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Inhibition of Mutated, Activated BRAF in Metastatic Melanoma

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ABSTRACT

BACKGROUND

The identification of somatic mutations in the gene encoding the serine–threonine protein kinase B-RAF (BRAF) in the majority of melanomas offers an opportunity to test oncogene-targeted therapy for this disease.

METHODS

We conducted a multicenter, phase 1, dose-escalation trial of PLX4032 (also known as RG7204), an orally available inhibitor of mutated BRAF, followed by an extension phase involving the maximum dose that could be administered without adverse effects (the recommended phase 2 dose). Patients received PLX4032 twice daily until they had disease progression. Pharmacokinetic analysis and tumor-response assessments were conducted in all patients. In selected patients, tumor biopsy was performed before and during treatment to validate BRAF inhibition.

RESULTS

A total of 55 patients (49 of whom had melanoma) were enrolled in the dose-escalation phase, and 32 additional patients with metastatic melanoma who had BRAF with the V600E mutation were enrolled in the extension phase. The recommended phase 2 dose was 960 mg twice daily, with increases in the dose limited by grade 2 or 3 rash, fatigue, and arthralgia. In the dose-escalation cohort, among the 16 patients with melanoma whose tumors carried the V600E BRAF mutation and who were receiving 240 mg or more of PLX4032 twice daily, 10 had a partial response and 1 had a complete response. Among the 32 patients in the extension cohort, 24 had a partial response and 2 had a complete response. The estimated median progression-free survival among all patients was more than 7 months.

CONCLUSIONS

Treatment of metastatic melanoma with PLX4032 in patients with tumors that carry the V600E BRAF mutation resulted in complete or partial tumor regression in the majority of patients. (Funded by Plexxikon and Roche Pharmaceuticals.)

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Metastatic melanoma is an aggressive disease for which there are few effective therapies. The two therapies approved by the Food and Drug Administration, high-dose interleukin-2 and dacarbazine, are each associated with response rates of only 10 to 20% and a small percentage of complete responses; neither is thought to improve overall survival.[1,2] In randomized trials, the median survival among patients treated with dacarbazine was less than 8 months.[3,4]

A search for mutations in a component of the mitogen-activated protein (MAP) kinase pathway in a large panel of common cancers revealed that 40 to 60% of melanomas, and 7 to 8% of all cancers, carry an activating mutation in the gene encoding the serine–threonine protein kinase B-RAF (*BRAF*).[5-15] Ninety percent of reported *BRAF* mutations result in a substitution of glutamic acid for valine at amino acid 600 (the V600E mutation). This BRAF mutation constitutively activates BRAF and downstream signal transduction in the MAP kinase pathway. *BRAF* mutations are also found in 40 to 70% of papillary or anaplastic thyroid cancers[6-8,16-18] and in a small percentage of several other types of tumor.

PLX4032 (Plexxikon; RG7204, Roche Pharmaceuticals) is a potent inhibitor of BRAF with the V600E mutation. Preclinical studies showed that PLX4032 and its analogue PLX4720 inhibit the kinase activity of BRAF with the V600E mutation at low nanomolar concentrations, abrogate signaling through the MAP kinase pathway, and block proliferation of cells carrying BRAF with the V600E mutation in vitro at high nanomolar concentrations.[17,18] Orally administered PLX4720 inhibits the growth — and, at higher doses, induces the regression — of human melanoma tumors transplanted into immunocompromised mice. None of these effects are observed in normal tissues or in tumor cells that lack a *BRAF* mutation.

We conducted a trial of the use of PLX4032 in patients with metastatic cancer. The primary goals were to define the safety and pharmacokinetic characteristics of treatment with continuous, twice-daily administration of PLX4032, to determine the maximum dose that could be administered until adverse effects prevented further dose increases (i.e., the recommended phase 2 dose), and to determine the objective response rate, the duration of response, and the rate of progression among patients who had melanoma tumors with the V600E BRAF mutation and who were given the recommended phase 2 dose of PLX4032.

METHODS

STUDY DESIGN

The study was sponsored by Plexxikon and Roche Pharmaceuticals, which provided the study drug. The study was designed by two academic authors and one industry author. All authors made the decision to submit the manuscript for publication. All authors analyzed the data, prepared the manuscript, and vouch for the completeness and accuracy of the data and analyses. The study was conducted in accordance with the protocol.

Dose-Escalation Phase

PLX4032 was initially in a crystalline formulation. In the dose-escalation phase of the study, which involved several consecutively enrolled groups of three to six patients, the first group received 200 mg of PLX4032 by mouth daily; subsequent groups received the drug at higher doses, according to a dose-escalation scheme. This formulation was found to have poor bioavailability (see the Results section), and enrollment for the dose-escalation phase was halted while the drug was reformulated as a highly bioavailable microprecipitated bulk powder, initially as a 40-mg capsule and subsequently as 80-mg and 120-mg capsules, as well as 240-mg tablets. Enrollment was resumed, with newly enrolled patients receiving the microprecipitated-bulk-powder formulation at a dose of 160 mg (two 80-mg capsules) twice daily, with subsequent escalation.

Patients received continuous therapy with PLX4032 unless unacceptable side effects or disease progression occurred. Doses were not escalated unless the patients receiving the highest current dose had been observed for at least 4 weeks and dose-limiting side effects had been reported in fewer than a third. Dose escalation in a given patient was permitted if the safety and adverse-effect profile had been established for the next highest dose. The recommended phase 2 dose was defined as the highest dose at which no more than one of six patients had dose-limiting side effects.

Extension Phase

Once the recommended phase 2 dose had been identified, an extension cohort was treated at this

dose. In this cohort, all patients had melanoma and a prospectively identified V600E BRAF mutation. The primary objective in the extension cohort was to determine the response rate. Secondary objectives were to define the toxicity and pharmacokinetics of PLX4032 more precisely and to obtain data on pharmacodynamic effects.

PATIENTS

Eligibility criteria included the provision of written informed consent, an age of 18 years or older, solid tumors confirmed histologically that were refractory to standard therapy or for which standard or curative therapy did not exist, Eastern Cooperative Oncology Group performance status score of 0 (able to be fully active and carry out all predisease activities without restriction) or 1 (unable to perform physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, such as light housework or office work),[19] life expectancy of 3 months or longer, absence of known progressing or unstable brain metastases, and adequate hematologic, hepatic, and renal function.

The dose-escalation phase of the trial was open to patients with any type of tumor, although patients who had melanomas with the V600E mutation in BRAF were overrepresented because of the selective activity of PLX4032 against such tumors in preclinical testing. For the extension cohort, eligibility was restricted to patients with melanomas harboring a V600E BRAF mutation, as ascertained by means of a polymerase-chain-reaction assay (TaqMan, Applied Biosystems). This assay involves hybridizing a probe specific to the 1799T→A substitution that results in the V600E BRAF mutation with DNA isolated from formalin-fixed, paraffin-embedded tumor tissue and determining the presence or absence of amplification after repeated chain-reaction cycles.[20]

STUDY ASSESSMENTS

Safety evaluations were conducted at baseline, day 8, day 15, day 29, and every 4 weeks thereafter. These evaluations included a physical examination, electrocardiography, laboratory studies that included a complete blood count, clinical chemical testing, and urinalysis. Adverse events were graded according to the Common Terminology Criteria for Adverse Events (version 3.0) (http://ctep.info.nih.gov/protocolDevelopment/electronic_applications/docs/ctcaev3.pdf). During the trial, squamous-cell carcinoma, keratoacanthoma type, was observed in several patients (see the Results section). As a result, the protocol was amended to ensure that patients underwent dermatologic evaluations at baseline and every 2 months during the study; computed tomographic (CT) scans of the chest were analyzed for the appearance of new lesions suggestive of a primary cancer.

CT studies were performed at 8-week intervals during therapy in all patients and at the end of the first 4 weeks of therapy in some patients. The findings were judged according to the Response Evaluation Criteria in Solid Tumors (RECIST), version 1.0. A complete response was defined as the disappearance of all target lesions, and a partial response as a decrease by at least 30% in the sum of the largest diameter of each target lesion, relative to the corresponding sum at baseline. Progressive disease was defined as an increase by at least 20% in the sum of the largest diameter of each target lesion, relative to the smallest corresponding diameter recorded since the start of treatment, or the appearance of one or more new lesions. Stable disease was defined as the absence of shrinkage sufficient for a partial response and the absence of enlargement sufficient for progressive disease, relative to the corresponding sum at baseline. Progression-free survival was defined as the time from the first day of treatment to the first documentation of disease progression or death, whichever occurred first.

Pharmacokinetic assessments were made on day 1 and day 15 of the first 4 weeks of therapy, and single plasma samples were obtained every 4 weeks during the study. Plasma samples were analyzed by means of high-performance liquid chromatography, with detection by means of mass spectroscopy.

Patients in the dose-escalation phase who were receiving a dose greater than 160 mg twice daily and patients in the extension cohort underwent ^{18}F-fluorodeoxyglucose–positron-emission tomography (FDG-PET) at baseline and on day 15 of the first 4 weeks of therapy. Selected patients underwent tumor biopsy before the start of therapy as well as on day 15. Biopsy specimens were immediately fixed in formalin for analysis of phosphorylated extracellular signal-regulated kinase (ERK), the protein cyclin D1, and the monoclonal antibody Ki-67 by means of immunohistochemistry. The sampled tumors were cutaneous or superficial lymph-node lesions; except in the case of

Table 1. Baseline Characteristics of the Patients, According to Study Cohort.*

Characteristic	Dose-Escalation Cohort (N=55)	Extension Cohort (N=32)
Age — yr		
Median	63	52
Range	23–89	23–83
Sex — no. (%)		
Male	34 (62)	19 (59)
Female	21 (38)	13 (41)
Tumor type — no. (%)		
Melanoma	49 (89)	32 (100)
Thyroid	3 (5)	0
Other	3 (5)	0
Extent of metastatic melanoma — no. (%)†		
Stage M1a	7 (14)	6 (19)
Stage M1b	6 (12)	2 (6)
Stage M1c	36 (73)	24 (75)
LDH >ULN — no. (%)		13 (41)
ECOG performance status score — no. (%)		
0	28 (51)	15 (47)
1	27 (49)	17 (53)
No. of previous chemotherapy regimens — no. (%)‡		
0	5 (10)	7 (22)
1	16 (33)	9 (28)
2	5 (10)	4 (12)
≥3	23 (47)	12 (38)

* ECOG denotes Eastern Cooperative Oncology Group, LDH lactate dehydrogenase, and ULN upper limit of the normal range.
† The extent of metastatic melanoma is described as the American Joint Committee on Cancer stage. These data were not reported for the six patients in the dose-escalation cohort who did not have melanoma.
‡ The number of previous therapies was not reported for the six patients in the dose-escalation cohort who did not have melanoma.

one patient, sequential biopsy specimens were not taken from the same lesion.

STATISTICAL ANALYSIS

For the primary end point of a partial or complete response in the extension cohort, we calculated that a sample of 32 patients would provide 95% confidence ($\alpha = 0.05$), with 80% power ($\beta = 0.20$), that an observed response rate of 40% would be consistent with a true response rate of more than 10%, which was considered justification for further study. For this report, January 31, 2010, was the cutoff date for the safety and efficacy follow-up.

RESULTS

PATIENTS

Fifty-five patients were enrolled in the dose-escalation phase of the study (see Fig. 2 in the Supplementary Appendix, available with the full text of this article at NEJM.org); 32 additional patients with metastatic melanoma that carried the V600E BRAF mutation were treated at the recommended phase 2 dose in the extension phase (Table 1). The majority of patients (49 of 55 [89%]) in the dose-escalation cohort had metastatic melanoma; 3 of the remaining 6 patients had papillary thyroid cancer that carried the V600E BRAF mutation. Screening for the V600E BRAF mutations was not a requirement for study entry during the dose-escalation phase, but throughout the trial, an increasing number of patients were prospectively identified as having the mutation (for a total of 16 of the 21 patients with melanoma who were enrolled in the groups receiving 240 mg twice daily to 1120 mg twice daily).

The initial crystalline formulation of PLX4032 was found to have no dose-limiting side effects or antitumor activity at doses of 200 mg daily to 1600 mg twice daily in consecutively enrolled groups of patients. Since the serum levels detected were lower than the levels predicted in preclinical models to be effective, a microprecipitated-bulk-powder formulation with substantially higher bioavailability was developed and used for the remainder of the study; the lowest dose was 160 mg twice daily, and escalated doses were 240, 320 or 360, 720, and 1120 mg twice daily (Fig. 2 in the Supplementary Appendix). Patients who had been receiving the crystalline formulation were switched to the microprecipitated-bulk-powder formulation. The protocol allowed the dose to be reduced if side effects developed.

Once the recommended phase 2 dose was established for the microprecipitated-bulk-powder formulation in the dose-escalation cohort, the extension cohort was enrolled. All patients in the extension cohort had melanoma carrying the V600E BRAF mutation (Table 1).

ADVERSE EVENTS

During the latter part of the dose-escalation phase of the trial, when the microprecipitated-bulk-powder formulation of PLX4032 was used, dose-limiting toxic effects were not observed until a dose of 720 mg twice daily was given. At the next-highest dose given to one group of patients,

Table 2. Drug-Related Adverse Events of Grade 2 or Higher Reported in More Than 5% of the 87 Study Patients, According to the Dose of PLX4032 Given Twice Daily.*

Event	<240 mg (N=30)	240 mg (N=4)	320 or 360 mg (N=8)	720 mg (N=7)	960 mg (N=32)	1120 mg (N=6)	Total (N=87)
				number (percent)			
Arthralgia							
Grade 2	0	1 (25)	2 (25)	0	10 (31)	1 (17)	14 (16)
Grade 3	0	0	0	0	1 (3)	1 (17)	2 (2)
Rash							
Grade 2	1 (3)	0	0	1 (14)	7 (22)	1 (17)	10 (12)
Grade 3	0	0	0	0	1 (3)	3 (50)	4 (3)
Squamous-cell carcinoma, keratoacanthoma type							
Grade 2	0	0	0	0	0	0	0
Grade 3	1 (3)	2 (50)	3 (38)	0	10 (31)	2 (33)	18 (21)
Nausea							
Grade 2	1 (3)	0	1 (11)	1 (14)	4 (12)	1 (17)	8 (9)
Grade 3	0	0	0	0	1 (3)	0	1 (1)
Fatigue							
Grade 2	0	0	0	0	2 (6)	1 (17)	3 (3)
Grade 3	0	0	0	0	2 (6)	2 (33)	4 (5)
Photosensitivity reaction							
Grade 2	0	0	0	1 (14)	4 (12)	1 (17)	6 (7)
Grade 3	0	0	0	0	1 (3)	0	1 (1)
Palmar–plantar dysesthesia							
Grade 2	0	0	0	0	2 (6)	1 (17)	3 (3)
Grade 3	0	0	0	0	2 (6)	0	2 (2)
Pruritus							
Grade 2	0	0	0	0	4 (12)	0	4 (5)
Grade 3	0	0	0	0	0	1 (17)	1 (1)
Lymphopenia							
Grade 2	0	0	2 (25)	0	2 (6)	0	4 (5)
Grade 3	0	0	0	0	0	1 (17)	1 (1)

* Adverse events were graded according to the Common Terminology Criteria for Adverse Events (version 3.0) (http://ctep.info.nih.gov/protocolDevelopment/electronic_applications/docs/ctcaev3.pdf). Four patients had a grade 4 adverse event: two had elevated γ-glutamyltransferase levels, one had fatigue, and one had reversible pancytopenia of uncertain attribution.

1120 mg twice daily, four of the six patients had dose-limiting side effects: three patients with grade 3 rash (two of whom also had grade 3 fatigue) and one patient with grade 3 arthralgia (Table 2). A dose of 960 mg twice daily was evaluated and determined to be tolerated in the first six patients given the dose, so it was established as the recommended phase 2 dose for the extension cohort. Those six patients were included as the first six patients in the extension cohort. In the extension cohort, 13 patients (41%) required a dose reduction during therapy (to 720 mg twice daily in 10 patients, to 600 mg twice daily in 1 patient, and to 480 mg twice daily in 2 patients). The most common PLX4032-related grade 2 or 3 side effects observed were arthralgia, rash, nausea, photosensitivity, fatigue, cutaneous squamous-cell carcinoma, pruritus, and palmar–plantar dysesthe-

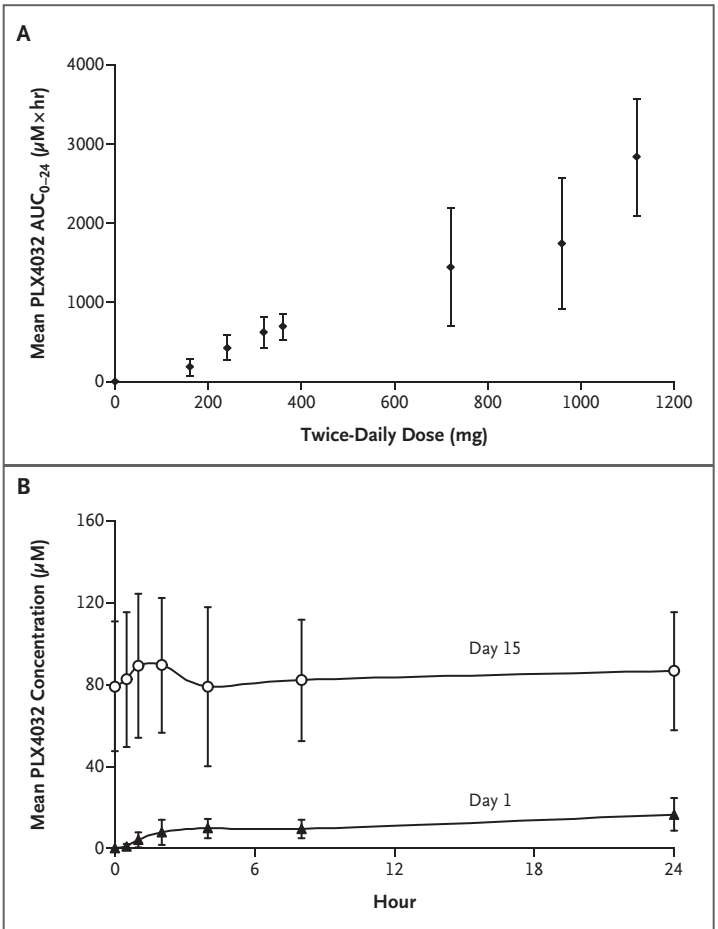


Figure 1. Mean PLX4032 Concentrations over a 24-Hour Period.

Data are shown for the microprecipitated-bulk-powder formulation. Panel A shows the mean area under the plasma concentration–time curve (AUC_{0-24}), according to the twice-daily dose. Panel B shows the mean concentration after the administration of a single dose, on day 1, or multiple doses at the steady state, on day 15, at the recommended phase 2 dose of 960 mg twice daily. I bars indicate standard deviations.

Figure 2 (facing page). Representative Findings of the Effect of PLX4032 at the Recommended Phase 2 Dose in Study Patients with Melanoma That Carried the V600E Mutation.

The recommended phase 2 dose was 960 mg twice daily. Panel A (hematoxylin and eosin) shows immunohistochemical analyses of the expression of phosphorylated extracellular signal-regulated kinase (ERK), cyclin D1, and Ki-67 in tumor-biopsy specimens obtained at baseline and on day 15 of treatment. Panel B shows the uptake of ^{18}F-fluorodeoxyglucose (FDG) at baseline and on day 15 of treatment, as assessed by means of positron-emission tomography (PET). Panel C shows computed tomographic images of lesions (arrows) in lung, liver, and bone (with each pair of images shown for a different patient) at baseline and at 8 weeks.

sia (Table 2). In total, 89% of all side effects were grade 1 or 2. Rashes were evenly distributed among the face or neck, trunk, and extremities.

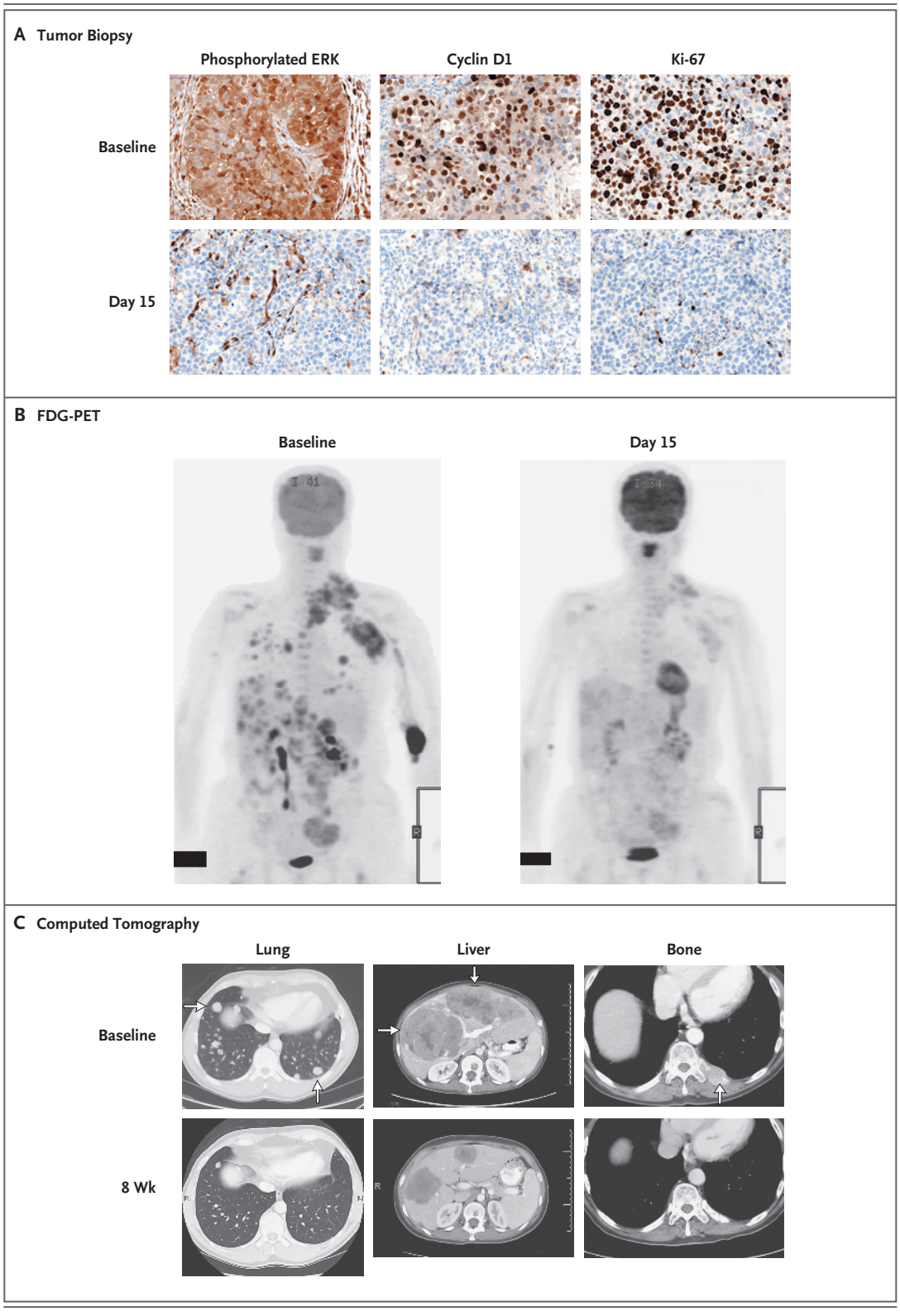
Eight patients in the dose-escalation cohort (15%) and 10 patients in the extension cohort (31%) had cutaneous squamous-cell carcinomas, with a total of 35 carcinomas. These were reviewed centrally, and all but one either were keratoacanthoma type or had features of a keratoacanthoma. The median time to the appearance of a cutaneous squamous-cell carcinoma was 8 weeks; the majority of the carcinomas were resected, and in no case did they lead to discontinuation of treatment. No squamous-cell carcinomas at other organ sites were observed during the study.

PHARMACOKINETICS

Pharmacokinetic analyses revealed that exposure increased with the dose throughout the range of doses of the microprecipitated-bulk-powder formulation that were administered, with exposure being proportional to dose for doses of 240 mg twice daily through 960 mg twice daily. At the recommended phase 2 dose, 960 mg twice daily, the mean (±SD) area under the plasma concentration–time curve over a 24-hour period (AUC_{0-24}) was 1741±639 μM×hour (Fig. 1A), and the mean maximum concentration at steady state was 86±32 μM (Fig. 1B). The mean PLX4032 level on day 15, after repeated dosing, was six to nine times the mean level on day 1, and its mean half-life was approximately 50 hours (range, 30 to 80). With the twice-daily dosing regimen, all patients were exposed to relatively constant daily levels of the drug at steady state.

PHARMACODYNAMICS

Tumor-biopsy specimens that had been obtained at baseline and at day 15 were available for seven of the patients in the extension cohort who were receiving PLX4032 at the recommended phase 2 dose. Tumor levels of phosphorylated ERK, cyclin D1, and Ki-67 were markedly reduced at day 15 as compared with baseline in all specimens tested (Fig. 2A). This finding suggests that PLX4032



inhibited the MAP kinase pathway, resulting in decreased cyclin D1 levels and decreased proliferation. In virtually all patients, a marked decrease in tumor uptake of FDG was noted at day 15 (Fig. 2B).

TUMOR RESPONSE

Dose-Escalation Phase

No responses were observed at doses of 160 mg twice daily of the microprecipitated-bulk-powder formulation or at any dose of the crystalline formulation. Of the patients receiving doses of 240 mg or more twice daily, 16 had melanoma with tumors that harbored the V600E BRAF mutation. Among these 16 patients, a partial or complete response was seen in the 1 patient receiving 240 mg twice daily, 2 of the 4 patients receiving 320 or 360 mg twice daily, 4 of the 6 patients receiving 720 mg twice daily, and 4 of the 5 patients receiving 1120 mg twice daily. The overall response rate was 69% (11 of 16 patients), with 10 partial responses and 1 complete response (Fig. 1A in the Supplementary Appendix). Responses were seen at all sites of metastatic disease, including the liver, small bowel, and bone (Fig. 2C). The duration of the response ranged from 2 to more than 18 months, with 4 patients still having a partial or complete response at the data cut-off date (Fig. 1B in the Supplementary Appendix). In addition to the patients with melanoma, the 3 patients with papillary thyroid cancer had a partial or complete response, with the response lasting 8 months in 1 patient (who was progression-free for 12 months) and stable disease lasting 11 and 13 months in each of the other 2 patients.

A total of 5 patients with metastatic melanoma whose tumors did not have a *BRAF* mutation received doses of 240 mg or more twice daily. None had evidence of tumor regression during the study; 4 had progressive disease within the first 2 months of treatment.

Extension Phase

The extension cohort consisted solely of patients who had melanoma with the V600E BRAF mutation. All were treated at the recommended phase 2 dose of 960 mg twice daily. A total of 26 of the 32 patients had a response (81%), with a complete response in 2 patients and a partial response in 24 patients (Fig. 3A). Among patients with symptomatic metastatic disease, improvement of symptoms, such as a reduced need for narcotics for pain in 3 patients, was reported within 1 to 2 weeks. As in the dose-escalation cohort, we observed tumor responses in visceral organs and bone metastases as well as more typically responsive sites such as the lungs and lymph nodes. Responses were also routinely observed in patients with elevated lactate dehydrogenase levels (10 partial responses among the 13 patients) and in patients who had received more than one previous type of therapy (11 partial responses among the 16 patients). To date, 16 of the 32 patients are still in the study; the estimated median progression-free survival among these patients is more than 7 months, on the basis of a Kaplan–Meier analysis. The estimated median overall survival has not been reached.

DISCUSSION

Our trial shows that therapy targeting tumors containing activating V600E BRAF mutations can induce complete or partial tumor regression in patients. PLX4032 induced complete or partial tumor regression in 81% of patients who had melanoma with the V600E BRAF mutation. Responses were observed at all sites of disease, including the bone, liver, and small bowel. During the dose-escalation phase of the trial, we also saw responses in patients who were receiving doses below the recommended phase 2 dose. These efficacy data are particularly encouraging in light of the high disease burden in most of our patients and the presence of symptomatic disease in many of them.

Most side effects related to PLX4032 appeared to be proportional to the dose and exposure to the drug. Cutaneous side effects, fatigue, and arthralgia predominated. In the extension cohort, at the dose of 960 mg twice daily, approximately 40% of patients required a short- or long-term reduction in dose to 720 mg, 600 mg, or 480 mg twice daily, many for grade 2 side effects. Squamous-cell carcinoma, keratoacanthoma type, developed in a total of 10 of 32 patients (31%); we also observed squamous-cell carcinoma, keratoacanthoma type, in patients in the dose-escalation cohort. The characteristic rapid eruption of individual, dome-shaped, nonpigmented lesions and histologic findings were present in each case. Usually, squamous-cell carcinoma, keratoacanthoma type, are well-differentiated tumors with very low invasive potential and no metastatic potential; our data do not allow us to determine their behavior in patients receiving PLX4032. Recent data show that BRAF inhibitors can activate the MAP kinase pathway in cells that lack a BRAF

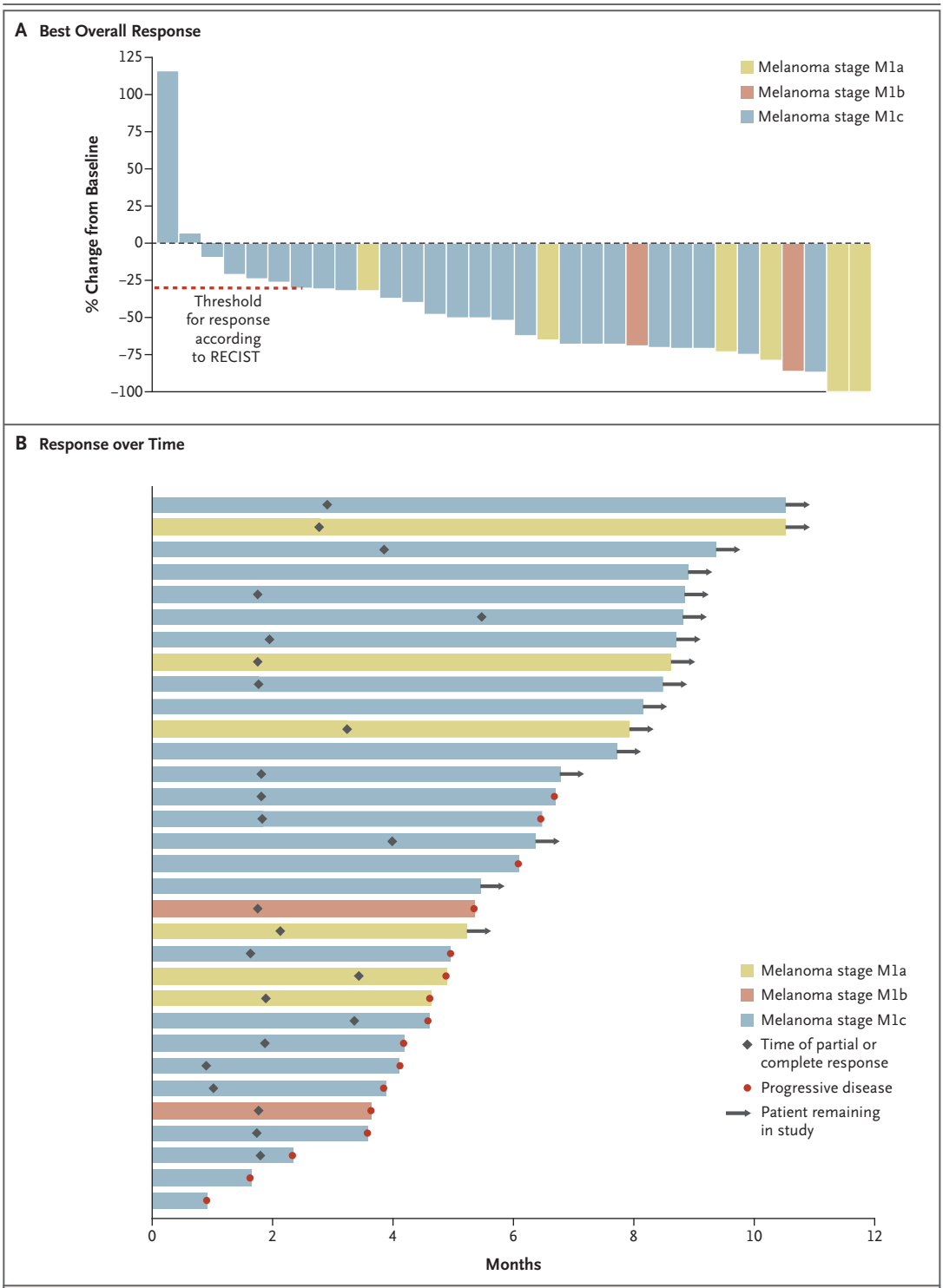


Figure 3. Antitumor Response in Each of the 32 Patients in the Extension Cohort.

All 32 patients had melanoma tumors that carried the V600E mutation of the v-raf murine sarcoma viral oncogene homologue B1 (BRAF). All were treated at the recommended phase 2 dose of 960 mg twice daily. Panel A shows the best overall response for each of the 32 patients, measured as the change from baseline in the sum of the largest diameter of each target lesion. Negative values indicate tumor shrinkage, and the dashed line indicates the threshold for a partial response according to Response Evaluation Criteria in Solid Tumors (RECIST) (i.e., shrinkage by 30%). Two patients had a complete response. Panel B shows the duration and characteristics of the responses in each patient.

mutation.[21-23] This activation may pertain to some of the side effects seen with PLX4032.

Though the early response to PLX4032 seems to occur reliably, responsive tumors can develop resistance to treatment. Among the patients in the dose-escalation cohort who had a response to treatment, the duration of the response ranged from 2 to more than 18 months. The mechanism of secondary tumor resistance is not yet known. We also observed that in some patients with V600E BRAF mutations, the tumors showed resistance without evidence of an early response. The mechanism of this primary refractory state is currently under investigation. To date, we have not seen "gatekeeper" *BRAF* mutations in resistant tumors, although this issue requires more investigation.

Sorafenib, which inhibits BRAF (both the wild type and the V600E mutant) and v-raf-1 murine leukemia viral oncogene homologue 1 (RAF1), has been studied in melanoma. In animal models of melanoma, sorafenib has not shown selective activity against tumors carrying BRAF mutations, and in clinical trials, sorafenib used either alone or in combination with chemotherapy has not had significant antimelanoma effects.[24-27] It is possible that the non-BRAF effects of sorafenib mediate side effects that limit the likelihood of achieving a drug concentration that is high enough to inhibit the V600E BRAF mutation.

It is now clear that melanomas can be categorized by specific molecular changes that drive their proliferation.[28] The overriding hypothesis is that inhibition of the activated pathway in the individual tumor will lead to tumor regression. There is recent preliminary evidence that imatinib can induce regression in 33% of the small proportion of melanomas driven by mutations in KIT (the v-kit Hardy–Zuckerman 4 feline sarcoma viral oncogene homologue).[29] In our study, PLX4032 induced responses in the vast majority of melanomas caused by *BRAF* mutations, which constitute 40 to 60% of all melanomas. We do not yet know whether treatment with PLX4032 will improve overall survival; an ongoing phase 3 trial (ClinicalTrials.gov number, NCT01006980) is addressing that question.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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