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Clinical Trials: Targeted Therapy

Clinical Cancer Research

Ensartinib (X-396) in ALK-Positive Non–Small Cell Lung Cancer: Results from a First-in-Human Phase I/II, Multicenter Study

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Abstract

Purpose: Evaluate safety and determine the recommended phase II dose (RP2D) of ensartinib (X-396), a potent anaplastic lymphoma kinase (ALK) tyrosine kinase inhibitor (TKI), and evaluate preliminary pharmacokinetics and antitumor activity in a first-in-human, phase I/II clinical trial primarily in patients with non–small cell lung cancer (NSCLC).

Patients and Methods: In dose escalation, ensartinib was administered at doses of 25 to 250 mg once daily in patients with advanced solid tumors; in dose expansion, patients with advanced *ALK*-positive NSCLC were administered 225 mg once daily. Patients who had received prior ALK TKI(s) and patients with brain metastases were eligible.

Results: Thirty-seven patients enrolled in dose escalation, and 60 enrolled in dose expansion. The most common treatment-related toxicities were rash (56%), nausea (36%), pruritus (28%), vomiting (26%), and fatigue (22%); 23% of patients experienced a treatment-related grade 3 to 4 toxicity (primarily rash and pruritus). The maximum tolerated dose was not reached, but the RP2D was chosen as 225 mg based on the frequency of rash observed at 250 mg without improvement in activity. Among the *ALK*-positive efficacy evaluable patients treated at $\geq$200 mg, the response rate (RR) was 60%, and median progression-free survival (PFS) was 9.2 months. RR in ALK TKI-naïve patients was 80%, and median PFS was 26.2 months. In patients with prior crizotinib only, the RR was 69% and median PFS was 9.0 months. Responses were also observed in the central nervous system, with an intracranial RR of 64%.

Conclusions: Ensartinib was active and generally well tolerated in patients with *ALK*-positive NSCLC. *Clin Cancer Res; 24(12); 2771–9. ©2018 AACR.*

Introduction

Chromosomal rearrangements involving the gene encoding the anaplastic lymphoma kinase (ALK) are detected in 3% to 8% of non–small cell lung cancers (NSCLC; refs. 1–5). The resultant ALK fusion proteins are a validated therapeutic target in NSCLC, and testing for ALK is now the accepted standard of care in the United States. Crizotinib was the first ALK inhibitor approved to treat patients with locally advanced or metastatic NSCLC whose tumors express ALK fusion proteins. Results from two phase III studies in previously treated or untreated patients with advanced, ALK-positive NSCLC showed that crizotinib, compared with chemotherapy, significantly prolonged progression-free survival (PFS; median treated 7.7 vs. 3.0 months and untreated 10.9 vs. 7.0 months) and increased response rates [RRs (65% vs. 20% treated and 74% vs. 45% untreated; refs. 6, 7)]. Despite experiencing initial responses, the majority of patients eventually had disease progression, typically in less than 12 months, and new strategies to overcome resistance remain an area of active investigation (8, 9).

Mechanisms of acquired resistance to crizotinib include both "on-target" genomic alterations, including mutations in the ALK tyrosine kinase domain and amplification of the *ALK* fusion, as well as activation of bypass signaling pathways, including EGFR, IGF-1R, c-KIT, and SRC. Additionally, in a retrospective analysis of

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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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Translational Relevance

The anaplastic lymphoma kinase (ALK) has become a validated therapeutic target in patients with non–small cell lung cancer (NSCLC), with approved tyrosine kinase inhibitors (TKI) in both the first- and second-line settings. Despite experiencing initial responses, most patients will become resistant and have disease progression. Mechanisms of resistance include secondary *ALK* mutations, amplification of *ALK*, and activation of bypass signaling networks. Additionally, a common site of disease progression is within the central nervous system (CNS). Thus, ensartinib was developed to improve on the target specificity and binding, as well as to improve activity in the CNS. This first-in-human phase I/II study evaluated the safety and efficacy of ensartinib in advanced *ALK*-positive NSCLC. Ensartinib was generally well tolerated, with rash being the most commonly observed toxicity, and demonstrated good clinical activity in patients who had received a prior ALK TKI, those who were ALK TKI-naïve, and those with CNS disease.

two clinical trials, the central nervous system (CNS) was the most common site of progression in patients receiving crizotinib (10). Thus, the development of second-generation ALK inhibitors has primarily focused on improved binding to the ALK kinase domain and improved CNS activity.

Ensartinib (X-396) is a novel, aminopyridazine-based small molecule that potently inhibits ALK. Ensartinib is 10-fold more potent than crizotinib at inhibiting the growth of *ALK*-positive lung cancer cell lines (11). Additionally, ensartinib potently inhibited *ALK* fusions engineered to have point mutations, L1196M and C1156Y, that are associated with crizotinib resistance. Ensartinib also demonstrated potent antitumor activity in H3122 lung cancer xenografts that harbored the *EML4–ALK* E13; A20 fusion, with favorable pharmacokinetic (PK) and safety profiles (11).

A phase I/II trial was conducted in patients with ALK-positive NSCLC, where the primary objectives were to evaluate the safety and determine the recommended phase II dose (RP2D). Key secondary objectives included characterizing preliminary PK and antitumor activity.

Patients and Methods

Biochemical kinase activity and selectivity

Biochemical assays were performed by Reaction Biology Corporation according to the procedures as previously described (12).

Distribution studies

Biodistribution studies were conducted in four groups of Sprague-Dawley rats (3 females and 3 males) treated orally with ensartinib at 50 mg/kg. Mice were sacrificed at 0.5, 4, 12, and 24 hours postdose to quantify concentration of ensartinib in the plasma and the skin. All animal experiments were reviewed and approved by the animal review board.

Study population

Eligible patients in dose escalation had advanced solid tumors. Dose expansion was limited to patients with advanced NSCLC whose tumors were positive for *ALK* as determined by fluorescence *in situ* hybridization (FISH) or immunohistochemistry (IHC) from local testing. *ALK* FISH-positive was defined as the FDA-cleared recommended threshold of 15% cells showing split signal; a second confirmatory test was required if the positive signal was between 10% and 15%. For *ALK* positivity by IHC, strong granular cytoplasmic staining (3+) was required. *ALK* rearrangements were confirmed centrally via FISH for all patients. Additional eligibility criteria included age $\geq$18 years, an Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 1, adequate organ function and, in dose expansion, measurable disease according to Response Evaluation Criteria in Solid Tumors (RECIST), v1.1. Prior therapy with crizotinib and/or second-generation ALK tyrosine kinase inhibitors (TKI) was allowed without a limit on prior therapies. A minimum washout period of 10 days was required between termination of treatment and administration of ensartinib. However, in the case of ALK TKIs, a 5-day window was allowed. Patients with asymptomatic CNS lesions were allowed. CNS lesions with evidence of tumor growth at least 1 month post whole brain radiation therapy (WBRT) can be target lesions as evidenced by gadolinium-enhanced MRI. Target lesions may not have been treated with stereotactic radiosurgery (SRS). Additional eligibility criteria are included in the Supplementary Methods.

This study was approved by the review boards at all participating institutions, and all subjects provided written informed consent. The study was conducted in accordance with good clinical practice and the Declaration of Helsinki and its amendments and was registered through ClinicalTrials.gov (NCT01625234).

Study design

Dose escalation used an accelerated titration scheme to determine the maximum tolerated dose. The starting dose of ensartinib was 25 mg administered orally once daily without food and was continuously doubled until 1 patient experienced a drug-related grade $\geq$2 adverse event (AE). At that point, accelerated titration was stopped and a 3 + 3 design was used for further dose escalation. In order to collect sufficient PK data, additional patients could be enrolled at lower doses during dose escalation. Once the RP2D was determined, the safety and antitumor activity of ensartinib were further evaluated in dose expansion. Cycle length was 28 days.

A dose limiting toxicity (DLT) was defined as any of the following ensartinib-related events that occurred during the first treatment cycle: grade 4 neutropenia persisting for >5 days or febrile neutropenia, grade 4 thrombocytopenia or grade 3 thrombocytopenia with bleeding, grade $\geq$3 nonhematologic toxicity with the exception of grade 3 rash, diarrhea, nausea, or vomiting if controlled and resolved within 48 hours, or a treatment delay of $\geq$14 days due to unresolved toxicity.

Dose expansion included five cohorts: (i) patients who were ALK TKI-naïve, (ii) patients who progressed on prior crizotinib and had not received other ALK TKIs, (iii) patients who had progressed on at least one second-generation ALK TKI and may or may not have had prior crizotinib, (iv) patients with CNS metastases, where at least one target lesion was $\geq$3 mm in diameter, and (v) patients with leptomeningeal disease.

Study assessments

Safety. AEs were graded according to the National Cancer Institute Common Terminology for Adverse Events, version 4.03. All

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patients who developed a DLT or received ≥80% of the planned doses during cycle 1 and completed the required safety evaluations were evaluable for the DLT assessment. Patients removed from the study during the first cycle not meeting these criteria were replaced. To test the difference in frequency and severity of specific gastrointestinal toxicities (e.g., nausea and vomiting) between a fed and fasted state, a subset of patients was assigned to take ensartinib with or without food during cycle 1. After the first cycle, patients were allowed to choose how they preferred to take ensartinib.

Pharmacokinetics. On days 1 and 22 of cycle 1, plasma samples were obtained from all patients in dose escalation and a subset of patients in the dose expansion phase before dosing of ensartinib, and at 0.5, 1, 2, 3, 4, 6, and 8 hours after dosing. Plasma samples were also collected prior to dosing on days 2, 8, and 15 of cycle 1. Plasma concentration–time profiles and PK parameters for ensartinib were obtained. To explore the potential effect of food on ensartinib absorption, half of the patients in dose expansion phase were instructed to take the daily dose with food while the other half took the study medication under fasting conditions during cycle 1. After cycle 1, the patients were allowed to choose.

Efficacy. At baseline, all patients underwent tumor imaging with CT and, if appropriate, MRI. Brain imaging was required for patients with known or suspected brain metastases. Restaging scans, both CT and, if applicable, MRI, were obtained at approximately 8-week intervals. Patients entered into the leptomeningeal disease cohort also had an MRI of the brain and spine at cycle 2 day 1. Systemic disease was assessed according to RECIST v1.1 by the investigator, although patients with clinical progression were considered to have disease progression in the absence of objective progression (13). Response for CNS metastases was also evaluated based on RECIST criteria, with some modifications (e.g., target lesion may be ≥3 mm).

Statistical analysis

All patients who received at least one dose of ensartinib were evaluable for safety, and patients who completed at least one cycle of treatment at a dose ≥200 mg and had a post-baseline response assessment were evaluable for efficacy. Confidence intervals were calculated using the Wilson method, and PFS and duration of response (DOR) were estimated using Kaplan–Meier methodology. PFS was calculated from the on-treatment date to the first date disease progression was observed. DOR was calculated from the first date a tumor response was observed per RECIST v1.1 to the first date of disease progression. For those patients that remained on study at the time of data pull, PFS and DOR were censored at the data cutoff date of February 15, 2017. Enrollment in dose expansion is ongoing.

Results

Biochemical kinase activity and selectivity and distribution studies

Ensartinib was tested against wild-type *ALK* and 17 *ALK* variants in the Reaction Biology panel. Ensartinib potently inhibited all evaluated *ALK* variants, with *in vitro* IC_{50} values <4 nmol/L. The wild-type and F1174, C1156Y, L1196M, S1206R, and T1151 mutants are particularly sensitive to ensartinib, with IC_{50} values of <0.4 nmol/L, while the IC_{50} for the G1202R mutant is approximately 10-fold higher (3.8 nmol/L). Besides ALK, ensartinib also potently inhibits (IC_{50} <1 nmol/L) TPM3-TRKA, TRKC, and GOPC-ROS1. Kinases inhibited at higher concentrations (IC_{50}: 1–10 nmol/L) are EphA2, EphA1, EphB1, and c-MET (Supplementary Table S1).

The mean concentration of ensartinib in plasma and skin at multiple time points postdose is listed in Supplementary Table S2. At 12 hours after a single dose, the concentration of ensartinib was 9.0 × higher in the skin than in the plasma.

Patient characteristics

As of February 15, 2017, 97 patients (37 in dose escalation and 60 in dose expansion) were enrolled across 13 sites in the United States (Fig. 1). Baseline demographics are presented in Table 1. The median age was 56 (range, 21–83). Eighty (82%) patients had received at least one prior systemic therapy for advanced disease, including chemotherapy and ALK TKIs, while 58 (60%) patients had ≥2 previous lines of therapy. Thirty-five (36%) patients had brain metastases at baseline. Fourteen patients had not received any prior radiation, 10 patients had prior stereotactic radiosurgery (SRS), and 11 patients had prior WBRT. The majority (92%) of patients had NSCLC; in dose escalation, 4 patients had head and neck cancer, 2 had colorectal cancer and 1 patient each had small cell lung and breast cancer. Note that only 1 patient with leptomeningeal disease was enrolled as of the cutoff date and that patient discontinued drug due to an unrelated AE and was not evaluable for response. No further efficacy data for that patient will be presented.

Adverse events

Dose escalation proceeded according to Fig. 1, where patients received 25 to 250 mg of ensartinib once daily. Two patients experienced DLTs. One patient at 200 mg had grade 3 fluid overload and grade 1 elevated troponin I, and another at 250 mg had grade 3 erythematous rash. All DLTs resolved after holding treatment, and treatment was resumed in both patients at a lower dose level. Although 250 mg was tolerable, the recommended phase II dose of ensartinib was chosen to be 225 mg based on the higher frequency of grade 3 rash observed at 250 mg without improvement in clinical activity. At the end of dose escalation, 6 of 10 patients in the 250 mg cohort experienced some form of rash (2 had grade 3 events), whereas 2 of the 3 patients in the 225 mg cohort experienced a rash (no grade 3 events). When this decision was made, 4 patients had a partial response in the 250 mg cohort, while all 3 patients in the 225 mg had a partial response. An example of the rash is depicted in Supplementary Fig. S1.

Treatment-related AEs, as reported by the investigator, occurred in 83 of 97 (86%) patients. The most common treatment-related AEs were rash (56%), nausea (36%), pruritus (28%), vomiting (26%), and fatigue (22%); the majority of these were grade 1–2 (Table 2). Treatment-related grade 3 to 4 AEs occurred in 22 of 97 patients (Table 2, 23%). The frequency and severity of nausea and vomiting were less when ensartinib was taken with food. In the 47 patients that received either 200 or 225 mg doses and fasted prior to taking ensartinib during cycle 1, 24 (38%) experienced nausea or vomiting during cycle 1 with five events being grade 2. Of the 27 patients that received either 200 or 225 mg doses and took ensartinib with food during cycle 1, 7 patients (26%) experienced nausea or vomiting, and all events were grade 1. All patients who received 250 mg doses were fasted and thus not included in the above analysis as to not skew the results.

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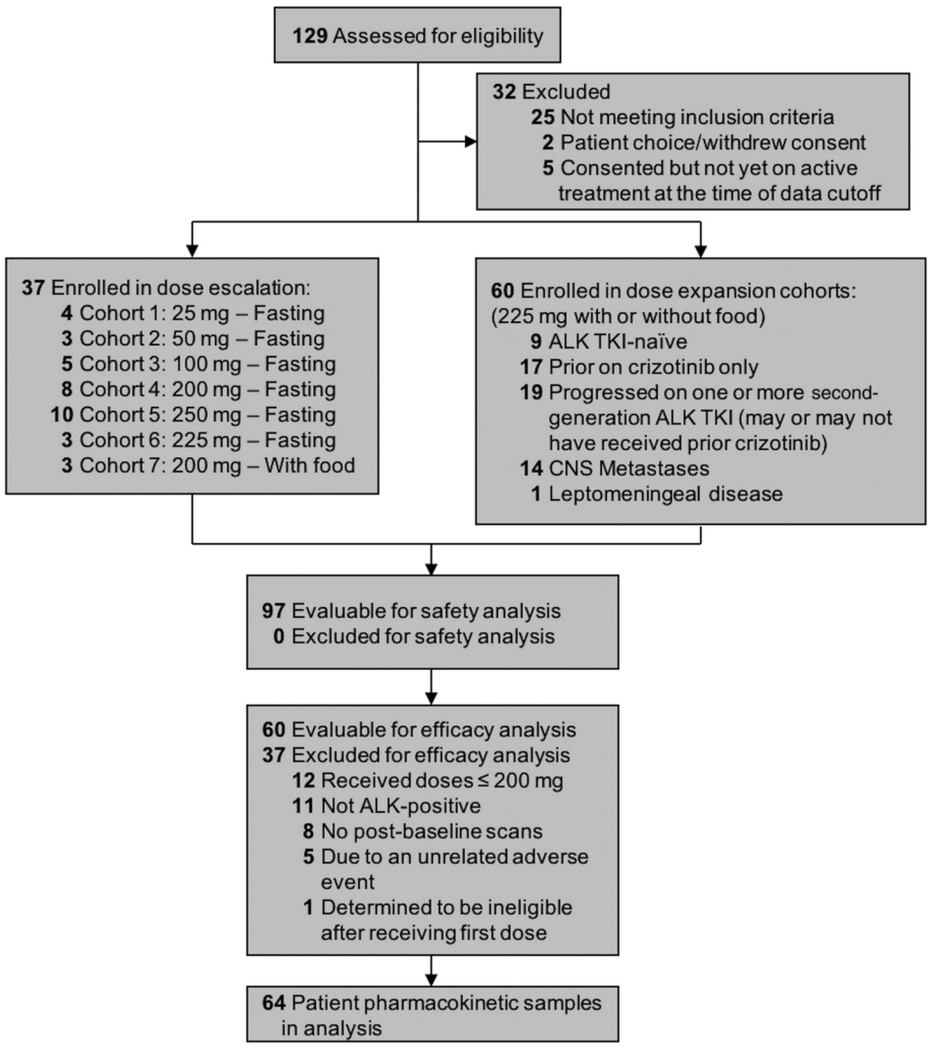

Figure 1.
CONSORT diagram. Clinical trial flow diagram that depicts the number of patients who provided consent, received study therapy in both dose escalation and expansion, and were evaluable for safety and response. The diagram also depicts the number of pharmacokinetic samples analyzed.

Fourteen patients (14%; 14/97) required at least one dose reduction and 15 patients (15%; 15/97) required at least one dose interruption due to an ensartinib-related toxicity. Of the 64 patients that received 225 mg doses of ensartinib, 16 (25%) required at least one dose reduction and 13 (20%) required at least one dose interruption due to a treatment-related toxicity. Ensartinib was permanently discontinued in 5 patients (5.2%) due to a toxicity considered related by the investigator: thrombotic microangiopathy ($n = 1$), hyperbilirubinemia ($n = 1$), and rash ($n = 3$). Five patients died ($n = 3$, respiratory failure; $n = 1$, disease progression; $n = 1$ stroke) while on study, though no deaths were considered by the sponsor to be related to ensartinib.

Pharmacokinetics

PK data were obtained from 36 patients in dose escalation and 28 in dose expansion. Under a fasted state, the mean day 22 C_{max} and area under the curve (AUC) increased dose proportionally between 50 and 200 mg, slightly more than dose proportionally from 200 to 225 mg, and significantly more than dose proportionally from 225 to 250 mg (AUC increased by 36% with an 11% increase in dose). Supplementary Fig. S2 shows the mean concentration–time curves for patients at 225 mg on days 1 and 22. For fasted patients, the mean C_{max} and AUC on day 22 are 2.3- and 3.2-fold higher, respectively, than those on day 1, suggesting significant accumulation. The key PK parameters (Supplementary Table S3) at the RP2D of 225 mg show that food (consumed within 30 minutes after taking ensartinib) has minimal impact on the absorption of ensartinib.

Efficacy

Tumor response. Ninety-seven patients were enrolled on study, of whom 12 were treated at doses <200 mg, 11 were *ALK*-negative, 8 (2 with clinical progression, 2 were recently enrolled in dose expansion, 1 requested to discontinue ensartinib, and 3 unrelated deaths before follow-up scans) had not had their first post-baseline imaging, 5 discontinued drug prior to 28 days due to unrelated AEs, and 1 patient was determined ineligible after receiving the first dose. The patients included in the efficacy evaluable group were those patients with at least one post-baseline response assessment and received ≥200 mg doses of

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Table 1. Patient demographic and clinical characteristics

Characteristic	Patients ($n = 97$)	ALK-positive evaluable[a] patients ($n = 60$)
Sex, *n* (%)		
Male	48 (49)	28 (47)
Female	49 (51)	32 (53)
Age, y		
Median	56	55
Range	21–83	21–80
Race, *n* (%)		
White	74 (76)	47 (78)
Black/African American	5 (5)	1 (2)
Asian	13 (13)	8 (13)
American Indian/Alaska Native	1 (1)	0
Other/unknown	4 (4)	4 (7)
Tumor type, *n* (%)		
NSCLC	89 (92)	60 (100)
Head and neck	4 (4)	0
Colorectal	2 (2)	0
Small cell	1 (1)	0
Breast	1 (1)	0
ECOG performance status, *n* (%)		
0	31 (32)	24 (40)
1	66 (68)	36 (60)
Smoking history, *n* (%)		
Current	4 (4)	2 (3)
Former	38 (39)	21 (35)
Never	55 (57)	37 (62)
Number of prior treatments, *n* (%)		
0	17 (18)	13 (22)
1	22 (23)	13 (22)
2	21 (22)	13 (22)
3	10 (10)	6 (10)
≥4	27 (28)	15 (25)
Prior ALK TKI treatment, *n* (%)		
ALK TKI naïve	35 (36)	15 (25)
Prior crizotinib only	41 (42)	29 (48)
Prior crizotinib and ceritinib	12 (12)	8 (13)
Prior crizotinib and alectinib	1 (1)	1 (2)
Prior crizotinib, ceritinib, and alectinib	7 (7)	6 (10)
Prior crizotinib, ceritinib, and brigatinib	1 (1)	1 (2)
Positive for ALK genomic alterations, *n* (%)	79 (81)	60 (100)
Brain metastases, *n* (%)		
Both target and nontarget lesions	11 (11)	9 (15)
Target lesions only	8 (8)	5 (8)
Nontarget lesions only	16 (16)	15 (25)
Prior radiation, *n* (% CNS patients)	$n = 35$	$n = 29$
None	14 (40)	12 (41)
Prior WBRT	11 (31)	9 (31)
Prior SRS	10 (29)	8 (28)

[a]Efficacy evaluable: Patients at ≥200 mg who completed 1 cycle of treatment and had a post-baseline response assessment.

ensartinib. A range of doses was chosen to include those patients who were treated at the RP2D (i.e., 225 mg), those who received the maximum evaluated dose of 250 mg, and those who received 200 mg to demonstrate that clinical activity was still observed at the dose level corresponding to the first dose level reduction from the RP2D.

For the 60 evaluable *ALK*-positive patients, 36 patients had a partial response (PR), a RR of 60% [95% confidence interval (CI), 47.4–71.4]; 13 achieved stable disease (SD) after at least 2 cycles of treatment, a disease control rate (DCR) of 81.7%, and 11 had progressive disease (PD) as best response. Figure 2 shows that tumor regression occurred in a majority (80.0%) of patients. Of the 15 ALK TKI-naïve patients, 12 had a PR, a RR of 80.0% (95% CI, 54.8–93.0), and 1 had SD, a DCR of 86.7%. Interestingly, the 2 ALK TKI-naïve patients with PD were FISH-positive via local testing, but negative when analyzed using NGS. Among the 29 patients with crizotinib as their only prior ALK TKI, 20 achieved a PR, a RR of 69.0% (95% CI, 50.8–82.7), and 8 had SD, a 96.6% DCR. For those with prior crizotinib and at least one second-generation ALK TKI ($n = 16$), four had a PR, a RR of 25% (95% CI, 10.2–49.5), and 4 had SD, a 50.0% DCR. A waterfall plot showing best tumor response for this cohort is included in Supplementary Fig. S3, which is color coded to represent which second-generation ALK TKIs that patient received. The median (range) of washout from prior ALK TKI therapy for the 24 responders that had at least one prior ALK TKI was 25 days (5–721 days).

In the 14 patients with baseline CNS target lesions, 2 achieved complete intracranial response (both had not received prior radiation), and 7 had an intracranial PR (5 had not received prior radiation and 2 had received prior WBRT), a RR of 64.3% (95% CI, 38.8–83.7). Additionally, 4 patients exhibited intracranial SD (1 without prior radiation and 3 with prior SRS), a 92.9% intracranial DCR. Figure 3 shows that intracranial tumor regression occurred in a majority (78.6%) of these patients.

Progression-free survival and duration of response. The overall median PFS for the *ALK*-positive evaluable patients was 9.2 months (Fig. 4A; 95% CI, 5.6–11.7). In the ALK TKI-naïve patients, the median PFS was 26.2 months (Fig. 4B; 95% CI, 9.2–not estimable). Of the patients with prior crizotinib only, the median PFS was 9.0 months (Fig. 4B; 95% CI, 5.6–11.7). Within the subgroup that received prior crizotinib and a second-generation ALK TKI, the median PFS was 1.9 months (Fig. 4B; 95% CI, 1.7–5.7).

The median DOR for *ALK*-positive evaluable patients ($n = 35$) at data cutoff was 12.8 months (95% CI, 5.6–24.4). Note that 1 responding patient was not included because the patient had not yet had a confirmatory scan. The median DOR for ALK TKI-naïve patients ($n = 12$) was 24.4 months (95% CI, 7.6–not estimable). Of those with prior crizotinib only ($n = 19$), the median DOR was 7.4 months (95% CI, 3.7–12.9). In the subgroup treated with crizotinib and a second-generation ALK TKI ($n = 4$), the median DOR was 4.4 months (95% CI, 0.4–not estimable). Among the

Table 2. Treatment-related AEs[a] reported in ≥10% of patients ($n = 97$)

Adverse event	All grades	Grade ≥3[b]
Rash	54 (56%)	12 (12%)
Nausea	35 (36%)	1 (1%)
Pruritus	27 (28%)	5 (5%)
Vomiting	25 (26%)	1 (1%)
Fatigue	21 (22%)	2 (2%)
Decreased appetite	18 (19%)	1 (1%)
Edema	15 (15%)	1 (1%)
Dry skin	14 (14%)	1 (1%)
Elevated aspartate aminotransferase	12 (12%)	1 (1%)
Constipation	11 (11%)	0
Diarrhea	11 (11%)	0

[a]As reported by the investigator.

[b]Other related grade 3 events reported were: lymphocytopenia ($n = 3$), dehydration ($n = 1$), anemia ($n = 1$), dizziness ($n = 1$), hyponatremia ($n = 1$), burning sensation ($n = 1$), hypertension ($n = 1$), extremity pain ($n = 1$), and urinary tract infection ($n = 1$). One grade 4 event, thrombotic microangiopathy, was reported ($n = 1$).

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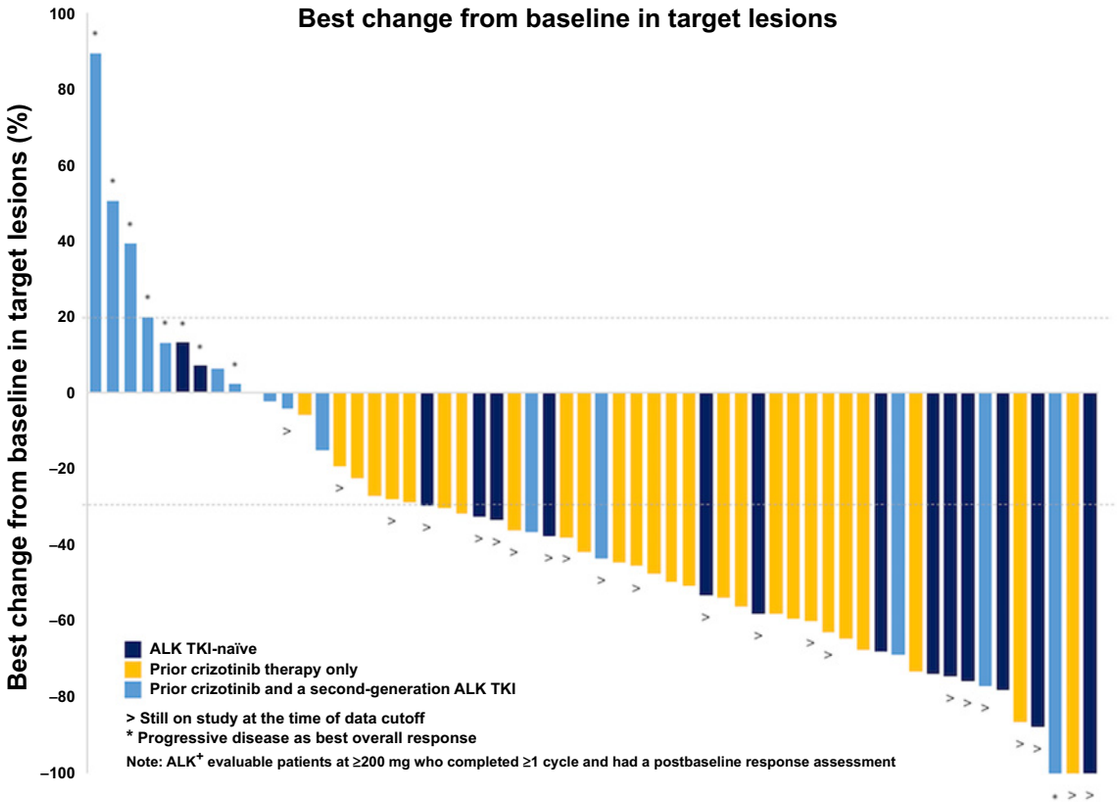

Figure 2.

Waterfall plot of best systemic response. Best percentage change from baseline in sum of target lesions is presented for the *ALK*-positive evaluable patients at ≥200 mg enrolled on study. Dashed lines are RECIST v1.1 criteria for partial response and progressive disease. Thirty-six patients (of 60) had a partial response, and 80% had tumor regression. The navy bars are those patients who were ALK TKI-naïve, whereas the yellow bars represent patients who had prior crizotinib therapy only, and the light blue bars represent patients who had prior crizotinib and a second-generation ALK TKI. (Note: Two patients had incomplete follow-up scans; therefore, a change in tumor size is not available.)

responding ALK-positive evaluable patients at doses ≥200 mg with CNS target lesions at baseline ($n = 9$), the median intracranial DOR was 5.7 months, ranging from 0.9 to 21 months.

Discussion

Ensartinib demonstrated clinical activity with high RR and DCR in patients with *ALK*-positive NSCLC. Ensartinib was not only effective in patients who were ALK TKI-naïve (RR = 80%), but also in patients with prior crizotinib as their only previous ALK TKI (RR = 69%). Encouraging efficacy was also observed in half of the patients who received a second-generation ALK TKI post crizotinib (RR = 25%, DCR = 50%). The CNS activity of ensartinib is also notable, given that 64.3% of NSCLC patients with CNS target lesions at baseline treated at doses ≥200 mg achieved an intracranial response. Moreover, the median DOR in the CNS at data cutoff was 5.7 months (with 5 of 9 patients still responding), which is important considering that the CNS is a common site of disease progression while on crizotinib (14, 15).

Overall, ensartinib was well tolerated, indicated by the low proportion of patients discontinuing the study due to an unacceptable drug-related toxicity and by most related AEs being grades 1 to 2. The most common toxicity associated with ensartinib was rash, which was generally managed with the use of topical medications and, for more severe toxicity, holding the dose until improvement and then resuming treatment at a reduced dose. The mechanism of rash related to ensartinib is unclear. But it has been reported that ALK is expressed in the epidermis of normal skin and that crizotinib inhibits growth of normal human epidermal keratinocytes *in vitro* (16, 17). The distribution study in rodents showed that the concentration of ensartinib in the skin was 9.0× higher than in the plasma 12 hours after a single dose (Supplementary Table S2). In comparison, alectinib was 5.7× higher in the skin than in the plasma (18). Taken together, these could help explain why ensartinib resulted in a higher frequency of rash compared with other ALK TKIs. This hypothesis is preliminary, however, and future studies are needed to fully characterize this mechanism.

Other second- and third-generation ALK inhibitors have entered the clinic and represent treatment options for patients who have progressed on crizotinib. Ceritinib, alectinib, and brigatinib have shown robust activity in *ALK*-positive NSCLC patients who have progressed or are intolerant to crizotinib, thus leading to approval in several countries, including the United

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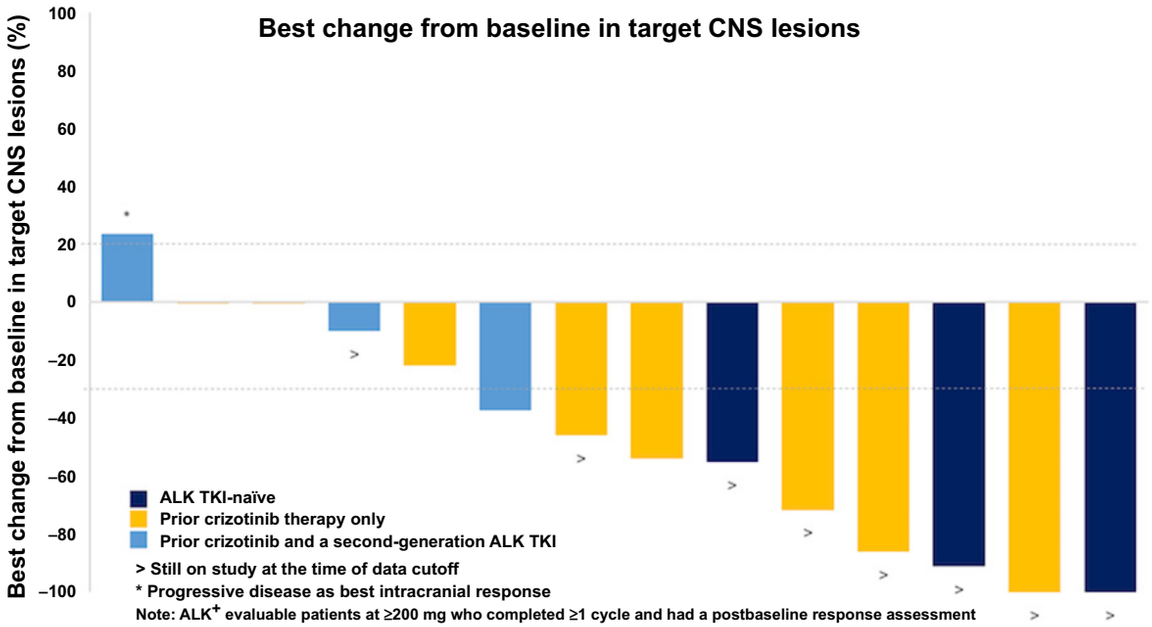

Figure 3.

Waterfall plot of best CNS response. Best intracranial percentage change from baseline in target CNS lesions is presented for the *ALK*-positive evaluable patients at doses ≥200 mg enrolled on study. Dashed lines indicate RECIST v1.1 cutoffs for partial response and progressive disease. Of the 14 patients with target CNS lesions, two had a complete response, 7 had a partial response, and 4 had stable disease. The navy bars are those patients who were ALK TKI-naïve, whereas the yellow bars represent patients who had prior crizotinib therapy only, and the light blue bars represent patients who had prior crizotinib and a second-generation ALK TKI.

States. The ASCEND-4 trial reported an RR of 73%, intracranial response of 57%, and median PFS of 16.6 months for first-line ceritinib (19). Additionally, 38% of patients experienced an SAE, with 10% of patients discontinuing therapy and 66% of patients with a dose interruption (19). The ALEX trial reported an 82% RR, 81% intracranial response, and median PFS of 25.7 months with first-line alectinib (20). Twenty-eight percent of patients had an SAE, while AEs leading to dose reduction, interruption, or discontinuation were reported in 16%, 19%, and 11%, respectively (20). In the crizotinib-refractory population, brigatinib achieved a 54% RR, with a median PFS of 12.9 months (21).

Currently, there are no randomized studies that compare next-generation ALK TKIs in the setting of crizotinib resistance; therefore, the ability to directly compare these agents is limited. However, the preliminary antitumor activity of ensartinib appears to be similar to that reported with the other second-generation ALK TKIs (10, 22). Additionally, ensartinib demonstrated *in vitro* activity against all *ALK* variants and a number of *ALK* mutants. Ensartinib may have other potential advantages as well, particularly with respect to toxicity. In terms of drug-related gastrointestinal toxicities, diarrhea was reported in 11% of patients treated with ensartinib, whereas, in some studies, 41% and 75% of patients who received brigatinib or ceritinib, respectively, experienced diarrhea (10, 23). Additionally, vomiting occurred in a majority of patients treated with ceritinib, but was observed in only a quarter of the patients with ensartinib, which was less frequent and severe when taken with food (i.e., meal consumed within 30 minutes of taking ensartinib) without affecting PK (see PK parameters in Supplementary Table S3). Furthermore, the frequency and severity of elevated aminotransferases reported as treatment-related AEs were low with ensartinib, and lower than what has been observed to date with other next-generation ALK inhibitors (10, 23–25). Lastly, the frequency and severity of early pulmonary toxicities that have been reported with brigatinib and ceritinib have not been observed with ensartinib (10, 23). Taken together, ensartinib appears to have a different toxicity profile from the other second-generation ALK TKIs, with most grade 3 events being rash and pruritus.

Although there are other next-generation ALK TKIs currently approved or in development, there is a need for multiple agents. While the RRs and CNS activity appear to be similar, these agents have different profiles with respect to toxicity and activity against different *ALK* mutations. It has been shown that patients can respond to other ALK TKIs, including ensartinib, after progressing on a prior ALK TKI. The best way to sequence these agents has yet to be determined and will be evaluated in upcoming trials.

It should be noted that there are some limitations to the current study. This is an ongoing study, with enrollment continuing and 23 of the 60 efficacy evaluable patients still on study. Additionally, as it was not a comparative trial, caution must be exercised in making comparisons with other ALK TKI trials.

In summary, ensartinib was generally well tolerated and demonstrated good clinical activity in patients with prior crizotinib, in patients who were ALK TKI-naïve, and in patients with CNS target lesions at baseline. Based on the findings from this trial, a randomized phase III study, eXalt3, comparing ensartinib with crizotinib in advanced *ALK*-positive, TKI-naive NSCLC patients, was started in June 2016 (NCT02767804).

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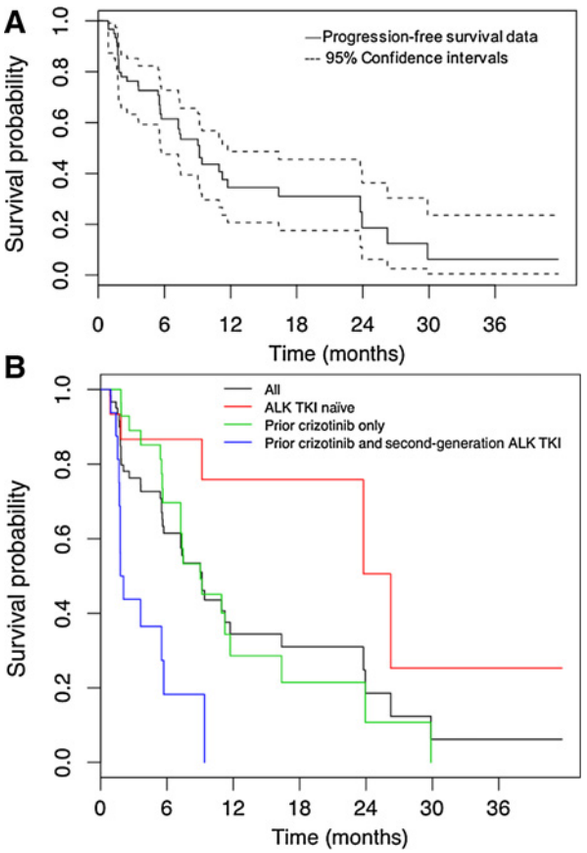

Figure 4.

PFS curves. Kaplan–Meier estimate of PFS with 95% confidence intervals for all *ALK*-positive, evaluable NSCLC patients who received ≥200 mg of ensartinib, where the median PFS was 9.2 months. **A,** Kaplan–Meier estimates of PFS are also shown for *ALK*-positive, evaluable patients who were ALK TKI-naïve (red line), received prior crizotinib only (green line), and received prior crizotinib and a second-generation inhibitor (blue line), where the median PFS for those groups was 26.2, 9.0, and 1.9 months, respectively (**B**).

Disclosure of Potential Conflicts of Interest

L. Horn is a consultant/advisory board member for AbbVie, AstraZeneca, Bristol-Myers Squibb, Merck, Roche-Genentech, and Xcovery, and reports receiving commercial research support from Boehringer Ingelheim. J.R. Infante is an employee of and has ownership interests (including patents) at Janssen Pharmaceuticals. K.L. Reckamp is a consultant/advisory board member for ARIAD and reports receiving commercial research grants from Xcovery. G.R. Blumenshein is a consultant/advisory board member for AbbVie, ARIAD, Bayer, Bristol-Myers Squibb, Celgene, Clovis, and Merck, and reports receiving commercial research grants from Adaptimmune, AstraZeneca, Bayer, Bristol-Myers Squibb, Celgene, Genentech, GlaxoSmithKline, Immatics, Kite Pharmaceuticals, Macrogenetics, Merck, Novartis, and Xcovery. T.A. Leal is a consultant/advisory board member for Novartis and Takeda. R.E. Sanborn is a consultant/advisory board member for ARIAD and Takeda. J.W. Neal is a consultant/advisory board member for ARIAD/Takeda, AstraZeneca, Lilly, and Boehringer Ingelheim, and reports receiving commercial research grants from ArQule, Boehringer Ingelheim, Exelixis, Genentech/Roche, Merck, Nektar, and Novartis. G. Dukart has ownership interests (including patents) in and is a consultant/advisory board member for Xcovery. K. Harrow and C. Liang have ownership interests (including patents) in Xcovery Holding. C.M. Lovly is an employee of and has ownership interests (including patents) at Foundation Medicine, and is a consultant/advisory board member for ARIAD, AstraZeneca, Cepheid, Novartis, and Takeda. H.A. Wakelee reports receiving commercial research grants from Genentech/Roche, Novartis, Pfizer, and Xcovery. No potential conflicts of interest were disclosed by the other authors.

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