BGB-283 was reported in a patient with *KRAS*-mutant NSCLC (37).

The lack of clinical efficacy with LY3009120 was unexpected, given the preclinical efficacy and PK level demonstrating exposure levels predicted to be therapeutic in *RAS*- and *RAF*-mutant tumors (19, 20). Furthermore, there have been occasional responses seen in patients treated with other pan-RAF inhibitors. The toxicity profile was in line with other inhibitors of the MAPK pathway, although the rate of cutaneous toxicity may have been slightly lower than that seen with potent/specific BRAF inhibitors, and MEK and ERK inhibitors. However, the rate of rash was similar to that seen with RAF265 (29), the only other pan-RAF inhibitor with significant clinical data published to date. One explanation for the disappointing clinical activity was poor selection of patients for cohort expansion. The original preclinical data demonstrated activity in RAS mutant and BRAF *V600E* mutant cancer, including secondarily resistant BRAF *V600E* mutant melanoma cell lines (19, 20). However, work with this compound and other

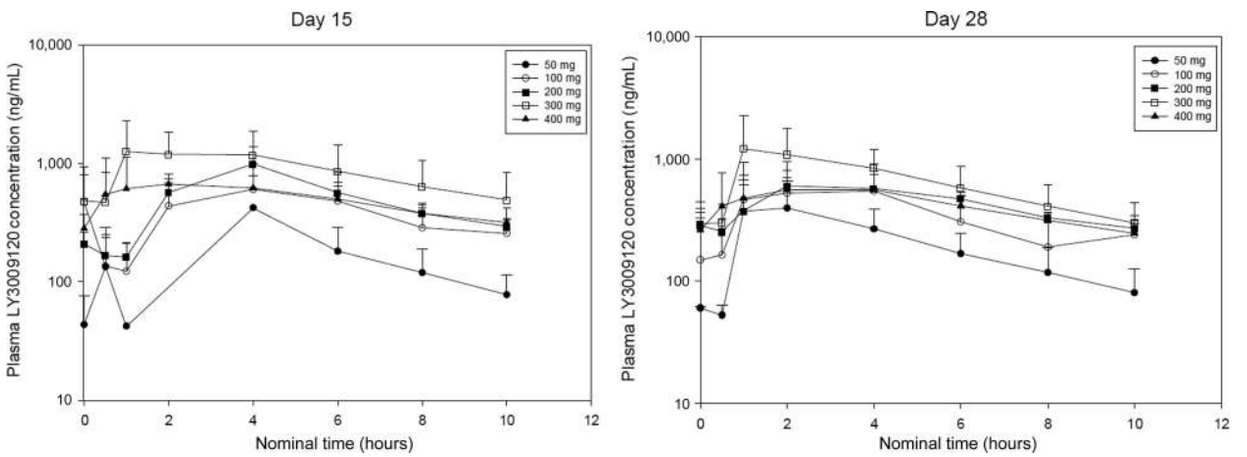

Figure 3.
Mean plasma LY3009120 concentration–time profiles following multiple dose administration (50–400 mg). The mean pharmacokinetic profile is shown for LY3009120 after twice a day administration of 50, 100, 200, 300, and 400 mg. Samples were collected on days 15 ($n = 22$; left) and 28 ($n = 18$; right) of cycle 1.

pan-RAF inhibitors has subsequently shown that sensitivity with pan-RAF inhibition is highest in BRAF microdeletions and Class 3 BRAF mutations more broadly (20). Pan-RAF inhibitors have also more recently shown promising preclinical efficacy when combined with a MEK inhibitor (38). Because this was not known at the time of trial launch and just being determined when a decision was made to close the trial, these patients were not actively recruited. In addition, among those who were recruited, only a limited number of RAS- and/or BRAF-mutant samples were available, limiting interpretability. However, perhaps the best explanation for the observed lack of effectiveness of LY3009120 is that, despite reasonable plasma exposure levels, there was no observed target engagement in the tumor environment, possibly due to insufficient time on target because of the short half-life of LY3009120. This possible explanation is supported by the limited but compelling pharmacodynamic data showing no major impact on MAPK signaling in pretreatment versus posttreatment tumor biopsy; it is unclear why there were no differences and whether higher doses would lead to effective target-tumor inhibition. However, we were only able to assess pERK IHC staining in two samples, making interpretation difficult. Given the toxicity results during the higher escalation doses, and lack of discernible pharmacodynamic effects, the decision was made not to pursue further development of LY3009120.

Disclosure of Potential Conflicts of Interest

R.J. Sullivan is a paid consultant at Merck, Novartis, Array Biopharma, Replimune, and Bristol Myers Squibb. K.T. Flaherty is a member of Board of Directors at Clovis Oncology, Vivid Biosciences, Loxo Oncology; is a member of Scientific Advisory Board at Fount Therapeutic, Aeglea, Shattuck Labs, Tolero Pharmaceuticals, Apricity, Oncoceutics, Fog Pharma, Neon Therapeutics, Tvardi, xCures, Monopteros, Vibliome, Sanofi, Amgen, Asana Biosciences, Adaptimmune; is a consultant at Novartis, Genentech, Bristol Myers Squibb, Merck, Takeda, Verastem, Boston Biomedical, Pierre Fabre, Debiopharm; is a member of Corporate Advisory Board at X4 Pharmaceuticals, PIC Therapeutics; reports of receiving commercial research grant from Novartis, Sanofi; and has ownership interest (including patents) in Loxo Oncology, Clovis Oncology, Fog Pharma, Tvardi, xCures, Monopteros, Vibliome, Strata Oncology, Vivid Biosciences, X4 Pharmaceuticals, PIC Therapeutics, Fount Therapeutics, Shattuck Labs, Apricity, and Oncoceutics. G.I. Shapiro is a member of advisory board at Astex, Lilly, Bayer, Ipsen, Cybrexa Therapeutics, Angiex, Daiichi Sankyo, Seattle Genetics, Pfizer, Almac, Roche, Bicycle Therapeutics, Merck-EMD Serono, Fusion Pharmaceuticals, G1 Therapeutics, Sierra Oncology; reports of receiving commercial research grant from Merck-EMD Serono, Merck & Co., Sierra Oncology, Pfizer, and Array Biopharma. J. Rodon is an adviser at Kelun, Peptomyc, Merck Sharpe Dome, Spectrum, Bayer Pharmaceuticals, and Roche Pharmaceuticals. M.J. Millward is a member of advisory board at Novartis, Roche, AstraZeneca, Merck Sharp & Dohme, Pfizer, Bristol Myers Squibb; and has provided expert testimony for Roche, Bristol Myers Squibb, and AstraZeneca. L. Gao is a Principal Research Scientist at Eli Lilly & Company. M.D. Willard is a Senior Clinical Research Scientist at Eli Lilly & Company. A.E Schade is a Distinguished Medical Fellow at Eli Lilly & Company. D. L. Flynn is a at Deciphera Pharmaceuticals; and has ownership interest (including patents) in Deciphera Pharmaceuticals. M.D. Kaufman is a Vice President Chemical & Pharmaceutical Development at Deciphera Pharmaceuticals; and has ownership interest (including patents) in Deciphera Pharmaceuticals. J.R. Henry is a Sr Research Fellow at Eli Lilly & Company; has ownership interest (including patents) in Eli Lilly & Company. K.A. Benhadji is a Vice President at Eli Lilly & Company, is a Senior Vice President at Taiho Oncology; and has ownership interest (including patents) in Eli Lilly & Company. I. Conti is a Se Medical Director at EMD Serono Research and Development Institute, and was a Sr Medical advisor at Eli Lilly & Company till April 2017. R.V. Tiu is a Senior Medical Advisor/Medical Advisor at Eli Lilly & Company; is a Senior Medical Director & Global Medical Lead at Astellas Pharma US; and has ownership interest (including patents) in Eli Lilly & Company. No potential conflicts of interest were disclosed by the other authors.

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