Original Article

Ensartinib in advanced ALK-positive non-small cell lung cancer: a multicenter, open-label, two-staged, phase 1 trial

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Background: Ensartinib, a potent second-generation tyrosine kinase inhibitor (TKI) that targets anaplastic lymphoma kinase (ALK), MET and ROS1, was evaluated in a phase I clinical trial in patients with advanced, ALK-rearranged non-small cell lung cancer (NSCLC).

Methods: Patients with advanced, ALK or ROS1-positive NSCLC were recruited from 2 centers in China. This study consisted of dose escalation and expansion stages. Patients were treated with oral ensartinib [dosage of escalation stage was from 150, 200, 225 to 250 mg per day, expansion stage was recommended phase II dose (RP2D)] in continuous 28-day cycles. The primary objectives were safety, dose limited toxicity (DLT), maximum tolerated dose (MTD), and RP2D based on tolerability. Key secondary objectives included pharmacokinetic (PK) and anti-tumor activity.

Results: Forty-eight patients were enrolled, 37 (77.1%) were ALK TKI-naïve, 11 (22.9%) patients had previously received crizotinib, ceritinib or alectinib. Ensartinib was well tolerated and common treatment-related adverse events (TRAEs) included rash (87.5%), transaminase elevation (60.4%), pruritus (45.8%) and creatinine elevation (35.4%). The top 3 grade 3–5 TRAEs were rash (14.6%), elevated alanine aminotransferase (ALT) (12.5%) and aspartate transaminase (AST) (4.2%). Two DLTs were observed in 250 mg, so MTD and RP2D was 225 mg per day. Ensartinib was moderately absorbed (median Tmax: 3.00–4.00 h) and slowly eliminated (mean T1/2: 21.0–30.2 h). The area under the curve (AUC) of ensartinib reached saturation at 200 to 225 mg and no major accumulation after daily administration. For all patients, the objective response rate (ORR) and disease control rates (DCR) were 64.6 % and 81.3%, median progression-free survival (mPFS) was 16.79 months. In subgroup analysis, the ORR and mPFS was 81.3% and 45.5%, 25.73 and 4.14 months in TKI-naïve and -treated ALK+ patients, respectively. The intra-cranial ORR and mPFS for patients with measurable brain metastases were 66.7% and 22.90 months. ALK abundance may predict the efficacy of ensartinib. Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis showed specific signaling pathways enrichment in long and short progression-free survival (PFS) groups.

Conclusions: Ensartinib was well tolerated under 225 mg (MTD) and demonstrated promising anti-tumor activity in ALK+ NSCLC patients, including those with CNS metastases and those previously TKI-treated.

Trial Registration: ClinicalTrials.gov NCT02959619.

Keywords: Ensartinib; anaplastic lymphoma kinase (ALK); non-small cell lung cancer (NSCLC); central nervous system metastases (CNS metastases); phase I study

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Introduction

Rearrangement of anaplastic lymphoma kinase (ALK) is observed in 3–11.6% of non-squamous non-small cell lung cancer (NSCLC) patients. ALK-positive NSCLC is associated with a more aggressive phenotype, a shorter time to recurrence, a poor prognosis (1-3) and are usually younger age with light or non-smoking history (4). About 30% of these patients were found with central nervous system (CNS) metastasis when first diagnosed (5). ALK inhibitors have shown efficacy and safety superiority than chemotherapy in ALK-positive NSCLC (6). Crizotinib is the first-generation ALK-tyrosine kinase inhibitor (TKI) (7,8). Despite the its initial efficacy, patients will eventually failed on crizotinib after about 12 months, with brain being the most frequent progression site and mostly disease progressed due to acquired secondary ALK mutations, such as L1196M, G1269A, C1156Y, L1152R, G1202R, S1206Y, 1151Tins, F1174C, and D1203N (9,10).

Highlight box

Key findings

- We report the results of safety, tolerability, pharmacokinetic (PK), efficacy and possible pharmacodynamics (PD) biomarkers of ensartinib of a phase I dose escalation and expansion trial in Chinese advanced ALK or ROS1 positive non-small-cell lung cancer (NSCLC) patients.

What is known and what is new?

- Ensartinib showed efficacy for patients with disease progression on crizotinib and superior efficacy to crizotinib in both systemic and intracranial disease.
- The recommended phase II dose (RP2D) decision, systemic PK and safety in dose escalation, and PD biomarkers of ensartinib in Chinese patients were firstly revealed.

What is the implication, and what should change now?

- We showed systemic PK, safety of different doses, pharmacodynamics and efficacy of ensartinib, which together with the results of Phase 2 and Phase 3 trials promoted the approval of ensartinib in ALK positive NSCLC patient treatment in China.

In order to overcome acquired resistance and manage CNS localizations, several second- (ceritinib, alectinib and brigatinib) and third-generation (lorlatinib) TKIs have been developed. These ALK-TKIs have different potencies in inhibiting secondary ALK mutations, and can potentially overcome resistance (11).

Ensartinib (X-396) is a novel second-generation, aminopyridazine-based ALK-TKI which improve the activity on CNS metastases. Ensartinib can inhibit wild-type and ALK variants (F1174, C1156Y, L1196M, S1206R, T1151 and G1202R) as well as TPM3-TRKA, TRKC, ROS1, EphA2, EphA1, EphB1 and c-MET (12). In a phase I/II study, the recommended phase II dose (RP2D) of ensartinib was established to be 225 mg QD, with common drug-related adverse events including rash (56%), nausea (36%), pruritus (28%), and vomiting (26%); the objective response rate (ORR) was 60% and the median progression-free survival (mPFS) was 9.2 months (13). However, there was no Chinese patients enrolled in this trial, considering the ethnic differences, further studies are warranted. Therefore, phase I/II/III trials to assess the safety, efficacy, pharmacokinetic (PK) and possible biomarkers of ensartinib in Chinese advanced ALK-positive NSCLC patients were launched. In phase II and III study (14,15), ensartinib showed antitumor activities in patients failed on crizotinib, and superiority against crizotinib in systemic and intracranial efficacy, respectively.

Here, we report the results of safety, tolerability, PK, efficacy and possible PD biomarkers in the phase I dose escalation and expansion trial of ensartinib in Chinese advanced ALK or ROS1 positive NSCLC patients. We present the following article in accordance with the TREND reporting checklist (available at https://jtd.amegroups.com/article/view/10.21037/jtd-22-1606/rc).

Methods

Patients and study design

Patients were eligible for enrollment if they were between

18 and 70 years of age, and a pathological confirmed NSCLC, harbored ALK fusion or ROS1 mutation (confirmed by immunochemistry, FISH or gene sequence), had a performance status of 0 to 1 on the Eastern Cooperative Oncology Group (ECOG) scale, and had adequate bone marrow and organ function. The primary objectives of this trial were safety, RP2D determination of ensartinib based on tolerability and PK, secondary objectives including PK, efficacy and possible PD biomarkers. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The two-center, open-label, two-staged, phase I trial was approved by the ethic committee of Sun Yat-Sen University Cancer Center (No. A2016-048-01) and the ethic committee of Zhejiang University School of Medicine Second Affiliated Hospital (No. 2017-018-IH). Informed consent was taken from all individual participants. This study was registered through ClinicalTrials.gov (NCT02959619).

Procedures

This study consisted of dose escalation and dose expansion phases. Dose escalation was based on a PK-guided 3+3 design. The starting dose was 150 mg, which was based on previous study (13). Patients orally received ensartinib administration at 150, 200, 225 and 250 mg dose levels once daily in 28-day cycles. Initially, each dose escalation cohort recruited 3–12 patients (determined by investigator and sponsor meeting based on safety or PK consideration). Dose limited toxicity (DLT) was assessed during the first 28-day cycle to determine maximum tolerated dose (MTD). After determination of the MTD and recommended dose, additional patients were enrolled in dose expansion stage and receive ensartinib at RP2D in 28-day cycles until disease progression or unacceptable toxicity. A DLT was defined as any of the following drug-related adverse events that occurred during the first treatment cycle: grade 4 neutropenia last >5 days or febrile neutropenia, grade 4 thrombocytopenia or grade 3 thrombocytopenia with bleeding, grade 3–5 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 >14 days due to unresolved toxicity. The MTD was defined as the highest dose level at which less than 1/3 patients experienced a DLT. The recommended dose of ensartinib would be 250 mg daily if no more than 1 DLT occurred, otherwise the MTD would be the recommended dose.

Assessment

Safety and efficacy

The safety and tolerability of ensartinib were assessed by evaluating vital signs, physical examination findings, performance status score, clinical laboratory testing (serum chemistry, hematology test, urinalysis, etc.), auxiliary examinations electrocardiogram (ECGs), visual history. Adverse events (AEs) were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.03.

At baseline, all patients underwent tumor imaging with CT and, if appropriate, magnetic resonance imaging (MRI). Brain imaging was required for patients with known or suspected brain metastases. Response evaluation was obtained at 8-week intervals, and assessed according to RECIST v1.1 by the investigators.

PK analyses

Patients were administered with a single dose of ensartinib in cycle 0 and received 7-day wash-out period, then administered continuously for multiple doses in cycle 1. PK analysis was performed in cycle 0 for single dose analysis and cycle 1 for multiple dose analysis. Systemic plasma samples were obtained from all patients in dose escalation and a subset of patients in the dose expansion phase before dosing of ensartinib (–0.5 h), and at 0.5, 1, 2, 3, 4, 8, 12, 16, 24, 36, 48, 72, 96, 120 and 144 h after dosing for single dose. On day 28 of cycle 1, systemic plasma samples were collected for multiple doses at same time points. Plasma samples were also collected prior (–0.5 h) to dosing on days 8, 15, and 22 of cycle 1. The concentration of ensartinib in plasma was determined by using a liquid chromatography-mass spectrometry (LC-MS) method (API 5500, Applied Biosystems, Waltham, MA, USA). PK parameters in plasma were calculated by non-compartmental analysis.

Biomarker analysis

Formalin-fixed paraffin-embedded (FFPE) tissues was collected voluntary at baseline, DNA was extracted using the QIAamp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany). Peripheral blood samples were collected at baseline and C1D31 respectively, plasma and white blood cells DNA were extracted using the QIAamp Circulating Nucleic Acid Kit (Qiagen, Hilden, Germany) and the Hipure Blood&Tissue DNA Kit (Magen Biotechnology, Guangzhou, China). The DNA obtained was captured by

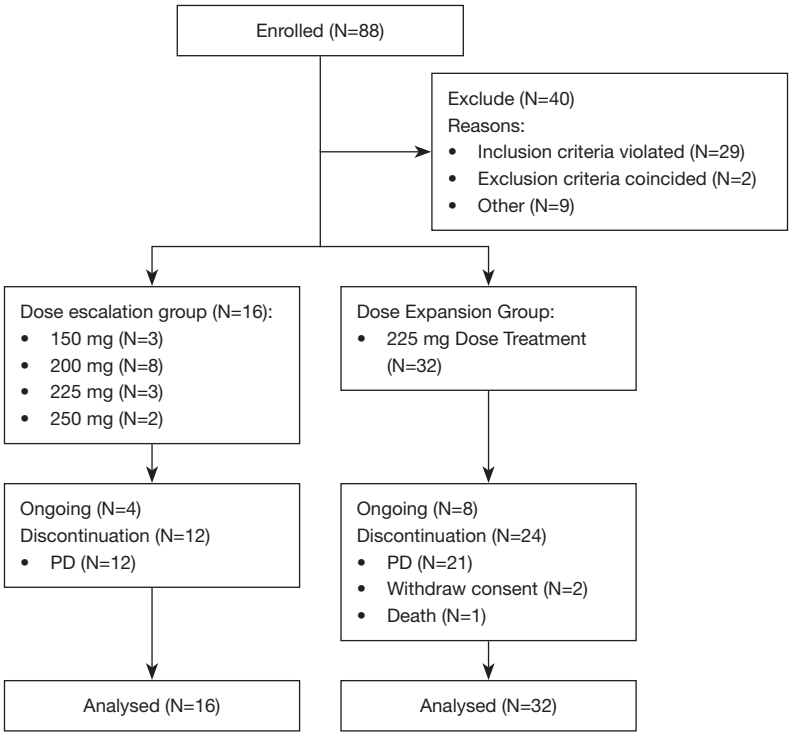

Figure 1 Patient flow diagram. PD, progressive disease.

SureSelect XT-HS Target Enrichment System (Agilent Technologies, Santa Clara, CA, USA) using a 212-gene panel (Table S1, Repugene Technology, Hangzhou, China) for all samples. The constructed libraries were sequenced with mean sequencing depths approximately 20,000 times for blood samples and 4,000 times for tissue samples using the Illumina HiSeq-X10 platform (Illumina, San Diego, CA, USA). Then, the samples and white blood cells were paired to filter out clonal hematopoiesis variants and germline mutations. MuTect2 was used for single-nucleotide variation and insert-deletion variant detection and LUMPY (version 0.2.13) was used for gene fusion detection (16). The copy number variation was detected using the CNVkit software (v0.9.5). Mutation load of each patient was the sum of the number of detected mutation and ALK mutation abundance was defined as the variant allele fractions of ALK mutations.

Statistical analysis

All patients who signed informed consent were included in full analysis set (FAS), safety analysis set (SS) included patients received at least one dose of ensartinib and has at least one post-baseline safety evaluation. Efficacy analyses were conducted in efficacy analysis set (10), which included patients who received at least one dose the study drugs and has at least one post-baseline tumor assessment. Toxicities were described by frequency and grade with the maximum grade over all cycles used as the summary measure per patient. Progression-free survival (PFS) was estimated using the Kaplan-Meier method and compared using the log-rank test. Mutation load between patients with TP53 mutations and patients without TP53 mutations was compared using Mann-Whitney U test. Genetic factors related to PFS were determined using Univariate Cox regression analyses. The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses was performed on genes detected with nonsynonymous mutations in long-PFS and short-PFS group using the R cluster Profiler package. Enriched pathways with P values <0.05 were considered significant. The analyses were conducted with SAS 9.4 software (SAS Institute, Cary, NC, USA).

Results

Patients characteristics

As showed in *Figure 1*, forty-eight patients (16 in dose

Table 1 Patients' demographic and baseline characteristics

Characteristics	Ensartinib dose cohorts (mg)									
	150 (N=3)		200 (N=8)		225 (N=35)		250 (N=2)		Total (N=48)	
	No.	%	No.	%	No.	%	No.	%	No.	%
Median age, years (range)	52.3 (40–64)		49 (37–77)		46.8 (23–65)		44 (41–49)		47.5 (23–77)	
Gender										
Female	1	33.3	3	37.5	20	57.1	1	50	25	52.1
Male	2	66.7	5	62.5	15	42.9	1	50	23	47.9
ECOG performance status										
0	1	33.3	3	37.5	18	51.4	2	100	21	43.8
1	2	66.7	5	62.5	17	48.6	0	0	27	56.2
Smoking status										
Yes	1	33.3	2	25	9	25.8	1	50	12	25
No	2	66.7	6	75	26	74.2	1	50	36	75
ALK positive	3		8		30		2		43	
ROS1 positive					5				5	
Ensartinib as first-line treatment	1	33.3	6	75	23	17.1	2	100	15	31.3
No prior ALK-TKIs treatment	1	33.3	6	75	28	17.1	2	100	37	77.1
Prior ALK-TKIs treatment	2	66.7	2	25	7	82.9	0	0	11	22.9
Median No. of metastatic organs (range)	2 (1–3)		3 (1–5)		3 (1–6)		3 (1–5)		3 (1–6)	
No. of prior metastatic anticancer regimens										
<3	3	100	8	100	32	91.4	2	100	45	93.7
≥3	0	0	0	0	3	8.6	0	0	3	6.3
No. of patients with brain metastases	1	33.3	4	50	11	31.4	1	50	17	35.4

ECOG, Eastern Cooperative Oncology Group; ALK, anaplastic lymphoma kinase; TKI, tyrosine kinase inhibitor.

escalation and 32 in dose expansion) were enrolled from March 6, 2017 to October 31, 2019. Baseline demographics are presented in *Table 1*. The median age was 48 (range, 23–77). Of 48 enrolled patients, 43 patients were detected with ALK rearrangement and 5 patients harbored with ROS1 fusion. Eleven (22.9%) patients were prior ALK TKI treated, among them 2 (4.2%) received second-generation ALK inhibitor treatment. Sixteen (33.3%) patients had brain metastases at baseline (*Table 1*).

Safety, tolerability and RP2D

As showed in *Table 2*, among all the treatment-related adverse events (TRAEs), 27% (13/48) were grade 3–5. The most common TRAEs were rash (87.5%), increased alanine aminotransferase (ALT) (60.4%), increased aspartate transaminase (AST) (54.2%), pruritus (45.8%), increased serum creatinine (35.4%), leukocytosis (29.2%), skin exfoliation (27.1%), vomiting (27.1%), dermatitis (25.0%), nausea (25.0%), neutrocytosis (20.8%). The most frequent grade 3–5 TRAEs were rash (14.6%) and increased ALT (12.5%). Most TRAEs were grade 1 to 2. Dose reduction and delay of ensartinib was seen in 9 and 13 patients, respectively. Serious AEs (SAEs) occurred in 10 (20.8%) patients. Rash (2 patients; 4.2%), facial edema (1 patient; 2.1%) and hepatic failure (1 patient; 2.1%) were considered treatment related. Two DLTs were observed in the 250 mg dose cohort (grade 3 rash) and the RP2D of ensartinib in Chinese NSCLC patients was determined to be 225 mg.

Table 2 Ensartinib related AEs of all grades and of grade ≥3 that occurred in all patients from screening visit until 28 days after last dose of ensartinib

TRAE	Ensartinib dose cohorts (mg)									
	150 (N=3)		200 (N=8)		225 (N=35)		250 (N=2)		Total (N=48)	
	No.	%	No.	%	No.	%	No.	%	No.	%
≥3 grade TRAE	0	0	2	25	9	25.7	2	100	13	27.1
Rash	2	66.7	6	75	32	91.4	2	100	43	87.5
≥3 grade rash	0	0	0	0	5	14.3	2	100	7	14.6
Increased ALT	1	33.3	5	62.5	22	62.9	1	50	29	60.4
≥3 grade increased ALT	0	0	2	25	4	11.4	0	0	6	12.5
Increased AST	1	33.3	5	62.5	19	54.3	1	50	26	54.2
≥3 grade increased AST	0	0	1	12.5	1	2.9	0	0	2	4.2
Pruritus	1	33.3	4	50	17	48.6	0	0	22	45.8
Leucocytosis	0	0	4	50	10	28.6	0	0	14	29.2
Neutrocytosis	0	0	2	25	8	22.9	0	0	10	20.8
Increased creatinine	0	0	4	50	12	34.3	1	50	17	35.4
Skin exfoliation	0	0	3	37.5	10	28.6	0	0	13	27.1
Dermatitis	0	0	3	37.5	8	22.9	1	50	12	25.0
Vomiting	0	0	0	0	12	34.3	1	50	13	27.1
Nausea	0	0	2	25	9	25.7	1	50	12	25.0
Constipation	0	0	0	0	3	8.6	0	0	3	6.3
Diarrhea	0	0	0	0	7	20	0	0	7	14.6
Fever	0	0	0	0	5	14.3	0	0	5	10.4
Dizziness	1	33.3	0	0	2	5.7	1	0	3	6.3
Albuminuria	0	0	0	0	5	14.3	0	0	5	10.4
Oral ulcer	1	33.3	2	25	5	14.3	1	50	9	18.8
Abdominal pain	0	0	0	0	5	14.3	0	0	5	12.5

Ensartinib-related adverse events include definitely related AEs and probably related AEs. TRAE, treatment related adverse events; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AEs, adverse events.

PK analyses

PK analysis was performed in cycle 0 for single dose analysis and cycle 1 for multiple dose analysis (Table S2). In single dose administration (150–250 mg), the area under the curve (AUC) reached the highest at 200 to 225 mg dose. Compared to the AUC of 225 mg, the AUC of 150, 200, 250 mg was 0.51, 0.96, 1.11 folds (R^2 of dose versus exposure linear regression graph for was 0.7707) at the steady state. The absorption of ensartinib was relatively slow with a median T_{max} of 3.00 to 4.00 hours; mean $T_{1/2}$ ranged from 21.0 to 30.2 hours; mean C_{max} ranged from 110 to 225 ng/mL. In multiple dose administration (150–225 mg), a steady-state concentration of ensartinib was reached after 8–15 days. The mean residence time (MRT) [± standard deviation (SD)] of 150, 200, 225 mg dose cohort was 2.76±0.626, 3.08±1.60, 3.43±2.03 h × ng/mL. Median time to reach maximum plasma concentration (T_{max}) was 2–3 h, with mean C_{max} ranging from 265 to 435 ng/mL. The $T_{1/2}$ ranged from 28.4 to 35.4 h. In single and multiple dose administration, the AUC_{0-t}, AUC_{0-tau}, C_{max}, C_{trough}

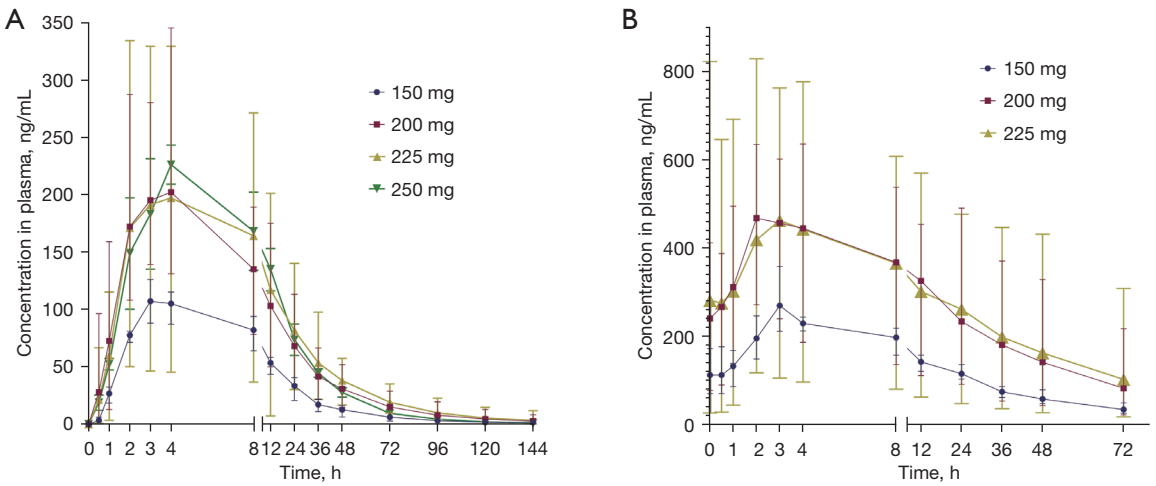

Figure 2 Mean plasma concentration time curve (linear graph) of different dose groups of ensartinib in single dose and multiple dose cohorts. (A) Single dose time-concentration Curve. (B) Multiple dose time-concentration curve.

tend to increase with ascending dose, but the accumulation pattern of ensartinib was not completely linear (*Figure 2*). No clear correlation was noted between ensartinib exposure and age, sex, ethnic organ, and body-mass index within every dose cohort (data not shown).

Anti-tumor activity

A summary of the confirmed best overall response on the basis of investigator review is provided in Table S3 and *Figure 3*. In all patients (43 ALK+ and 5 ROS1+), the ORR and disease control rates (DCR) were 64.6 % (1 CR and 30 PR) and 81.3% (8 SD), respectively. The median PFS was 16.79 months [95% confidence interval (CI), 8.11 to 25.47 months]. For patients treated with ensartinib ≥225 mg (37 patients), the ORR and DCR were 62.2% (95% CI, 45.8–78.6%) and 78.4% (95% CI, 64.5–92.3%), while ORR and DCR for patients with ensartinib <225 mg (11 patients) were 72.7% (95% CI, 41.3–100%) and 90.9% (95% CI, 70.7–111%). The median PFS in 150 and 200 mg cohorts was 2.07 months (ranged, 1.15 to 16.79 months) and 26.88 months (95% CI, 2.29–51.46 months), while in 225 mg and 250 mg cohorts the mPFS was 16.62 months (95% CI, 6.11–26.14 months) and 22.82 months (ranged,18.23 to 38.37 months).

In 43 ALK positive patients, the ORR and DCR were 72.1% (95% CI, 58.1–86.1%) and 83.7% (95% CI, 72.2–95.2%), respectively. The mPFS was 18.23 months (95% CI, 8.77–27.70 months) (Table S4). In ALK TKI-naïve patients, the confirmed ORR was 81.3% (26/32) compared to 45.5% (5/11) in ALK TKI-resistant patients, mPFS was 25.73 months (95% CI, 20.41–31.04 months) and 4.14 months (95% CI, 0.00–8.75 months) respectively. While in 5 ROS1 positive patients, the ORR and DCR were 0% and 60% (3 SD). Ensartinib showed disease control in patients with brain metastases (*Figure 3*). The ORR and DCR in 16 patients with baseline brain metastases were 68.8% (95% CI, 43.2–94.3%) and 68.8% (95% CI, 43.2–94.3%) respectively, median PFS was 7.59 months (95% CI, 6.75–8.43 months). In 3 patients with measurable brain metastasis lesions, the ORR were 66.7% (2 PR and 1PD), median intra-cranial PFS was 22.90 months (ranged, 3.65 to 30.36 months) .

Biomarker analyses

Baseline mutation landscape

All genes detected with nonsynonymous mutations at baseline were displayed in *Figure 4*. TP53 and KMT2C had the highest mutation rates, both detected in 5 patients (5/27, 19%). After one cycle of treatment, the number of mutant genes detected at C1D31 was greatly reduced (Figure S1). Notably, the frequency of ALK mutations remarkably decreased from 100% at baseline to 5% at C1D31.

The median ALK mutation abundance at baseline was taken as cutoff (median =0.000573148) to define high or low abundance. The median PFS was 26.25 months (95% CI, 4.50 to not estimable) and 8.46 months (95% CI, 4.25–

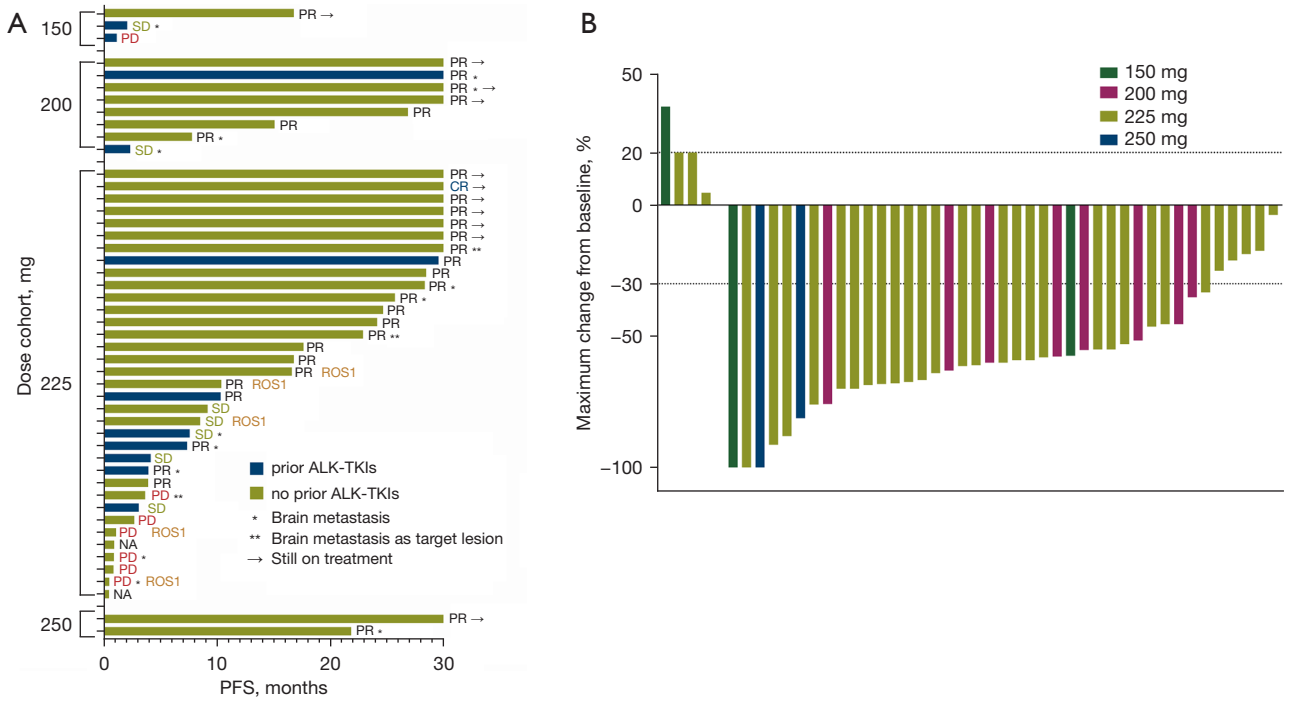

Figure 3 Time on treatment and maximum reduction of target lesions for patients in the 150- to 250-mg dose cohorts. (A) Time on treatment for patients in the 150- to 250-mg dose cohorts. All data were cut off on December 31, 2020. (B) Maximum reduction of target lesions from baseline for patients in the 150- to 250-mg dose cohorts. The best response for target lesions per patient was determined on the basis of RECIST v1.1 criteria. *, patients who reported baseline brain metastasis; **, brain metastasis as target lesion. PR, partial response; CR, complete response; SD, stable disease; PD, progressive disease; ALK, anaplastic lymphoma kinase; TKI, tyrosine kinase inhibitor; PFS, progression free survival.

21.9 months) in patients with low and high ALK mutation abundance (*Figure 4*). Although there was marginally statistical significance between two groups [hazard ratio (HR), 0.39; 95% CI, 0.14–1.09; log-rank test P=0.06], ALK abundance at baseline showed high predictive value of ensartinib anti-tumor activity.

Change of mutation landscape during the course of treatment

Among 43 patients with advanced disease, 21 patients (48.84%) underwent continuous ctDNA testing at baseline and C1D31. Change in the mutation profile was evident during the treatment (Figure S1). At baseline, nonsynonymous mutations were detected in 50 genes. At C1D31, only 2 baseline mutations remained, 21 de novo mutations occurred. At baseline, patients with TP53 mutation had higher gene mutation load (TP53 mutant group, 4.25±0.96 versus TP53 wild-type group, 1.76±1.44, P=0.010). At C1D31, the mutation load of the two group has not significant difference (TP53 mutations detected group, 1.25±1.26 versus TP53 mutations undetected group, 1.06±1.09, P=0.779).

KEGG analysis

Taking 9 months as the cut-off time, the patients were divided into long PFS group (PFS >9 m) and short PFS group (PFS >9 m). KEGG analysis was performed on the two groups of all genes detected with nonsynonymous mutations (Figure S2). Pathways that were only enriched in the long PFS group were screened as signaling pathways (MAPK, FoxO, HIF-1 and Rap-1), focal adhesion and virus carcinogenesis; and pathways that only enriched in the short PFS group were screened as p53 signaling pathway, apoptosis, notch signaling pathway and chemical carcinogenesis-receptor activation (Figure S3).

Discussion

In this phase I study, we found that ensartinib ≤225 mg once per day was safe and well tolerated in Chinese NSCLC

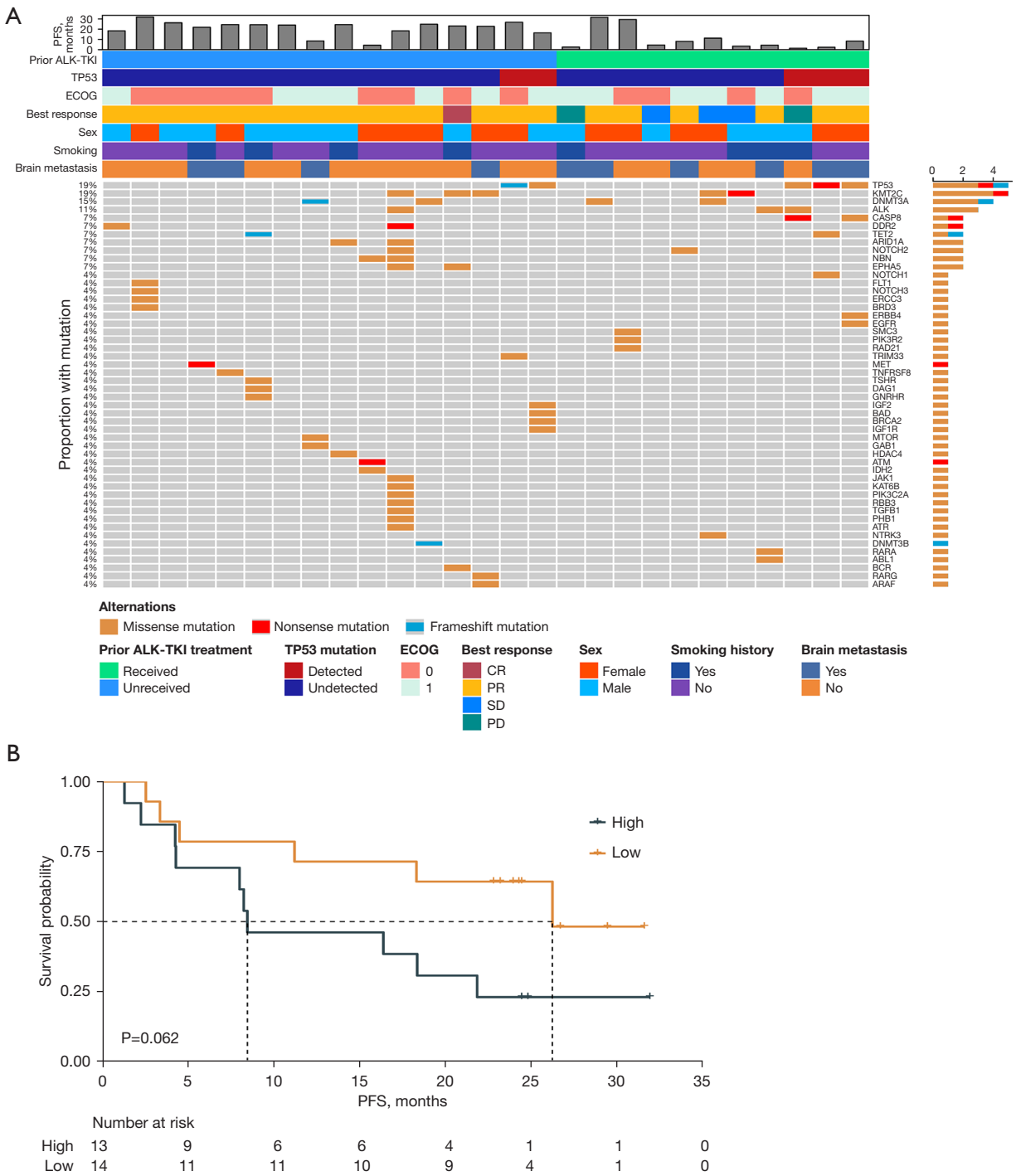

Figure 4 The baseline mutational profile and PFS in the group with high or low ALK fusion abundance. (A) The baseline mutational profile. All the genes with nonsynonymous mutations are displayed. (B) Kaplan-Meier estimate of progression-free-survival in the group with high ALK fusion abundance and group with low ALK fusion abundance. PFS, progression-free survival; ALK, anaplastic lymphoma kinase; TKI, tyrosine kinase inhibitor; ECOG, Eastern Cooperative Oncology Group; CR, complete response; PR, partial response; PD, progression disease; SD, stable disease.

patients. Results in dose escalation supported 225 mg once per day as the RP2D. Also, systemic PK results indicated that the AUC of ensartinib reached saturation at 225 mg. Safety analysis indicated that common TRAEs was rash, transaminase elevation and pruritus, which was consistent with phase II/III trials. Once again, ensartinib proved its anti-tumor activity in ALK+ NSCLC patients in TKI-naïve or resistant patients. The biomarkers analysis suggested patients with higher ALK mutation abundance at baseline were likely benefit less in ensartinib treatment.

The main TRAEs were grade 1 or 2 rash, which was consistent with reports of ensartinib from phase I/II/III multicenter studies (13-15). TRAEs could be successfully managed by dose suspension or reduction. Most patients did not require dose adjustment during the treatment. Ensartinib appears to have a different safety profile from other ALK-TKIs. Previous studies showed that gastrointestinal toxicities (nausea, diarrhea, and vomiting) were common in patients after crizotinib treatment. While diarrhea was reported low incidence in patients treated with ensartinib, which was significantly lower than those in patients who received brigatinib (17) or ceritinib (18). Moreover, the frequency and severity of elevated aminotransferases reported as TRAEs with ensartinib was also lower than those with other ALK inhibitors (17-20). The incidence of grade 3 or higher TRAEs reported in this study was consistent with crizotinib (21) and less than ceritinib (22) and brigatinib (18). Overall, the safety of ensartinib was acceptable.

The PK analysis demonstrated that the plasma exposure of ensartinib may not increase proportionally as dose escalated. Notably, the half-life of ensartinib in the present study was consistent with previous reported results of alectinib, brigatinib and lorlatinib but was shorter compared to crizotinib or ceritinib, respectively (23-26). A short half-life allows ensartinib quickly decrease in the plasma thus reducing the drug or active metabolites accumulation, which might improve safety and decrease TRAEs of ensartinib.

Ensartinib once again demonstrated satisfy efficacy in patients with ALK positive NSCLC patients. In this study, the included patients were prior treated with one or more lines of chemotherapy, or with ALK-TKIs, yet the ORR and clinical benefit rate (CBR) were impressive. The median PFS and ORR of ensartinib in ALK TKI-naïve patients were consistent with alectinib and might higher than ceritinib (20,22). A high risk of developing brain metastases was found in patients with ALK-positive NSCLC. About 30% of cases were found brain metastasis when first diagnosed (27). It's worth mentioning that, in this study, the ORR and DCR in 16 patients with baseline brain metastases were comparable with those of ceritinib, alectinib and brigatinib (9,23,28,29).

The gene mutation landscape showed that the most common co-mutation genes of ALK positive patients in our study were TP53 and KMT2C, which were reported functions in cell proliferation, tumor formation and DNA damage repair (30-34). At baseline, patients with TP53 mutation had higher gene mutation load, which was reported associated with primary resistance to crizotinib in ALK+ NSCLC (35). We found that patients with low ALK mutation abundance at baseline achieved significantly longer mPFS compared to those with high ALK mutation abundance. KEGG analysis showed that mutations enriched only in the short PFS group, which may results in therapeutic resistance and cancer progression through chronic inflammation (36,37). Due to limited sample size, the relationship among TP53 mutation, mutation load, ALK mutation abundance with mPFS in ensartinib treatment should be clarified in perspective studies with more subjects.

Conclusions

In conclusion, ensartinib demonstrated good clinical activity and an acceptable safety profile in Chinese ALK-positive NSCLC patients with or without prior ALK TKIs treatment, and with CNS metastases.

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Footnote

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Data Sharing Statement: Available at https://jtd.amegroups.com/article/view/10.21037/jtd-22-1606/dss

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://jtd.amegroups.com/article/view/10.21037/jtd-22-1606/coif). SW, LZ, and HZ report funding from Betta Pharmaceuticals Co., Ltd. SW reports that the fund was managed by Zhejiang University School of Medicine Second Affiliated Hospital. LD and LC report that they are employees of Betta Pharmaceuticals Co., Ltd. SX and TW report that they are employees of Hangzhou Repugene Technology Co., Ltd. The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the ethic committee of Sun Yat-Sen University Cancer Center (No. A2016-048-01) and the ethic committee of Zhejiang University School of Medicine Second Affiliated Hospital (No. 2017-018-IH). Informed consent was taken from all individual participants.

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Supplementary

Table S1 The list of 212 genes in the panel

ABL1	CASP8	EPHB2	HDAC6	MAP2K1	PARP3	RET	YY1
ABL2	CDK12	EPHB6	HDAC8	MAP2K2	PARP4	ROS1	ZNF143
ACVR1B	CDK2	ERBB2	HRAS	MAP2K4	PBRM1	RUNX3	
ACVR2A	CDK4	ERBB3	HRH2	MAPK1	PDGFRA	SHC1	
AKT1	CDK6	ERBB4	HSP90AA1	MAPK3	PDGFRB	SMC3	
AKT2	CDK7	ERCC1	HSPA4	MAPK8	PDK1	SOS1	
AKT3	CDK8	ERCC2	IDH1	MAPK8IP1	PIGF	SRC	
ALK	CDKN1A	ERCC3	IDH2	MAX	PIK3C2A	STAT3	
AR	CDKN1B	FGF10	IFNAR1	MAZ	PIK3C2B	STAT4	
ARAF	CDKN2A	FGF12	IFNAR2	MDM2	PIK3C2G	STAT5A	
ARID1A	CDKN2B	FGF14	IGF1	MDM4	PIK3C3	STAT5B	
ARID2	CDKN2C	FGF19	IGF1R	MET	PIK3CA	SYK	
ATM	CEBPB	FGF23	IGF2	MSH6	PIK3CB	TET1	
ATR	CYP2C19	FGF6	IL7R	MTA3	PIK3CG	TET2	
ATRX	CYP2D6	FGF7	ING1	MTOR	PIK3R1	TGFB1	
AURKA	CYP3A4	FGFR1	ING4	MUC1	PIK3R2	TGFBR2	
AURKB	DAG1	FGFR2	JAK1	MXI1	PIM1	TLR4	
AXL	DDR1	FGFR3	JAK2	MYC	PLK1	TNFAIP3	
BAD	DDR2	FGFR4	JAK3	NBN	PML	TNFRSF13B	
BCL2L11	DIRAS3	FLT1	KAT6A	NFKBIA	PRKCA	TNFRSF14	
BCL7A	DNMT1	FLT3	KAT6B	NOTCH1	PRKCB	TNFRSF8	
BHLHE40	DNMT3A	FLT4	KDM5A	NOTCH2	PTEN	TOP1	
BIRC5	DNMT3B	FOXM1	KDM5C	NOTCH3	PTK2	TOP2A	
BRAF	EDNRA	GAB1	KDM6A	NOTCH4	PTK2B	TOP2B	
BRCA1	EGFR	GNRHR	KDR	NRAS	RAD21	TP53	
BRCA2	EPHA2	GRB2	KIT	NTRK1	RARA	TRIM33	
BRD1	EPHA3	HDAC1	KMT2A	NTRK2	RARB	TSHR	
BRD3	EPHA5	HDAC2	KMT2B	NTRK3	RARG	UGT1A1	
BRD4	EPHA7	HDAC3	KMT2C	PARP1	RB1	VEGFA	
BTK	EPHB1	HDAC4	KRAS	PARP2	RCOR1	VEGFB	

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Table S2 PK analysis

Dose (mg)	N	Median, T_{max} (h)	Mean (CV%)									Mean ± SD, $t_{1/2}$ (h)
			λ_z (1/h)	C_{max} (ng/mL)	AUC_{0-t} (h*ng/mL)	AUC_{0-24} (h*ng/mL)	AUC_{0-inf} (h*ng/mL)	AUC_%Extrap (%)	CL/F (L/h)	V_z/F (L)	MRT (h)	
Cycle 0 single dose												
150	3	4.00	0.0232 (17.6)	110 (15.0)	2240 (25.8)	1400 (12.4)	2300 (26.5)	2.26 (30.5)	65.3 (26.5)	2810 (17.9)	25.9 (22.8)	30.2±5.34
200	8	3.50	0.0261 (22.5)	220 (32.5)	4590 (30.8)	2600 (26.9)	4700 (31.6)	1.69 (101.5)	42.5 (31.6)	1630 (36.9)	29.1 (18.2)	27.1±6.16
225	19	3.00	0.0246 (21.3)	206 (48.9)	5230 (39.9)	2720 (49.6)	5380 (38.9)	2.19 (81.8)	41.8 (38.9)	1700 (54.4)	31.9 (13.8)	28.8±6.91
250	2	4.00	0.0332	225	4810	3040	4840	0.525	51.7	1560	24.5	21.0
Cycle 1 multiple dose												
150	3	3.00	0.0236 (24.9)	265 (27.5)	98.4 (39.8)	158 (15.0)	3790 (15.0)	105 (12.4)	39.6 (15.0)	1670 (29.8)	2.76 ±0.626	29.9±6.95
200	8	2.00	0.0252 (27.8)	474 (35.7)	184 (60.2)	299 (47.7)	7170 (47.7)	89.4 (47.1)	27.9 (47.7)	1110 (45.7)	3.08 ±1.60	28.4±7.89
225	12	3.00	0.0204 (27.9)	435 (55.6)	190 (90.1)	290 (61.7)	6950 (61.7)	76.8 (48.6)	32.4 (61.7)	1590 (59.0)	3.43 ±2.03	35.4±12.2

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Table S3 Best ORR In All Patients (Cutoff Date: December 31, 2020)

Efficacy	Ensartinib Dose Cohorts(Mg)									
	150 (N=3)		200 (N=8)		225 (N=35)		250 (N=2)		Total (N=48)	
	No.	%	No.	%	No.	%	No.	%	No.	%
CR					1	2.9			1	2.1
PR	1	33.3	7	87.5	20	57.1	2	100	30	62.5
SD, Months										
2-6	1	33.3	1	12.5	2	5.7			4	8.3
≥6					4	11.4			4	8.3
PD	1	33.3			6	17.1			7	14.6
NA					2	5.7			2	4.2
ORR (95% CI)	33.3		87.5		60.0 (42.9 to 77.1)		100		64.6 (50.5 to 78.6)	
DCR (95% CI)	66.7		100		77.1 (62.5 to 91.8)		100		81.3 (69.8 to 92.7)	
CBR (95% CI)	33.3		87.5		71.4 (55.7 to 87.2)		100		72.9 (59.9 to 86.0)	
Median PFS, Months	2.07		26.88		16.62		22.82		16.79	
(95% CI)	(1.15, 16.79)		(2.29 to 51.46)		(7.11 to 26.14)		–		(8.11 to 25.47)	
(Minimum, Maximum)	–		(2.33, 43.17)		(0.46, 36.99)		(18.23, 38.37)		(0.46, 43.17)	
Median OS, Months	–		–		–		–		42.88	
(95% CI)	–		–		–		–		(23.37 to 62.38)	
(Minimum, Maximum)	(21.98, 45.60)		(7.33, 43.17)		(0.53, 40.90)		(27.07, 38.37)		(0.53, 45.60)	

Abbreviations: CR, Complete Response; PR, Partial Response; SD, Stable Disease; PD, Progressive Disease; CBR, clinical benefit rate (CR + PR + SD ≥6 Months); ORR, Objective Response Rate (CR + PR); DCR, Disease Control Rate (CR + PR + SD); PFS, Progression-Free Survival; OS, Overall Survival; Month =30.4375 days.

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Table S4 Best ORR In Different Cohorts (Cutoff Date: December 31, 2020)

Efficacy	ALK+ (N=43)		ALK + TKI-naïve (N=32)		ALK + TKI-resistance (N=11)		ROS1+ (N =5)		Brain metastasis (N=16)	
	No.	%	No.	%	No.	%	No.	%	No.	%
CR	1	2.3	1	3.1						
PR	30	69.8	25	78.1	5	45.5			11	
SD, months										
2-6	4	9.3			4	36.4			2	
≥6	1	2.3			1	9.1	3	60		
PD	5	11.6	4	12.5	1	9.1	2	40	3	
NA	2	4.7	2	6.3						
ORR (95% CI)	72.1 (58.1 to 86.1)		81.3 (67.0 to 95.5)		45.5 (10.4 to 80.5)		0		68.8 (43.2 to 94.3)	
DCR (95% CI)	83.7 (72.2 to 95.2)		81.3 (67.0 to 95.5)		90.9 (70.7 to 111.2)		60		81.3 (59.8 to 102.7)	
CBR (95% CI)	74.4 (60.8 to 88.0)		81.3 (67.0 to 95.5)		54.5 (19.5 to 89.6)		60		68.8 (43.2 to 94.3)	
Median PFS, Months	18.23		25.73		4.14		8.51		7.59	
(95% CI)	(8.77 to 27.70)		(20.41 to 31.04)		(0.00 to 8.75)		(0.00 to 24.45)		(6.75 to 8.43)	
(Minimum, Maximum)	(0.46, 43.17)		(0.46, 43.17)		(1.15, 41.69)		(0.49, 16.62)		(0.49, 41.69)	
Median OS, Months	42.88		43.17		21.98		16.62		22.34	
(95% CI)	(23.43 to 62.32)		(26.31 to 60.04)		(5.56 to 38.40)		(0.00 to 49.78)		(12.36 to 32.32)	
(Minimum, Maximum)	(6.64, 45.60)		(8.61, 45.60)		(6.64, 42.88)		(0.53, 39.43)		(0.526, 42.88)	

Abbreviations: CR, Complete Response; PR, Partial Response; SD, Stable Disease; PD, Progressive Disease; CBR, Clinical Benefit Rate (CR+ PR + SD ≥6 Months); ORR, Objective Response Rate (CR + PR); DCR, Disease Control Rate (CR + PR + SD); PFS, Progression-Free Survival; OS, Overall Survival; Month = 30.4375days.

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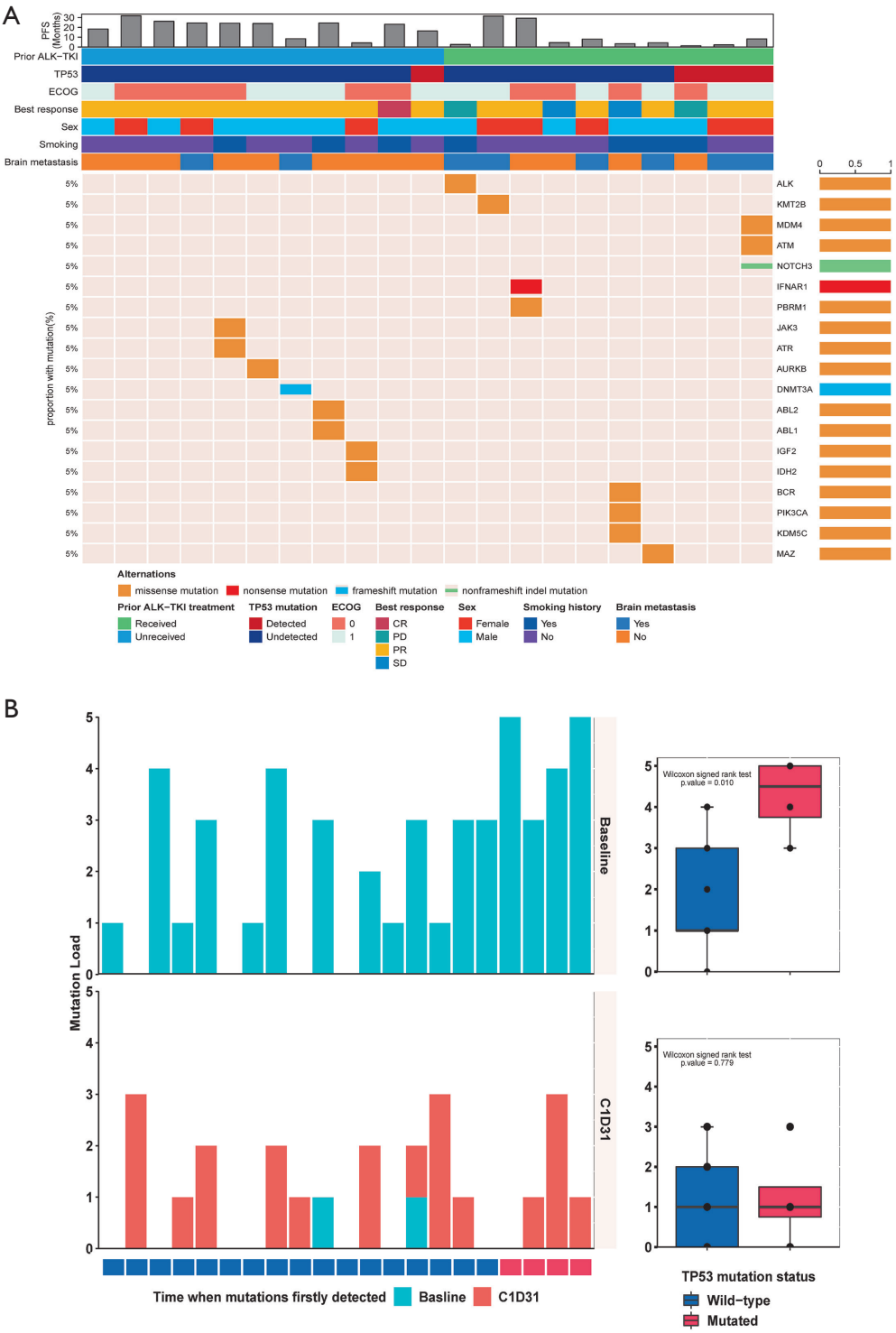

Figure S1 The mutation landscape at C1D31 and change of mutation load during ensartinib treatment. All the genes detected at C1D31 with nonsynonymous mutations(A). CR, complete response; PD, progression disease; PFS, progression-free survival; PR, partial response; SD, stable disease. Change of mutation load during ensartinib treatment. Change of mutation load during ensartinib treatment(B). The Bar graphs on the left show the mutation spectrums for each patient at baseline and C1D31. The box plots on the right display the mutation load of patients with and without baseline TP53 mutations.

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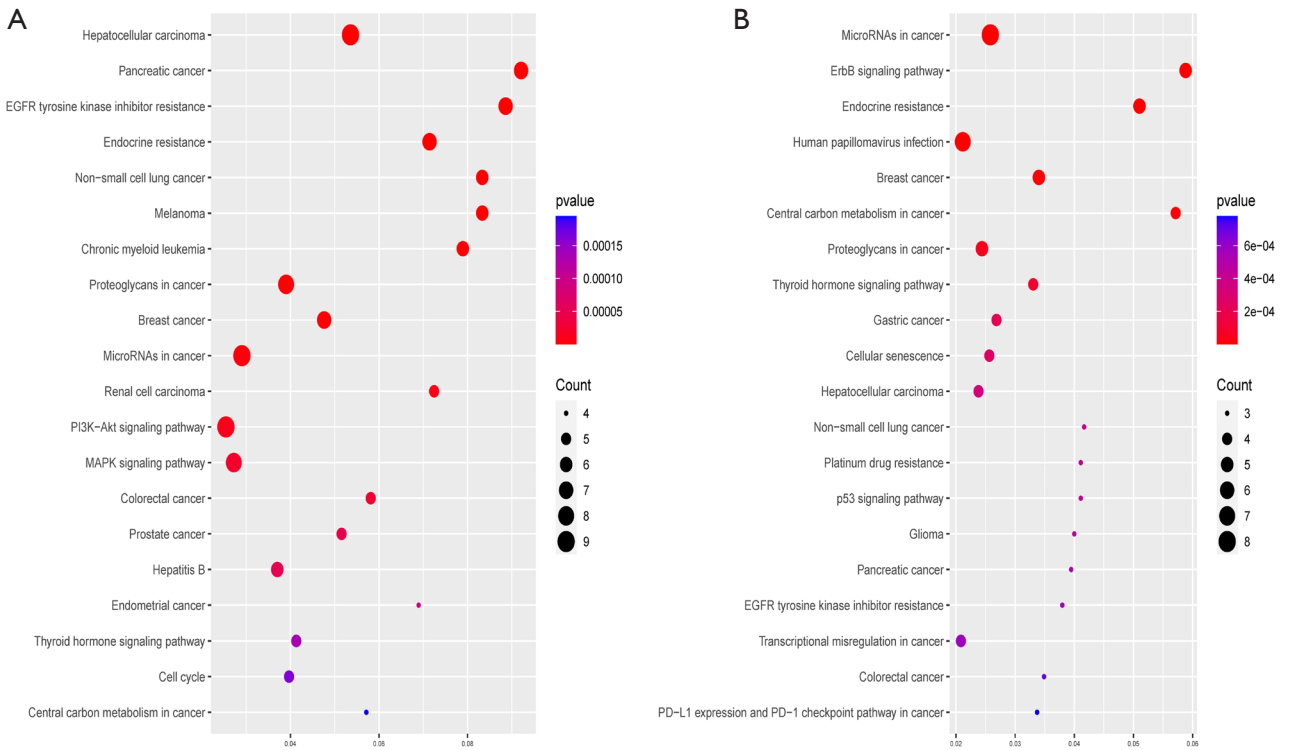

Figure S2 KEGG pathway enrichment analysis of genes detected with nonsynonymous mutations in long PFS and short PFS group. The pathway enriched in long PFS group (A) and the pathway enriched in short PFS group (B)

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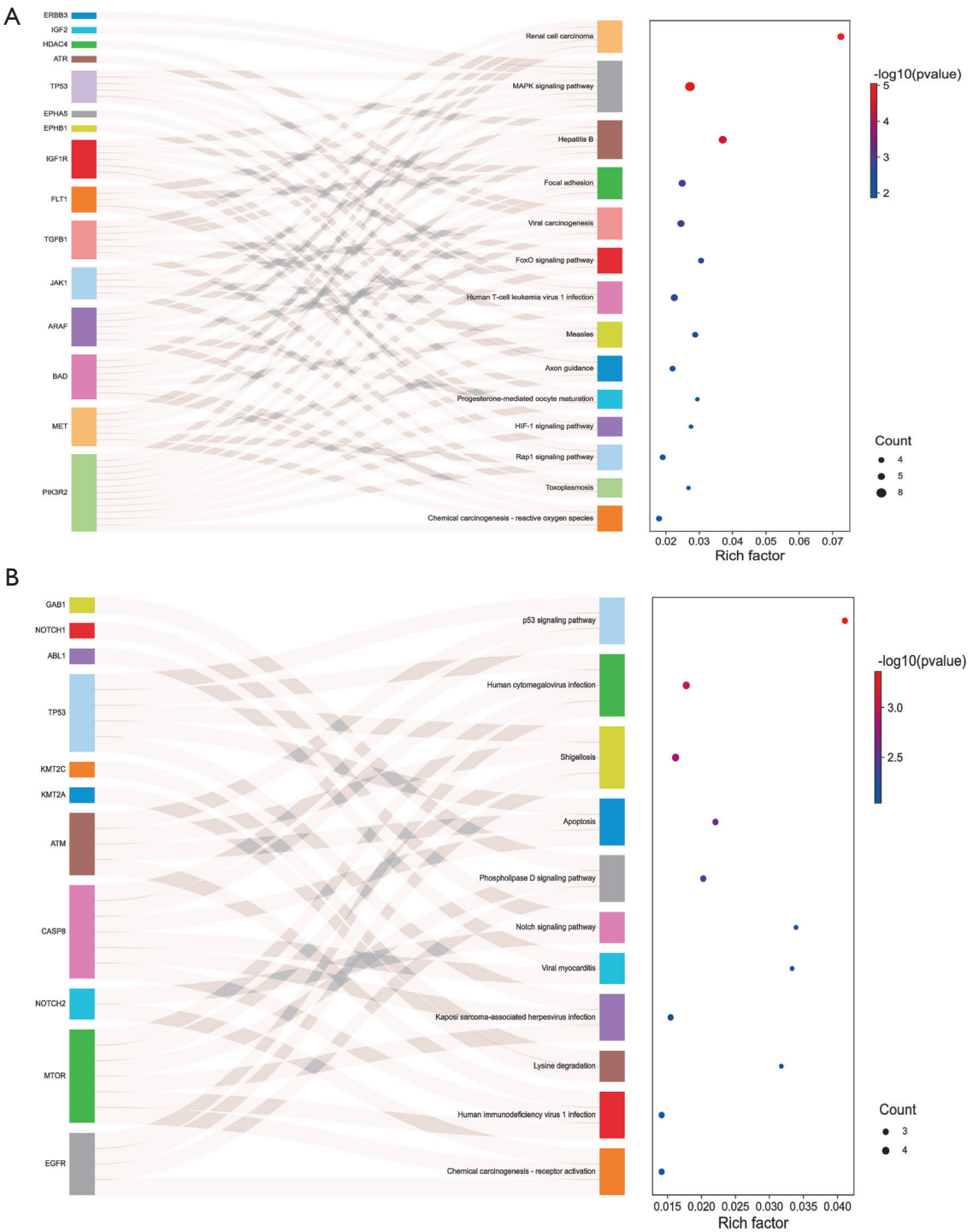

Figure S3 Specifically enriched pathways in long PFS and short PFS groups. The pathway enriched only in long PFS group but not in short PFS group(A) and the pathway enriched only in short PFS group but not in long PFS group (B).

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