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Reviewer A

Comment:
Ensartinib 225mg dose was already approved as a first-line treatment for ALK-positive non-small cell lung cancer in china. I think that further studies are not desired warranted to confirm the safety and PK of ensartinib in the Chinese ALK-positive NSCLC patients.
The key PK parameters and curative effect for ALK-positive NSCLC patients in China were presented at the 2018 ASCO annual meeting.
Results from a Phase III trial comparing ensartinib to Pfizer's Xalkori (crizotinib) was published in JAMA Oncology in September 2021, showed that ensartinib benefited patients more than Xalkori. This data is not attracting.
Reply: This is the first phase 1 trial to recruit Chinese NSCLC patients. Although the phase 2 study had been published earlier, the results of this phase 1 trial are also an indispensable part for scientists or clinicians to fully understand all-round of ensartinib.

Reviewer B

Ma et al report an interesting phase 1 trial of ensartinib. This is important study given the approval of ensartinib in China. However, the trial needs to follow standard reporting guidelines for phase 1 clinical trials. More comprehensive analysis of safety and TRAE is needed, as a phase 1 trial. The lack of discussion of TRAEs which were generally of greater incidence compared to eXalt-3 is a significant omission from the current paper. In addition, the exploratory analyses (biomarker and KEGG analysis) are very much exploratory and should not be emphasized in the discussion.

Major comments
1. Up to date introduction section
Although the design and recruitment of this trial likely predated the findings of eXalt-3, the Introduction section should be updated to describe the current state of the literature. This includes the regulatory approval of ensartinib in China. The rationale for this study should also be more strongly elucidated – were any patients of Chinese ethnicity included on the prior phase 1 trial?

Reply 1: We have modified our text as advised in the Introduction section (see Page 6, line 15-24 and Page 7, line 1-2).
Changes in the text:
In a phase I/II study, the recommended phase II dose (RP2D) of ensartinib was established to be 225mg with the most frequently toxicities including rash (56%),

nausea (36%), pruritus (28%), and vomiting (26%); the objective response rate (ORR) was 60% and the median PFS was 9.2 months. 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 and possible biomarkers of ensartinib in Chinese advanced ALK-positive NSCLC patients were launched. These studies promoted the approval of Ensatinib in China. In the phase 2 and 3 study, ensartinib showed efficacy for patients with disease progression on crizotinib and superior efficacy to crizotinib in both systemic and intracranial disease. While the reports of dose escalation and RP2D decision, systemic PK and safety of different doses, pharmacodynamics (PD) of Chinese patients were still lacking to complete the story (Page 6, line 15-24).

Here, we report the results of safety, tolerability, PK, efficacy and possible PD biomarkers of ensartinib of a phase I dose escalation and expansion trial in Chinese advanced ALK or ROS1 positive NSCLC patients (Page 7, line 1-2).

2. Primary endpoints

Reporting guidelines for clinical trials and phase 1 trials should be adhered to. For example, this includes primary endpoint, secondary endpoints etc. should be made clear. Pre-specified statistical analysis plan should be described in much more detail. More detailed definitions of toxicities which would meet DLT criteria should be provided. Protocol defined imaging intervals (not approximations) should be provided.

Reply 2: We have revised the manuscript adhere to the reporting guidelines. We updated endpoints, statistical analysis plan, definitions of toxicities and more protocol details in our study. We have modified our text as advised in Abstract section (see Page3, line 9-10) and the Methods section(see Page8, line 1-7,line 15-16 and Page 9, line 17-20).

Changes in the text:

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(Page3, line 9-10).

A DLT was defined as any of the following ensartinib-related 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. The objective of this trial was to determine the DLT, MTD and RP2D of ensartinib based on safety, tolerability and PK analyses(Page8, line 1-7).

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 (EAS), which included patients who received at least one dose the study drugs and has at least one post-baseline tumor assessment(Page 9, line 17-20).
Response evaluation scans were obtained at 8-week intervals, and assessed according to RECIST v1.1 by the investigators (Page 8, line 15-16).

3. MTD, TRAE, safety

As a phase 1 trial, the primary aim is to determine the MTD and RP2D. Consequently, much more detailed information and discussion of TRAEs and safety is required. In particular, a comprehensive comparison with the prior global phase 1/2 trial and phase 3 trial is needed. In fact, the incidence of rash was much higher in this trial (87.5%) compared to eXalt-3 (67.8%). Elevated AST/ALT, pruritis etc were all much higher – however this is not discussed nor even mentioned.

Reply 3: We have modified our text as advised (see Page 12, line 24 ; Page 13, line 1-8, 21; Page 14, line 1-5).

Changes in the text:

In this phase I study, we found that ensartinib ≤225mg once per day was safe and well tolerated in Chinese NSCLC patients. The dose escalation results stated the rationale of choosing 225mg daily as the RP2D. Also, systemic PK results indicated that the AUC of ensartinib reached saturation at 225mg which support our decision too. 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 also reported for ensartinib from phase I/II/III multicenter studies [13-15]. All instances were successfully managed by withholding or reducing the dose.

Ensartinib once again demonstrated satisfactory efficacy in patients with ALK positve NSCLC patients(Page 12, line 24 ; Page 13, line 1-8, 21).

It’s worth mentioning that in this study, the ORR and DCR in 16 patients with baseline brain metastases were higher than those of crizotinib, ceritinib, alectinib and brigatinib[9, 23, 28, 29]. In our study, ensartanib was found partially penetrated the blood-brain barrier after administration, thus ensartinib showed potential of targeting brain metastases lesion. This was consistent with previous results[14, 15]. At baseline, patients with TP53 mutation had higher gene mutation load, which was reported associated with primary resistance to crizotinib in ALK+ NSCLC[35](Page 14, line 1-5).

4. Exploratory analyses

Analyses such as the biomarker analyses in Fig 3 and KEGG analysis in Fig 4 is exploratory at best – and would be more suited to Supplementary. As a phase 1 trial with different doses, the study is underpowered to detect significant associations. In particular, the KEGG analysis uses an arbitrary cutoff of 9 months, and the lack of orthogonal validation of findings and analysis which was not prespecified in the

analysis plan – taken together means this should not be highlighted as key findings.

Reply 4: We have moved Figure 4 to Supplementary Materials as Supplementary FigureS7. In order to better understand the patient's genetic background and explain the correlation between ALK fusion abundance and efficacy of ensartinib, we retained Figure3 in our paper.

Minor comments

1. Fig 2B – waterfall plot should be ordered in descending order
2. Fig 3A – text is too small

Reply: Changes as advised had been complited in Fig2B and Fig 3A.