Clinical Cancer Research

A Phase I, Dose Escalation Study of Oral ASP8273 in Patients with Non–small Cell Lung Cancers with Epidermal Growth Factor Receptor Mutations

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Abstract

Purpose: Acquired *EGFR* T790M mutations are the most frequently identified resistance mechanism to EGFR tyrosine kinase inhibitors (TKI) in patients with *EGFR*-mutant lung cancers. ASP8273 is a third-generation EGFR TKI with antitumor activity in preclinical models of EGFR-mutant lung cancer that targets mutant EGFR, including *EGFR* T790M.

Experimental Design: In this multicohort, phase I study (NCT02113813), escalating doses of ASP8273 (25–500 mg) were administered once daily to non–small cell lung cancer (NSCLC) patients with disease progression after prior treatment with an EGFR TKI. *EGFR* T790M was required for all cohorts, except the dose escalation cohort. Primary endpoints were safety/tolerability; secondary endpoints were determination of the RP2D, pharmacokinetic profile, and preliminary antitumor activity of ASP8273. Evaluation of the use of EGFR mutations in circulating free DNA (cfDNA) as a biomarker of ASP8273 treatment effects was an exploratory endpoint.

Results: A total of 110 patients were treated with ASP8273 across dose escalation ($n = 36$), response–expansion ($n = 36$), RP2D (300 mg; $n = 19$) and food–effect ($n = 19$) cohorts. The most common treatment-emergent adverse events included diarrhea, nausea, fatigue, constipation, vomiting, and hyponatremia. Across all doses, in patients with *EGFR* T790M, the response rate was 30.7% ($n = 27/88$; 95% CI, 19.5%–44.5%), and median progression-free survival was 6.8 months (95% CI, 5.5–10.1 months). *EGFR* mutations in cfDNA, both the activating mutation and *EGFR* T790M, became undetectable in most patients in the setting of clinical response and reemerged upon disease progression.

Conclusions: ASP8273 was well tolerated and promoted antitumor activity in patients with *EGFR*-mutant lung cancer with disease progression on prior EGFR TKI therapy. *Clin Cancer Res; 23(24); 7467–73. ©2017 AACR.*

Introduction

Mutations in the *EGFR* gene result in constitutive activation of EGFR signaling causing cell survival, proliferation, and metastatic spread (1–3). EGFR tyrosine kinase inhibitors (TKI) are the recommended first-line treatment for *EGFR*-mutant lung cancers with superior outcomes compared with standard cytotoxic chemotherapy (4–6). The clinical activity of these agents is limited by drug resistance most commonly due to acquisition of a second *EGFR* mutation, *EGFR* T790M (7, 8). To overcome this mechanism of resistance, mutant-selective, irreversible *EGFR* inhibitors have been developed for patients with lung cancer after progression on an EGFR TKI with evidence of *EGFR* T790M (9).

Oncogenic mutations are typically identified in tumor tissue; however, recent advances in technology have resulted in the ability to perform "liquid biopsies" by assessing circulating free tumor DNA (cfDNA) within plasma. These techniques identify relevant genetic alterations from low levels of tumor-shed cfDNA within plasma (10); concordance between cfDNA plasma assays and tissue genotyping has been high (11–13). Limitations of plasma assays include decreased sensitivity compared with tissue testing, and results can be dependent on tumor volume and the sites of disease (14). Similar to tissue molecular genotyping, *EGFR* mutations identified within plasma serve as biomarkers that predict response to EGFR TKI treatment (12, 15, 16). Plasma assays of *EGFR* exon 19 deletions (ex19del), *EGFR* L858R, and T790M point mutations are now approved for clinical use (17). Quantitative changes in the *EGFR* mutations may also have predictive value. Failure to clear cfDNA-harboring *EGFR* mutations predicts shorter response and inferior outcomes with EGFR TKI treatment (15, 18). Similarly, a decline in the activating

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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doi: 10.1158/1078-0432.CCR-17-1447

Translational Relevance

EGFR tyrosine kinase inhibitors (TKI) have shown clinical activity in patients with non–small cell lung cancer (NSCLC) harboring EGFR activation mutations; however, acquired resistance often develops, limiting antitumor activity. ASP8273 is an irreversible, once-daily, orally available TKI with activity against both activating and resistance EGFR mutations. We assessed the clinical pharmacology as well as safety/tolerability and clinical response of ASP8273. In addition, utilizing cell-free DNA assay, we were able to confirm inhibition and track the emergence of drug resistance. ASP8273 was generally well tolerated with a linear pharmacokinetic profile and a recommended phase II dose of 300 mg. Patients with NSCLC harboring *EGFR*-T790M mutations had most pronounced antitumor activity following treatment with ASP8273, which was followed by variable reemergence of disease progression. As *EGFR* T790M–mediated resistance to first- and second-generation EGFR TKIs is the dominant resistance mechanism identified to date, these data provide insight into these mechanisms and support the need for further studies.

mutations and *EGFR* T790M occurs when treated with a third-generation EGFR TKI and can begin to rise again with the emergence of resistance (19).

ASP8273 is a small-molecule, irreversible, selective TKI that inhibits the kinase activity of mutant EGFR, including *EGFR* T790M. On the basis of preclinical activity, ASP8273 was evaluated in two separate phase I/II studies in patients with *EGFR*-mutant lung cancer in Japan and the United States; this article details the final results of the ASP8273 phase I study conducted in the United States.

Materials and Methods

Study oversight and design

This study was designed by the study sponsor in collaboration with the investigators and was conducted in accordance with the Declaration of Helsinki ethical principles, Good Clinical Practices, principles of informed consent, and requirements of public registration of clinical trials (ClinicalTrials.gov identifier, NCT02113813). Site-specific Institutional Review Boards approved the protocol. Written informed consent was obtained from each subject at enrollment.

This prospective, open-label, multicenter dose escalation phase I study was conducted across 10 sites in the United States and consisted of dose escalation (25–500 mg), response–expansion (100–400 mg), recommended phase II dose (RP2D; 300 mg), and food–effect (300 mg) cohorts (Supplementary Fig. S1). In the dose-escalation cohorts, subjects at each dose level (25–500 mg) were administered ASP8273 orally in a single-dose period (cycle 0; 2-day duration) followed by repeat-dose cycles consisting of once-daily treatment over 21 days (cycle 1 and subsequent cycles). Bayesian continual reassessment method (CRM) was used to guide the dose escalation or deescalation based on dose-limiting toxicity (DLT) incidence. Dose levels continued to be escalated using the dose escalation parameters until reaching the MTD (defined as the highest dose level at which the posterior mean DLT rate was <33%) or until establishing an RP2D dose. The starting dose level was 25 mg/day, and escalation increments of 100% were used until one patient experienced a DLT or 2 patients experienced a grade $\geq$2 drug-related adverse event at a given dose level during cycle 0 or 1. Thereafter, dose escalation increments were approximately 50%.

As the dose escalation cohorts were ongoing, additional subjects were enrolled in the response–expansion cohorts. The initial response–expansion cohort was opened if a partial or complete response at a dose level was observed or if pharmacokinetics data were in the efficacious range based on preclinical models, providing the dose level was deemed tolerable. Once the first response–expansion cohort was opened, each subsequent dose level also enrolled a response–expansion cohort after the dose level was cleared and deemed tolerable by the dose escalation committee. Each response–expansion cohort could have up to 6 patients. Any DLTs identified during the DLT period in a response–expansion cohort were included in the Bayesian CRM model to determine dose escalation and the MTD. The RP2D of ASP8273 was determined on the basis of consideration of safety, pharmacokinetic profile, and antitumor activity and was not to exceed the MTD.

Patients in the dose escalation cohorts who did not receive $\geq$80% of the planned doses of ASP8273 during cycle 1, for reasons other than treatment-related toxicity, were to be replaced and observed for signs of toxicity; however, no patients were replaced. Dose reductions were allowed in increments of 100 mg. Patients who did not experience a DLT continued on treatment until unacceptable toxicity, progression of disease, serious protocol deviation, or withdrawal of informed consent.

The RP2D cohort consisted of approximately 15 patients to assess ASP8273 antitumor activity and safety. Patients in the food–effect cohort were randomized to receive a single dose of the RP2D of ASP8273 at cycle 0 day 1 and cycle 0 day 4 under assigned food conditions, followed by repeated daily dosing of ASP8273 starting on cycle 1 day 1.

Patient selection

Patients had advanced (metastatic or unresectable) non–small cell lung cancer (NSCLC) harboring an *EGFR*-sensitizing mutation (e.g., ex19del, L858R) or an exon 20 insertion (ex20ins) and previous EGFR TKI treatment; the patients in the response–expansion, RP2D, and food–effect cohorts must have had an *EGFR* T790M mutation. Patients could not have symptomatic central nervous system metastases and could not require corticosteroid treatment. There was a 6-day washout of EGFR TKI therapy prior to study treatment initiation.

Endpoints and assessments

The primary endpoint was to assess the safety and tolerability of ASP8273 and to determine the MTD and/or the RP2D of ASP8273. Secondary endpoints included evaluating the antitumor activity of ASP8273 and determining the ASP8273 pharmacokinetic profile; exploratory endpoints included evaluation of potential biomarkers within cfDNA and tumor tissue.

During screening, patients underwent tumor imaging, including an MRI brain scan if indicated; restaging scans were obtained at 6-week intervals during treatment and were assessed according to the RECIST version 1.1. Adverse events (AE) were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 4.0. A DLT was defined as any grade 4 hematologic toxicity, grade 3 thrombocytopenia with

bleeding, or grade 3 febrile neutropenia. In addition, any grade ≥3 nonhematologic AE was considered a DLT with the exception of: (i) diarrhea, nausea, or vomiting that could be managed to grade ≤1 with supportive care; (ii) electrolyte abnormalities that did not recur and could be managed to grade ≤1; or (iii) grade 3 alanine transaminase/aspartate aminotransferase elevations that do not recur after drug is held.

Plasma samples were serially collected prior to study start and at each treatment cycle. cfDNA was extracted from plasma samples collected before and during treatment with ASP8273 and was analyzed by beads, emulsification, amplification, and magnetics (BEAMing) digital PCR for *EGFR* T790M, three coding variants of *EGFR* exon 19 deletions (2235-49D, 2236-50D, and 2240-57D), *EGFR* L858R, and *EGFR* C797S mutations. *EGFR* mutation status was also assessed centrally by reverse-transcriptase PCR using therascreen *EGFR* RGQ assay (Qiagen) in archival formalin-fixed, paraffin-embedded tissue and plasma samples obtained prior to treatment with ASP8273. *EGFR* mutation data were compared with tumor response data for utility as pharmacodynamic biomarker and to explore resistance mechanisms to ASP8273; concordance between tissue and plasma samples on *EGFR* mutation status was also investigated. These samples were not paired; archival tissue samples were collected any time after TKI failure, and plasma samples were collected on the first day of cycle 1.

Blood samples were also collected to assess the pharmacokinetic profile of ASP8273. During dose escalation, blood samples for pharmacokinetic analyses were collected for all subjects at various time points over a 48-hour period in cycle 0 and a 24-hour period in cycle 2. During response–expansion, blood samples for pharmacokinetic analyses were collected for all subjects at various time points over a 24-hour period on day 1 of cycles 1 and 2.

Statistical analysis

The sample size of the dose escalation cohort was dependent on the DLT incidence. The RP2D cohort sample size was based on toxicity, antitumor activity, and pharmacokinetic data and was based on a proposed response rate of 60% with a 95% confidence interval (CI) of 32%–84%. All patients who received ≥1 dose of study medication were included in the safety analysis set. Antitumor activity data were summarized for all patients who had both baseline and ≥1 postbaseline imaging assessment. Patients who discontinued study participation for any reason before the first radiographic assessment were counted as nonresponders. Progression-free survival (PFS) was estimated using the Kaplan–Meier method. The response rate was calculated using binomial proportions and exact 95% CIs. Pharmacokinetic and biomarker analyses were reported on all patients.

Results

From April 2014 to December 2015, 113 patients were enrolled at 10 centers in the United States; 110 patients received ≥1 dose of study drug across the dose escalation ($n = 36$), response–expansion ($n = 36$), RP2D ($n = 19$), and food–effect ($n = 19$) cohorts. The demographics and baseline disease characteristics of the 110 treated patients are listed in Table 1. All 110 patients dosed with ASP8273 were evaluable for safety and efficacy. Twelve patients did not have confirmed responses and were therefore not counted as responders. Of the 110 patients dosed with drug, only 93 had detectable mutant EGFR cfDNA in plasma to provide data for plasma/tissue concordance. Seventeen additional patients were excluded from the plasma response analysis because their plasma testing was negative, inadvertently not drawn, or not analyzed. Only 46 patients had EGFR cfDNA detected and sufficient plasma samples (>1) for longitudinal analysis.

Patients in the dose escalation cohorts received ASP8273 25 to 500 mg; no DLTs were observed from 25 to 200 mg. Four patients experienced a DLT; one in the 300 mg dose cohort and three in the 400 mg cohort: hyponatremia ($n = 2$), anorexia ($n = 1$), and diarrhea ($n = 1$). Although no patient in the 500 mg dose cohort had a DLT, 6 of 7 patients required dose interruptions or modifications due to treatment-emergent grade 3 AEs. On the basis of Bayesian CRM, the MTD was not reached, but the decision was made not to further dose escalate based on the toxicities identified

Table 1. Baseline demographics and disease characteristics (FAS)

	25 mg ($n = 1$)	50 mg ($n = 2$)	100 mg ($n = 12$)	200 mg ($n = 12$)	300 mg ($n = 63$)	400 mg ($n = 13$)	500 mg ($n = 7$)	Total ($n = 110$)
Median age, years (min, max)	82 (82, 82)	66 (55, 77)	68 (50, 85)	60 (38, 71)	64 (44, 81)	65 (55, 71)	64 (47, 72)	64 (38, 85)
Sex, *n* (%)								
Male	0	1 (50)	4 (33)	1 (8)	16 (25)	6 (46)	2 (29)	30 (27)
Female	1 (100)	1 (50)	8 (67)	11 (92)	47 (75)	7 (54)	5 (71)	80 (73)
Race, *n* (%)								
White	0	0	9 (75)	8 (67)	46 (73)	12 (92)	4 (57)	79 (72)
Black	0	0	1 (8)	1 (8)	6 (10)	1 (8)	0	9 (8)
Asian	1 (100)	2 (100)	2 (17)	2 (17)	9 (14)	0	2 (29)	18 (16)
Other	0	0	0	1 (8)	2 (3)	0	1 (14)	4 (4)
Prior EGFR TKI therapies for NSCLC, *n* (%)								
Erlotinib	1 (100)	2 (100)	12 (100)	11 (92)	54 (86)	12 (92)	7 (100)	99 (90)
Afatinib	1 (100)	0	2 (17)	4 (33)	12 (19)	4 (31)	0	23 (21)
Gefitinib	0	0	0	0	2 (3)	0	0	2 (2)
EGFR mutation status by local testing, *n* (%)								
Exon 19 deletion	0	1 (50)	8 (67)	9 (75)	33 (52)	8 (62)	3 (43)	62 (56)
Exon 21 L858R	1 (100)	1 (50)	4 (33)	2 (17)	15 (24)	3 (23)	2 (29)	28 (25)
Exon 18 G719x	0	0	0	0	4 (6)	0	0	4 (4)
Exon 20 insertion	0	0	0	0	1 (2)	1 (8)	0	2 (2)
T790M mutation status by local testing, *n* (%)								
Positive	1 (100)	0	10 (83)	8 (67)	58 (92)	6 (46)	5 (71)	88 (80)
Negative	0	2 (100)	1 (8)	3 (25)	1 (2)	4 (31)	2 (29)	13 (12)
Unknown	0	0	1 (8)	1 (8)	4 (6)	3 (23)	0	9 (8)

Abbreviation: FAS, full analysis set.

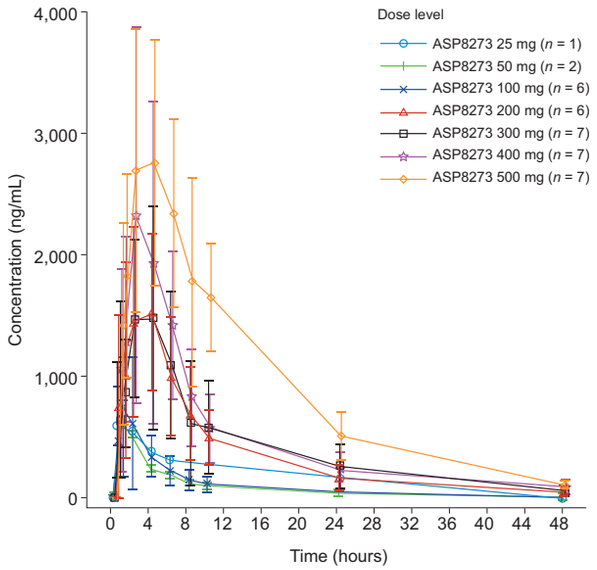

Figure 1.
Plasma concentration of ASP8273 in patients in the dose escalation cohorts after a single dose of ASP8273. The figure illustrates plasma concentrations of ASP8273 after a single dose over the subsequent 48 hours. Each colored line represents the mean concentration of ASP8273 at each time point in patients treated at a given dose level of ASP8273.

at the 500 mg dose level. All but one of the subjects tested for *EGFR* mutations in plasma had near elimination of *EGFR* T790M cfDNA in plasma by cycle 2 at all doses assessed (Supplementary Fig. S2). On the basis of the aggregate safety, antitumor effect, and pharmacokinetic data, the sponsor and study investigators set the RP2D 300 mg once daily.

Analyses of plasma samples confirmed that ASP8273 showed a linear pharmacokinetic profile and dose proportionality over the dose range of 100 to 500 mg (Fig. 1; Supplementary Table S1). Oral absorption was rapid with maximum concentrations ranging from 1 to 4 hours; elimination half-life of ASP8273 was 6 to 14 hours. Steady-state ASP8273 concentration was achieved by day 8 after once-daily dosing.

A total of 97% of patients ($n = 107/110$) had ≥1 treatment-emergent adverse event (TEAE); among these, 85% ($n = 93/110$) were considered treatment related. The most commonly reported TEAEs were diarrhea (47%), nausea (42%), and fatigue (32%; Table 2). Thirteen patients reported serious AEs that were considered related to study treatment, while 40 patients (36%) reported serious AEs not considered related to study drug. On the basis of central electrocardiogram review, no patient had changes from baseline QTcF values ≥60 msec or absolute QTcF values >480 msec. Ten patients died on study or during active follow-up, none of which were considered related to treatment. Of the 110 ASP8273-treated patients, 17 required a dose reduction [100 mg ($n = 1$), 300 mg ($n = 8$), 400 mg ($n = 6$), 500 mg ($n = 2$)], and 22 patients discontinued study therapy following a TEAE.

All 110 patients treated with ASP8273 were evaluable for response; antitumor activity was similar across dose levels of ASP8273 (Fig. 2A). The majority of patients had a decrease in the sum of their target lesions. At the time of data cutoff, February 10, 2016, 12 patients had disease assessments that were not confirmed, and 8 patients did not have scans. The overall response rate (ORR) across all doses was 28.2% ($n = 31/110$; 95% CI, 20–37.6); ORR at RP2D was 30.2% ($n = 19/63$; 95% CI, 19.2–43). In all patients, median PFS was 6 months (95% CI, 4.5–7.2 months); median PFS was 6.8 months (95% CI, 5.5–10.1 months; Fig. 2B) in the T790M-positive patients. In patients who harbored an *EGFR* T790M mutation, the response rate was 31% ($n = 18/58$; 95% CI, 19.5–44.5). In patients who were *EGFR* T790M negative, the response rate was 15.4% ($n = 2/13$; 95% CI, 1.9–45.4); median PFS for this population was 1.7 months (95% CI, 1.4–5.9 months).

Of the 110 patients enrolled, 93 (85%) with *EGFR* L858R or ex19del mutations were eligible for biomarker analysis of cfDNA. Mutant *EGFR* cfDNA was detectable in ≥1 plasma sample for 80 patients (86%); mutation concordance between cfDNA based on BEAMing detection and local tissue testing was 96% (95% CI, 80–99), 67% (95% CI, 54–78), and 79% (95% CI, 68–86) for *EGFR* L858R, ex19del, and T790M, respectively. Concordance between cfDNA based on BEAMing detection and central tissue testing was 100% (95% CI, 74–100), 71% (95% CI, 54–83), and 84% (95% CI, 69–92) for *EGFR* L858R, exon 19 deletion, and T790M, respectively.

Of the 93 patients eligible, 46 had detectable *EGFR* cfDNA and sufficient plasma samples for longitudinal analyses. In serially monitored patients who achieved a partial response (PR) as best overall response ($n = 19/46$, 41%) with ASP8273 (100–500 mg), treatment with ASP8273 consistently decreased *EGFR* activating and T790M mutations in cfDNA to near or below the level of detection after 1 cycle of treatment [<0.03% (ex19del/L858R); <0.04% (T790M)], and levels generally remained undetectable throughout the sustained PR. In serially monitored patients who achieved stable disease (SD) as best overall response ($n = 18/46$, 39%), *EGFR* activating and T790M mutations in cfDNA were generally reduced after 1 cycle of treatment; however, variability was observed with stable or increased levels of *EGFR*-activating mutations and T790M seen in some cases (Supplementary Fig. S3).

Of the 9 serially monitored patients who developed acquired resistance to ASP8273 (defined as progression after initial partial responses) for whom cfDNA data are available, *EGFR*-activating and T790M mutations reemerged in the plasma of 5 patients (Supplementary Fig. S4). In two of these cases, activating and T790M mutations decreased below detection during PR and remained below the limit of detection despite clinical disease progression. In one patient, T790M reemerged at the time of disease progression while the original activating mutation remained below the limit of detection; in another patient, the original activating mutation reemerged at the time of disease progression while T790M remained undetectable. BEAMing analysis for *EGFR* C797S was performed in a subset of 28 patients; C797S was detectable in 3 patients, all of whom had progression after initial PR. In those 3 patients, emergence of *EGFR* C797S coincided with reemergence of *EGFR* T790M and/or the activating *EGFR* mutation.

Discussion

This phase I study suggests that ASP8273 is tolerable and demonstrates antitumor activity in patients with *EGFR* T790M who have progressed on a prior EGFR TKI inhibitor. Toxicities seen with ASP8273 (e.g., diarrhea, nausea, and fatigue) are similar

Table 2. Adverse events of ASP8273 (25–500 mg) occurring in ≥15% of all patients (FAS)

Event, *n* (%)	25 mg (*n* = 1)	50 mg (*n* = 2)	100 mg (*n* = 12)	200 mg (*n* = 12)	300 mg (*n* = 63)	400 mg (*n* = 13)	500 mg (*n* = 7)	Total (*n* = 110)
Diarrhea								
Any grade	0	1 (50)	1 (8)	2 (17)	34 (54)	8 (62)	6 (86)	52 (47)
Grade ≥3	0	0	0	0	1 (2)	2 (15)	1 (14)	4 (4)
Nausea								
Any grade	0	1 (50)	3 (25)	7 (58)	24 (38)	7 (54)	4 (57)	46 (42)
Grade ≥3	0	0	1 (8)	0	1 (2)	1 (8)	0	3 (3)
Fatigue								
Any grade	0	0	4 (33)	3 (25)	14 (22)	10 (77)	4 (57)	35 (32)
Grade ≥3	0	0	0	1 (8)	1 (2)	1 (8)	0	3 (3)
Constipation								
Any grade	0	1 (50)	2 (17)	4 (33)	15 (24)	6 (46)	4 (57)	32 (29)
Grade ≥3	0	0	0	0	1 (2)	1 (8)	0	2 (2)
Vomiting								
Any grade	0	1 (50)	1 (8)	3 (25)	14 (22)	4 (31)	3 (43)	26 (24)
Grade ≥3	0	0	1 (8)	0	0	0	0	1 (1)
Hyponatremia								
Any grade	0	0	3 (25)	3 (25)	13 (21)	2 (15)	4 (57)	25 (23)
Grade ≥3	0	0	3 (25)	2 (17)	8 (13)	2 (15)	4 (57)	19 (17)
Decreased appetite								
Any grade	0	1 (50)	0	3 (25)	9 (14)	5 (38)	4 (57)	22 (20)
Grade ≥3	0	0	0	0	0	1 (8)	0	1 (1)
Dyspnea								
Any grade	0	1 (50)	1 (8)	4 (33)	10 (16)	5 (38)	1 (14)	22 (20)
Grade ≥3	0	0	1 (8)	0	2 (3)	0	0	3 (3)
Headache								
Any grade	0	0	2 (17)	5 (42)	11 (17)	2 (15)	2 (29)	22 (20)
Grade ≥3	0	0	0	1 (8)	0	0	0	1 (1)
Cough								
Any grade	0	1 (50)	1 (8)	4 (33)	11 (17)	4 (31)	0	21 (19)
Grade ≥3	0	0	0	0	0	0	0	0
Dizziness								
Any grade	0	0	1 (8)	4 (33)	14 (22)	1 (8)	1 (14)	21 (19)
Grade ≥3	0	0	0	0	1 (2)	0	0	1 (1)
Dry mouth								
Any grade	0	0	0	2 (17)	9 (14)	4 (31)	3 (43)	18 (16)
Grade ≥3	0	0	0	0	0	0	0	0
Paresthesia								
Any grade	0	0	0	1 (8)	12 (19)	5 (38)	0	18 (16)
Grade ≥3	0	0	0	0	0	0	0	0

Abbreviation: FAS, full analysis set.

to other drugs in class. Hyponatremia and paresthesias/neuropathy may occur more with ASP8273 compared with other drugs in class; neuropathies were not reported in the osimertinib or rociletinib phase I studies as drug-related AEs ≥10% (9, 20). Hyponatremia is common in the metastatic lung cancer patient population; however, in several cases, it was temporally considered related to drug initiation and resolved with discontinuation of study drug, suggesting it is a unique toxicity seen with ASP8273.

ASP8273 demonstrated antitumor activity in patients with *EGFR*-mutant lung cancers after prior treatment with EGFR TKIs. This study identified the ASP8273 RP2D to be 300 mg daily based on pharmacokinetics, pharmacodynamics, safety, and antitumor activity; the MTD was not established. The ORR was similar across studied dose levels; ASP8273 had most pronounced activity in patients with lung cancers harboring *EGFR* T790M, with a 31% ORR in this population. Some patients with *EGFR* T790M–negative lung cancers appeared to have benefited: 15.4% ($n = 2/13$) of *EGFR* T790M–negative patients had a PR, and 23.1% ($n = 3/13$) had SD as their best response. In this initial phase I study, the ORR in patients with *EGFR* T790M–positive disease was lower than the response rate seen in the phase I study of osimertinib, the currently approved *EGFR* T790M inhibitor (9).

The ability to detect EGFR mutations in cfDNA is of significant clinical importance. The recent approvals of plasma based tests for EGFR mutations in NSCLC demonstrate the novel clinical application of these technologies. Furthermore, the ability to monitor response and resistance to EGFR-targeted therapies constitutes an important future use of these approaches. A unique aspect of this study is the utilization of cfDNA as a biomarker to confirm inhibition of the drug target, predict response to treatment, and track emergence of drug resistance. Across all ASP8273 doses, ASP8273 decreased circulating *EGFR* T790M cfDNA to below the level of detection, confirming successful on-target inhibition. The presence of *EGFR* T790M in cfDNA predicted response to ASP8273 treatment and was highly correlated with the identification of *EGFR* T790M in tumor tissue; ASP8273 response rate was identical for patients who had *EGFR* T790M identified from plasma versus tumor tissue. In patients who responded to ASP8273, *EGFR* T790M and the *EGFR*-activating mutation within cfDNA were inhibited to undetectable levels that variably reemerged with disease progression. There were several patterns of progression identified; some patients had reemergence of both the activating *EGFR* mutation and T790M cfDNA; some patients had continued suppression of both mutations despite clinical

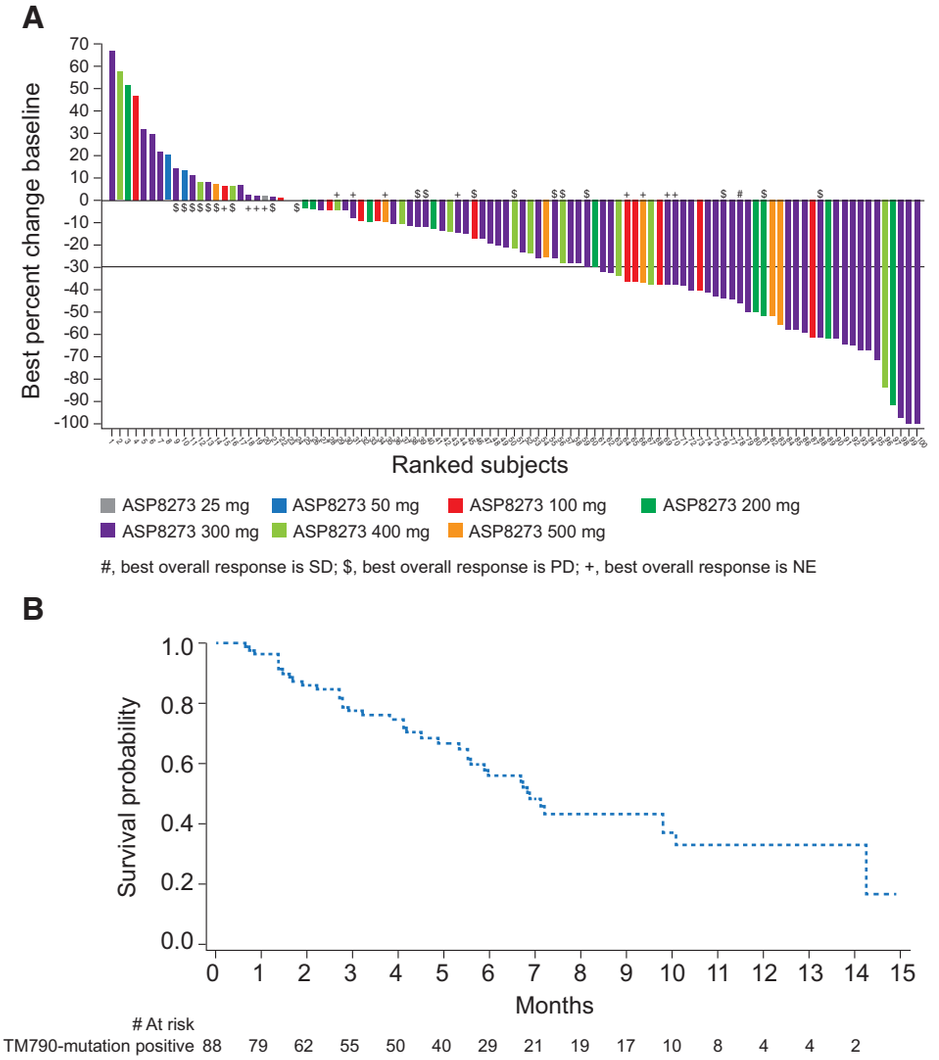

Figure 2.

A, Best percentage change in target lesions. Waterfall plot for best percent change in size of target lesions is shown for all patients. The color key indicates the daily dose of ASP8273. The solid line at −30% represents the boundary for determination of partial response. **B,** Progression-free survival. Kaplan–Meier estimates of progression-free survival in patients with *EGFR* T790M–positive, metastatic non–small cell lung cancer who received ASP8273 at doses of 25 to 500 mg orally daily; median progression-free survival was 6.8 months (95% CI, 5.5–10.1).

progression, while others had emergence of either *EGFR* T790M or activating *EGFR* mutation cfDNA. A few patients acquired an *EGFR* C797S mutation in plasma, which has previously been reported as a resistance mechanism in patients treated with osimertinib (19, 21). Various limitations, however, exist that must be addressed to further refine the use of these approaches in both clinical research and clinical practice. In this study, our ability to assess for novel mechanisms of resistance to ASP8273 in cfDNA was limited by our use of a digital PCR assay focused on recurring mutations in *EGFR*; further discovery efforts could leverage evolving technologies for next-generation sequencing of cfDNA (22). In addition, findings in this study demonstrate some cases where decreases in EGFR cfDNA occurred in the absence of observed reductions in tumor burden. Furthermore, in many cases, the depth of tumor response is not well correlated with EGFR cfDNA. Further research is needed to understand the association between cfDNA findings and mechanisms of resistance to ASP8273 therapy. Similarly, a greater understanding of the relationship between cfDNA and tumor burden or other clinical parameters will further enable the use of these technologies in these novel applications.

EGFR T790M–mediated resistance to first- and second-generation EGFR TKIs is the dominant resistance mechanism identified to date. Currently, osimertinib is the only drug approved for this indication; in the published phase I study, osimertinib had a high response rate and long median PFS on treatment (9). Compared with osimertinib, ASP8273 appears to have less significant skin toxicity (e.g., dry skin, rash, and pruritus). In addition, pneumonitis has been observed with treatment with osimertinib and, to date, there has not been a case of pneumonitis with ASP8273. Furthermore, early data suggest that these T790M inhibitors may be sequenced with additive benefit (23).

ASP8273, and the other third-generation EGFR TKIs, have demonstrated activity in pretreated patients. Studies that assess whether these agents are superior as an initial treatment option for patients with EGFR-mutant lung cancers are warranted. ASP8273 may have antitumor activity in patients with *EGFR* T790M–negative disease, and further study will be required to understand the appropriate population to treat with ASP8273. The purpose of utilizing these agents in the first-line setting would be to prevent *EGFR* T790M–mediated resistance from developing.

Disclosure of Potential Conflicts of Interest

H.A. Yu is a consultant/advisory board member for AstraZeneca. L. Horn is a consultant/advisory board member for Abbvie, AstraZeneca, Bayer, Bristol-Myers Squibb, Janssen, Lilly, Merck, Roche-Genentech, and Xcover. J. Weiss reports receiving commercial research grants from Astellas and AstraZeneca. H. West reports receiving speakers bureau honoraria from Genentech/Roche and is a consultant/advisory board member for Astellas, AstraZeneca, Boehringer-Ingelheim, and Genentech/Roche. R.J. Kelly is a consultant/advisory board member for Astellas. G.R. Oxnard is a consultant/advisory board member for AstraZeneca, Guardant, Inivata, Novartis, Sysmex, and Takeda. No potential conflicts of interest were disclosed by the other authors.

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Acknowledgments

We would like to acknowledge all investigators, coordinators, and study site personnel, as well as patients and their families for their participation in this study. This research was sponsored by Astellas Pharma, Inc. Financial support for the development of this manuscript, including writing and editorial assistance under the authors' guidance, was provided by Regina Switzer, Ph.D., of SuccinctChoice and was funded by the study sponsor.

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Received May 25, 2017; revised August 18, 2017; accepted September 20, 2017; published OnlineFirst September 27, 2017.

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