nature portfolio

Peer Review File

Intravenous liposomal irinotecan in metastatic triple-negative breast cancer after ≥ 2 prior lines of chemotherapy: a phase Ib study

Corresponding Author: Professor Binghe Xu

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
- Did any patients receive ADC with irinotecan as pay-load prior to treatment with single-agent irinotecan, how do their response rates differ if at all? In current treatment paradigm for mTNBC, irinotecan would not generally be used until after progression of SG, so further investigation needed to understand efficacy in this setting.

- Please disclose/describe racial/ethnic background of patients enrolled - what is the make-up of asian, caucasian etc. as this effects applicability of results to other patient populations

- Please introduce concept of UGT1A1 homozygosity/compound heterozygosity, single heterozygous, wild type expression prior to discussing exploratory analysis results for context

- Would not describe ER and/or PR 1-10% expression as a weakness of the analysis, as this cohort is being included more and more in clinical trials and is appropriate given disease behaves as if TNBC in this setting. Would consider this an inclusive strength of the trial.

- It is beneficial to know irinotecan can be used safely as single-agent for our mTNBC with some efficacy, however mPFS of around 5 months is not enough to impact the lives of our patients in the clinical setting, of course, as unfortunately we have found with all single-agent chemo options and we need to do better. Recommend suggesting future trial ideas with combinations with irinotecan, now that we understand safety data from this important phase I study to potentially increase efficacy and response? Can we use with ADCs, immunotherapy, other doublet-chemotherapy options, how could this be further evaluated?

Reviewer #2

(Remarks to the Author)
Thank you for this important phase 1b study of the liposomal irinotecan agent HE072 in patients with heavily pretreated triple-negative breast cancer (TNBC) as well as patients with HER2- breast cancer and CNS metastasis. The study demonstrates a similar toxicity profile with HE072 compared to Onivyde monotherapy from the NAPOLI-1 trial and, with respect to clinical outcomes, a similar median PFS and median OS to sacituzumab govitecan, which also exerts cytotoxic effects via SN-38, and was tested and approved in a patient population with similarly pretreated metastatic TNBC. Overall, the manuscript is well-written. Questions and comments are below:
General comments:
- This is a very interesting trial and results. The key question it raises is the optimal means of treating TNBC patients with irinotecan / SN-38, where the current treatment algorithm for pretreated TNBC includes SG, which you address in the Discussion but warrants additional focus.
1) Please clarify if patients with prior topo-1 inhibitor-based ADC were eligible, particularly SG; if so, please include in Table 1. On a related note, the supplementary appendix does clarify that patients were excluded if they had prior topoisomerase-1

treatment including irinotecan or other investigational agents; however, for the readers's clarity this bears mentioning at least once in the main manuscript as well.
2) Please include this concept and framing in the Introduction.
- The limitations should include that few TNBC had received prior ICI nor ADC, making this a less generalizable population.
- Please have the manuscript edited for English grammar - it is well-written, but there are minor mistakes or omissions throughout.
Regarding patient population and demographics table:
- There are two Table 1's included with the manuscript – one details patient demographics and disease characteristics, and the other summarizes TRAEs after HE072 in patients with HER2 negative breast cancer brain metastases (HER2- BCBM). The latter might be renamed and/or moved to the supplementary table section; it also does not appear to be referenced in the manuscript.
- In Table 1 (Demographics and baseline characteristics). How did the patients with ER>10% get included in the TNBC cohort? Would clarify in the table instead of in the footnote, address in Results, and clarify that they are not included in EAS cohort.
- In Table 1, the number of metastatic sites is labeled as ≤3 and >3; whereas in Figure S2 it is reported as <4 and ≥4; and in the methods section under Statistical Analysis (line 320) it is incorrectly reported as <3 and ≥4. Would recommend these labels be corrected and kept consistent.
- Consider including de-novo versus recurrent metastatic disease in Table 1.
- What is the breakdown of patients based on site of metastatic disease? (eg. how many had metastases to liver, bone, etc.; or visceral vs non-visceral)?
Regarding correlative data:
- Between Table 3 and Figure 2B, the Cmax value of free irinotecan of 162.8 ng/mL at 50 mg/m2 does not appear to agree with what is shown in the figure, which appears to be showing a value less than 150 ng/mL.
- In Table 3, the reported Cmax of SN-38 at 50 and 70 mg/m2 is 4.1 and 5.6 ng/mL, respectively; however, in the pharmacokinetics section, the units of the SN-38 Cmax at 70 mg/m2 are incorrectly reported as 5.6 µg/mL.
- Between Table 3 and Figure 2C, the SN-38 Cmax values of 4.1 and 5.6 ng/mL do not appear to agree with what is displayed in Figure 2C, which appear to show Cmax values less than 4 ng/mL.
- Exploratory analysis section does not stipulate that it refers to UGT1A1 alleles, forcing the reader to look down to Methods to figure out what genotyping is being studied. Please rectify.
- Please clarify which Topo 1 Ab was used for immunostaining.
Regarding clinical efficacy and safety:
- Among patients who received dose reductions, what were the dose reduction levels?
- In the efficacy section and in Table 4, compared to Figure 1 and the subgroup analysis in Figure S2, the denominators differ (89 in the efficacy section and in Table 4, 95 in Figure 1, and 101 in Figure S2). Please clarify.
- The text in Results/safety should focus on AEs in the RPh2D (70mg/m2) dose level, not the combined 50mg/m2 + 70mg/m2 cohorts.
- Regarding the determination of RPh2D, the explanation for why the 3rd dose level of 90mg/m2 was not pursued remains opaque. Please clarify.
- Efficacy, bottom page 5, reports early closure of the BCBM cohort because only 1/18 had ORR, but Figure S2 reports 2 responders out of 8. 1) Where did the other responder come from? 2) Who are the 8 patients if the evaluable subgroup included 12 TNBC with BCBM?

Reviewer #3

(Remarks to the Author)
The author presented an open-label Phase 1b trial to evaluate the safety, tolerability, and preliminary anti-tumour activity of irinotecan liposome in patients with advanced breast cancer. They reported that HE072 showed acceptable safety profile and promising antitumor activity in heavily pre-treated patients with mTNBC was observed, which warrants further validation.

1) There are however several methodological concerns due to insufficient information being provided for the statistical methods/analysis:
• The authors specified in their protocol that the study used a 3+3 design and incorporated 3 dose levels, with up to 36 patients being needed in the Dose Escalation study component (Part 1) and up to 100 patients in the Dose Expansion cohort(Part 2).
They reported: "Once a dose level has been determined to have not exceeded the MTD, up to six to nine additional subjects may be enrolled for dose expansion to provide additional safety information on that dose level." The total numbers at 50mg/m2 and 70mg/m2 dose levels show contradiction with what they have reported in the protocol – "at the same time, 50 mg/m2 and/or 70 mg/m2 will be selected to enrol a certain number of subjects in each group (in addition to the subjects in the dose escalation period, 12 to 15 subjects in each group or no more than 30 subjects in total)", and also deviates from their planned design (which we would expect to be 3 or 6 at the initial doses). Please explain the deviation from the planned 3+3 design and the contradiction.
• They mentioned that the total sample size was expected to be up to approximately 136 treated patients with 36 for the dose escalation cohort and 100 for the dose expansion cohort. They specified that the sample size was not determined based on statistical assumption, so how was the sample size of 100 determined? Please clarify.
• Please specify the number of patients in each cohort of expansion part in the CONSORT flow diagram (Figure S1). The losses or exclusions (with reasons) after allocation to each dose level should also be clearly presented.
• The author reported: "In the dose escalation and expansion part, 3+3 design was used to investigate three dose levels of HE072 (50 mg/m2, 70 mg/m2 and 90 mg/m2)". Please provide the rationale of planned dose levels.
• Definitions of analysis population sets for their key objectives, e.g., for safety, dose-escalation, and other key endpoints

should be reported (in the main paper or appendix).
- Please provide the definition of DLT evaluable patients in the Method part.
- Please specify the primary/secondary/exploratory endpoints in the Outcome part. The author didn't mention Duration of response (DoR) and time to response (TTR) in the Outcome part but they're in the table. Are they secondary endpoints?
- Please provide definition of analysis population sets (if any) in the main context of the paper. The definition of Safety analysis set and DLT evaluable set are currently missing in the main paper.

2) Statistical methods for additional analyses are inadequate. The authors mentioned in the protocol (provided as a supplementary document) that statistical analysis plan (SAP) would be a separate document and would include a more technical and detailed information on the subgroup analysis. However, the SAP was not provided with the submitted article. Hence, information on the implemented statistical approaches for subgroup analysis is missing and remain unclear in both the report and protocol.

3) The authors are requested to provide information on the following points:
• For each group, losses and exclusions after allocation to each dose level, together with reasons
• Composition of any decision making or safety review committee or group; summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details can be found (such as in a charter or protocol)
• Description of who had access to interim results and made the interim and final decision to terminate the trial (or part(s) of the trial, eg, end of dose-escalation), and measures to safeguard the confidentiality of interim information

Additionally, it is highly encouraged that the authors complete the CONSORT-DEFINE checklist (https://www.bmj.com/content/383/bmj-2023-076387/related) to ensure all essential contents required for the reporting of phase I trials are included. Completing this CONSORT checklist will ensure comprehensive reporting, enhance the manuscript quality, and facilitate peer review and reproducibility.

4) The abstract could be made more informative to include the dose escalation design. The introduction part shall also mention specific objectives (eg, relating to safety, activity, pharmacokinetics, pharmacodynamics, recommended dose(s)) of the trial.

5) The author reported: "As of June 5 2023, 89 (88.1%) of the 101 patients with TNBC were evaluable for response". However, according to Figure 1, TNBC (N=74) are included in the expansion cohort and EAS(N=95) are analysed, according to Figure 4 (Efficacy of of HE072 in patients with TNBC and Triple negative BCBM), TNBC (N=78) are with MTD. please explain the deviations.

6) I believe it's a typo in the footnote of Table 4: TOR-time to response, which shall be changed to TTR.

Reviewer #4

(Remarks to the Author)
The authors report their findings from a phase I study exploring the safety and preliminary efficacy of liposomal irinotecan (HE072) in patients with metastatic triple-negative breast cancer (mTNBC) after ≥ 2 prior lines of chemotherapy.

The authors report some activity of this agent in these patients in terms of ORR, duration of response and also include PFS and OS data. Further investigation is suggested based on the response rates seen here in a heavily pretreated population. The authors also justify the rationale for MTD.

While the title of the manuscript clearly focuses of patients with metastatic triple negative breast cancer, the authors have also included patients with brain metastases, which possibly takes the focus away from the primary results and may not actually be needed. Suggest removing these patients from the study or else include one section with a sub analysis and reporting these findings, although as mentioned I am not sure this is needed. Consider also adding the term "Intravenous" into the title to provide further clarification.

Consider adding more about the methods within the abstract, including dosing schedules, routes of administration and primary endpoint.

Consider revision of some language within the Introduction (Compared to other type of breast cancer, TNBC can't benefit from endocrine therapy and human epidermal growth factor receptor (HER) 2 targeting therapy and have limited treatment options.) and other sections ("The waterfall plot also showed very nice tumor shrinkage").

The Methods section should also be moved up, placed after the introduction and preceding the Results section. Also Line 276 when describing dose reductions, were they permanent or temporary?

Some additional clarification is required in the Results section. Line 78 refers to 18 pts with HER2 negative BCBM, and the accompanying tables state that some of these may have low ER/PR expression. Table 1 also reports only 87% were ER/PR<1%. Did any of these patients receive endocrine therapy? If not this should be stated within the manuscript.

The authors have provided a detailed description of adverse events and safety, reporting 45% incidence of Grade 3 or

higher AEs, however the abstract reports an acceptable safety profile. Were patient reported outcomes collected? Additional information regarding missed doses, dose reductions, dose delays, dose interruptions and dose discontinuation should be included. Consider adding an additonal table with this information.

Reviewer #5

(Remarks to the Author)
I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Reviewer #6

(Remarks to the Author)
I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
This study offers safety data for new treatment option for patients with mTNBC, area of great unmet need, with liposomal irinotecan (HE072) after ≥ 2 prior lines of chemo. ARound 25% patients had overall response- the disease control rate was 67.8%, median PFS and OS were 4.8 months and 14.1 months which are unfortunately on-par with average single-agent chemotherapy (maybe a bit better than single-agent gemcitabine etc. which is around 3 months mPFS).

Reviewer #2

(Remarks to the Author)
Thank you for your extensive revisions to this phase 1b study of the liposomal irinotecan agent HE072 in heavily pretreated triple-negative breast cancer (TNBC) as well as patients with HER2- breast cancer and CNS metastasis. The manuscript is well-written, and we appreciate the changes and responses you made to feedback from the reviewers. A few minor questions and comments are below:
- Regarding comparison to SG, we appreciate your explanation in your rebuttal regarding how the timing of approval in SG in China played a role in why no patients on this study received prior SG. However, you would do well to state this explicitly in the discussion of the paper as well.
- In Line 172, reference 7 is fine as a reference to a preclinical trial of liposomal vs non-liposomal irinotecan, but if there is preclinical work comparing HE072 to Onivyde as you state in the manuscript, it needs to be referenced here.
The English grammar overall is much improved, but there still remain minor mistakes and omissions. Suggestions include:
1. Line 68 – "…thus increasing…and allowing more irinotecan to accumulate in the tumor zone.
2. Line 80 - Results should be plural
3. Line 88 – …inhibitors, and 23.8%...
4. Line 123 – "of the R2PD" (missing space)
5. Line 175 – "…of an immune checkpoint inhibitor."...For patients after first-line therapy (the patient did not fail the therapy).
6. Line 176 – "…first-line therapy, few treatment options are limited…"
7. Line 183-184 – "In the ASCENT trial13, the antibody-drug conjugate SG significantly improved…"
8. Line 194 – "In total, nine patients with…"

Reviewer #3

(Remarks to the Author)
By addressing our concerns, the author has significantly improved the methodology standards of this article. No further comments then.

Reviewer #6

(Remarks to the Author)
I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Reviewer #7

(Remarks to the Author)

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Response to Reviewer #1:

- Did any patients receive ADC with irinotecan as pay-load prior to treatment with single-agent irinotecan, how do their response rates differ if at all? In current treatment paradigm for mTNBC, irinotecan would not generally be used until after progression of SG, so further investigation needed to understand efficacy in this setting.

Response: No patient in this trial previously received treatment with ADC with irinotecan as pay-load due to the following reasons: firstly, according to the protocol, patients who previously received treatment with irinotecan, topotecan, or any other topoisomerase I inhibitors would be excluded from this study; secondly, all the patients of this study were enrolled between May 2021 and December 2022, while sacituzumab govitecan was approved in China for treatment of locally advanced and metastatic TNBC in June 2022. We agree that further study is needed to evaluate the efficacy of HE072 in the patients who experienced progression following the treatment of sacituzumab govitecan.

- Please disclose/describe racial/ethnic background of patients enrolled - what is the make-up of Asian, Caucasian etc. as this effects applicability of results to other patient populations

Response: All 119 patients in this trial were Asian, of which 95.8% of patients (114/119) were Han nationality, others were Man (*n*=2), Hui (*n*=1), Zhuang (*n*=1) and Mengolia (*n*=1). We have added the limitation accordingly.

Change in manuscript:

Other limitations included patient recruitment solely in China, our findings extrapolation beyond Asian to other patient populations need to be determined. (Line 263-265, Page 9)

- Please introduce concept of UGT1A1 homozygosity/compound heterozygosity, single heterozygous, wild type expression prior to discussing exploratory analysis results for context

Response: We deeply appreciate your insightful suggestion, and have added the concept of UGT1A1 homozygosity/compound heterozygosity, single heterozygous, wild type expression prior to discussing exploratory analysis results.

Change in manuscript:

In this trial, the homozygosity/compound heterozygosity group included patients with UGT1A1*6/*6, UGT1A1*28/*28 or UGT1A1*6/*28; single heterozygous group included patients with UGT1A1*1/*6; wild type group included patients with UGT1A1*1. (Line 243-245, Page 9)

- Would not describe ER and/or PR 1-10% expression as a weakness of the analysis, as this cohort is being included more and more in clinical trials and is appropriate given disease behaves as if TNBC in this setting. Would consider this an inclusive strength of the trial.

Response: We are grateful for the valuable suggestion, and have removed the statement on ER and/or PR 1-10% expression from the limitation section.

Change in manuscript:

~~Second, ER and/or PR 1-10% were found in 11 patients, as the diagnosis of TNBC in our study was based on the ASCO-CAP guidelines with an immunohistochemical expression of <1% for the~~

~~definition of ER/PR negativity. However, it had been noted that HER2-negative, ER and/or PR 1-10% displays similar clinical characteristics and outcomes to TNBC 29, thus inclusion of this group in TNBC clinical trials might be acceptable.~~ (Line 257-261, Page 9)

- It is beneficial to know irinotecan can be used safely as single-agent for our mTNBC with some efficacy, however mPFS of around 5 months is not enough to impact the lives of our patients in the clinical setting, of course, as unfortunately we have found with all single-agent chemo options and we need to do better. Recommend suggesting future trial ideas with combinations with irinotecan, now that we understand safety data from this important phase I study to potentially increase efficacy and response? Can we use with ADCs, immunotherapy, other doublet-chemotherapy options, how could this be further evaluated?

Response: Thank you for your comments, mTNBC is a subtype of breast cancer with very poor prognosis and has very limited treatment options. New agents are highly anticipated. For heavily-pretreated mTNBC, in ASCENT trial when for mTNBC patients with at least 2 lines of treatment previously, TROP2 ADC Sacituzumab Govitecan achieved 5.5 months of PFS and for single-agent chemotherapy, the PFS was only 1.7 months. So a PFS of 5 months from HE072 monotherapy in this trial is very promising. However, this is only phase Ib study, future studies are planned to examine the efficacy of HE072 in combination with other therapy (platinum or immunotherapy) in patients with metastatic TNBC.

No changes in manuscript.

Response to Reviewer #2:

Thank you for this important phase 1b study of the liposomal irinotecan agent HE072 in patients with heavily pretreated triple-negative breast cancer (TNBC) as well as patients with HER2- breast cancer and CNS metastasis. The study demonstrates a similar toxicity profile with HE072 compared to Onivyde monotherapy from the NAPOLI-1 trial and, with respect to clinical outcomes, a similar median PFS and median OS to sacituzumab govitecan, which also exerts cytotoxic effects via SN-38, and was tested and approved in a patient population with similarly pretreated metastatic TNBC. Overall, the manuscript is well-written. Questions and comments are below:

General comments:

- This is a very interesting trial and results. The key question it raises is the optimal means of treating TNBC patients with irinotecan / SN-38, where the current treatment algorithm for pretreated TNBC includes SG, which you address in the Discussion but warrants additional focus.

1) Please clarify if patients with prior topo-1 inhibitor-based ADC were eligible, particularly SG; if so, please include in Table 1. On a related note, the supplementary appendix does clarify that patients were excluded if they had prior topoisomerase-1 treatment including irinotecan or other investigational agents; however, for the readers' clarity this bears mentioning at least once in the main manuscript as well.

Response: We appreciate your valuable suggestion, and have added the statement accordingly.

Change in manuscript:

Exclusion criteria were previous treatment with irinotecan, topotecan, or any other topoisomerase I inhibitors; previous treatment with…(Line 287-288, Page 10)

2) Please include this concept and framing in the Introduction.

Response: We appreciate your valuable suggestion, and have added the statement accordingly.

Change in manuscript:

Compared to other type of breast cancer, TNBC can't benefit from endocrine therapy and human epidermal growth factor receptor (HER) 2 targeting therapy and have limited treatment options. Sacituzumab govitecan (SG) has been approved for treatment of unresectable locally advanced or metastatic TNBC who have received at least two systemic therapies in China in June 2022…… (Line 57-59, Page 3)

- The limitations should include that few TNBC had received prior ICI nor ADC, making this a less generalizable population.

Response: Thank you for your suggestion, we have added the limitations accordingly.

Change in manuscript:

Secondly, few patients (20.8%) have previously received treatment with checkpoint inhibitors and no patient received treatment with SG, thus further investigations are needed to explore the efficacy of HE072 in these patients. (Line 261-263, Page 9)

- Please have the manuscript edited for English grammar - it is well-written, but there are minor mistakes or omissions throughout.

Response: Thak you for your kind reminder. We have checked throughout the manuscript carefully, and make the corrections accordingly.

Change in manuscript:

About 45% of patients diagnosed with advanced TNBC will develop distant metastasis to the brain and/or visceral organs, with a median overall survival (OS) of 13.5-15.2 months[2,3]. (Line 53-55, Page 3)

Compared to other types of breast cancer, TNBC can't benefit from endocrine therapy and human epidermal growth factor receptor 2 (HER2) targeting therapy and has limited treatment options. (Line 55-57, Page 3)

Despite the development of various targeted therapies in recent years, cytotoxic chemotherapy remains the backbone of treatment for TNBC. (Line 58-59, Page 3)

The incidence of breast cancer brain metastasis (BCBM) is increasing with the longer survival of patients with breast cancer. (Line 60-61, Page 3)

The limited ability to cross the blood-brain barrier (BBB) for the antitumor agents represents a great challenge for patients with BCBM. (Line 62-63, Page 3)

……

The exploratory endpoints were UGT1A1 gene polymorphism expressed as the frequencies of homozygosity/compound heterozygosity (UGT1A1*6/*6, UGT1A1*28/*28 or UGT1A1*6/*28), single heterozygous (UGT1A1*1/*6) and wild type and Topo I expression (negative/positive, based on the percentage of positive tumor cells, <30% interpreted as negative and ≥30% as positive[10]). (Line 335-329, Page 12)

Regarding patient population and demographics table:

- There are two Table 1's included with the manuscript – one details patient demographics and disease characteristics, and the other summarizes TRAEs after HE072 in patients with HER2 negative breast cancer brain metastases (HER2- BCBM). The latter might be renamed and/or moved to the supplementary table section; it also does not appear to be referenced in the manuscript.

Response: Thank you for your kind reminder. The latter table 1 was provided after article submission as the requested of the editor, and we have added the statement referenced the table in the revised manuscript and moved it to the revised supplementary appendix.

Change in manuscript:

The safety of HE072 in patients with HER2 negative BCBM was shown in Table S1. (Line 108-109, Page 4)

Change in supplementary appendix:

Table S1. Summary of TRAE (≥ 15%) and ≥ grade 3 TRAEs after HE072 treatment in patients with HER2 negative BCBM. (Page 11)

- In Table 1 (Demographics and baseline characteristics). How did the patients with ER>10% get included in the TNBC cohort? Would clarify in the table instead of in the footnote, address in Results, and clarify that they are not included in EAS cohort.

Response: We appreciate your insightful suggestion. Two patients with ER>10% were incorrectly enrolled in this trial. We have removed these two patients from EAS and provided the updated efficacy results in the revised manuscript.

Change in manuscript:

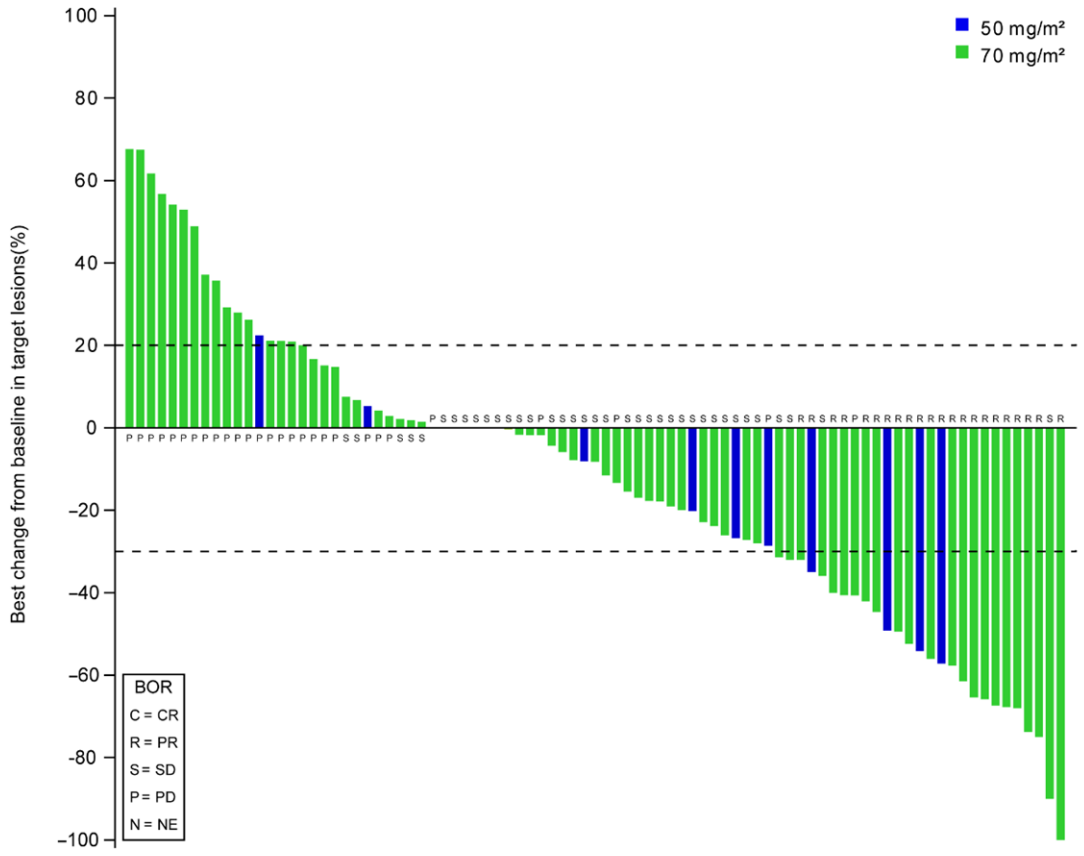
As of June 5 2023, 12 patients were not evaluable for response due to adverse events (*n*=6) or severe adverse events (*n*=3) or withdrawal of consent (*n*=3). Two patients with ER>10% had been excluded from efficacy evaluable set. Therefore, 87 (88.1%) of the 101 patients with TNBC were included in efficacy evaluable set. After a median follow-up of 10.1 months (range 8.8-10.8)……(Line 147-151, Page 6)

Table 4. Efficacy of HE072 in patients with TNBC and Triple negative BCBM (EAS)

	TNBC			Triple negative BCBM	
	50 mg/m²	70 mg/m²	Total	70 mg/m² (RECIST v1.1)	70 mg/m² (RANO-BM)
	n=11	*n*=~~78~~76*	*n*=~~89~~87*	*n*=12	*n*=12
Best overall response, *n* (%)					
Complete response	0	0	0	0	0
Partial response	4 (36.4)	18 (~~23.1~~23.7)	22 (~~24.7~~25.3)	1 (8.3)	0
Stable disease	3 (27.3)	34 (~~43.6~~44.7)	37 (~~41.6~~42.5)	5 (41.7)	4 (33.3)
Progressive disease	4 (36.4)	~~25.~~24 (~~32.1~~31.6)	~~29.~~28 (~~32.6~~32.2)	2 (16.7)	4 (33.3)
Not evaluable	0	~~1 (1.3)~~0	~~1 (1.1)~~0	4 (33.3)	4 (33.3)
ORR/CNS ORR, *n* (%)	4 (36.4)	18 (~~23.1~~23.7)	22 (~~24.7~~25.3)	1 (8.3)	0
95%CI	(10.9, 69.2)	(~~14.3~~14.7, ~~34.0~~34.8)	(~~16.2~~16.6, ~~35.0~~35.8)	(0.2, 38.5)	(0, 26.5)
DCR/CNS CBR, *n* (%)	7 (63.6)	52 (~~66.7~~68.4)	59 (~~66.3~~67.8)	6 (50.0)	4 (33.3)
95%CI	(30.8, 89.1)	(~~55.1~~56.8, ~~76.9~~78.6)	(~~55.5~~56.9, ~~76.0~~77.4)	(21.1, 78.9)	(9.9, 65.1)

(Table 4, Page 23)

Figure 3. Waterfall plot (a) and Kaplan-Meier progression-free survival (b) and overall survival (c) curves in the patients with TNBC. a. Based on BOR, 22 patients (25.3%) achieved PR, 37 patients (42.5%) achieved SD. b. The median PFS was 4·8 months (95%CI 3.2-5.8). c. The median OS was 14.1 months (95%CI 11.2-not reached), the OS rate was 59.3% (95%CI 47.7-69.1) at 12 months and 47.3% (95%CI 34.8-58.9) at 18 months.



(Figure legend and Figure 3, separate file)

- In Table 1, the number of metastatic sites is labeled as ≤3 and >3; whereas in Figure S2 it is reported as <4 and ≥4; and in the methods section under Statistical Analysis (line 320) it is incorrectly reported as <3 and ≥4. Would recommend these labels be corrected and kept consistent.
Response: We apologize for the careless expression, and have made the correction accordingly.

Change in manuscript:
Prespecified subgroup analyses (age [< 65 years, ≥ 65 years], number of previous systemic therapies [< 5, ≥ 5], ECOG PS [0, 1, 2], metastatic organs [<4, ≥ 4], previous treatment with PD-1/PD-L1 inhibitor [yes, no], CNS metastasis [yes, no]) of best overall response rate was planned. (Line 358-361, Page 12)

No. of metastatic sites				
~~≤3~~<4	10 (83.3)	67 (75.3)	77 (76.2)	14 (77.8)
~~>3~~≥4	2 (16.7)	22 (24.7)	24 (23.8)	4 (22.2)

(Table 1, Page 17)

- Consider including de-novo versus recurrent metastatic disease in Table 1.

Response: We have added the de-novo versus recurrent metastatic disease in Table 1.

Change in manuscript:

Disease status				
de-novo	0	0	0	10 (55.6)
Recurrent	12 (100)	89 (100)	101 (100)	8 (44.4)

(Table 1, Page 17)

- What is the breakdown of patients based on site of metastatic disease? (eg. how many had metastases to liver, bone, etc.; or visceral vs non-visceral)?

Response: We have added the number (percentage) of metastatic site in Table 1.

Change in manuscript:

Metastatic sites				
Lung	8 (66.7)	49 (55.1)	57 (56.4)	8 (44.4)
Bone	7 (58.3)	39 (43.8)	46 (45.5)	11 (61.1)
Lymph node	6 (50.0)	50 (56.2)	56 (55.4)	6 (33.3)
Liver	3 (25.0)	24 (27.0)	27 (26.7)	3 (16.7)
Brain	0	5 (5.6)	5 (5.0)	18 (100)
Others	7 (58.3)	44 (49.4)	51 (50.5)	5 (27.8)

Regarding correlative data:

- Between Table 3 and Figure 2B, the C_{max} value of free irinotecan of 162.8 ng/mL at 50 mg/m^2 does not appear to agree with what is shown in the figure, which appears to be showing a value less than 150 ng/mL.

Response: Thank you for your inquiry regarding the C_{max} value of free irinotecan. In this trial, the pharmacokinetic parameters were determined by noncompartmental analysis. According to the definition of pharmacokinetic parameter in noncompartmental analysis, individual C_{max} value was the actual maximum observed concentration measured, while the C_{max} value of 50 mg/m^2 dose group shown in Table 3 was calculated as the mean value of all individual C_{max} values in 50 mg/m^2 dose group. Since time of free irinotecan released from liposomal irinotecan was affected by many factors, individual C_{max} might occur in different timepoint. While the mean plasma concentration-time curve was drawn based on the mean concentration in the corresponding timepoint, that's why the calculated C_{max} value of free irinotecan seems to be inconsistent with the value shown in the Figure 2B.

No changes in manuscript.

- In Table 3, the reported C_{max} of SN-38 at 50 and 70 mg/m^2 is 4.1 and 5.6 ng/mL, respectively; however, in the pharmacokinetics section, the units of the SN-38 C_{max} at 70 mg/m^2 are incorrectly reported as 5.6 µg/mL.

Response: We apologize for the carelessness, and have made correction accordingly.

Change in manuscript:
The mean C_{max} and $AUC_{0-\infty}$ of total irinotecan and SN-38 with 70 mg/m^2 were 45.0 μg/mL and 1706.2 h*μg/mL, 5.6 ng/mL, and 427.9 h*ng/mL, respectively. (Line 128-130, Page 5)

- Between Table 3 and Figure 2C, the SN-38 C_{max} values of 4.1 and 5.6 ng/mL do not appear to agree with what is displayed in Figure 2C, which appear to show C_{max} values less than 4 ng/mL.
Response: Thank you for your inquiry regarding the C_{max} value of SN-38. In this trial, the pharmacokinetic parameters were determined by noncompartmental analysis. According to the definition of plasma parameter in noncompartmental analysis, individual C_{max} value was the actual maximum observed concentration measured, while the C_{max} value of 50 mg/m^2 dose group shown in Table 3 was calculated as the mean value of all individual C_{max} values in 50 mg/m^2 dose group. Since the metabolites process of irinotecan was affected by many factors, individual C_{max} value of SN-38 might occur in different timepoint. While the mean plasma concentration-time curve was drawn based on the mean concentration in the corresponding timepoint, that's why the calculated C_{max} value of SN-38 seems to be inconsistent with the value shown in the Figure 2C.

No changes in manuscript.

- Exploratory analysis section does not stipulate that it refers to UGT1A1 alleles, forcing the reader to look down to Methods to figure out what genotyping is being studied. Please rectify.
Response: We are grateful for the valuable suggestion, and have added the UGT1A1 accordingly.

Change in manuscript:
The frequencies of UGT1A1 homozygosity/compound heterozygosity, single heterozygous, and wild type were 10.9% (13/119), 26.9% (32/119) and 46.2% (55/119), respectively. (Line 174-175, Page 7)

- Please clarify which Topo 1 Ab was used for immunostaining.
Response: Topo I (C-21) antibody used in this trial was supplied by SANTA (SC-32736).

Change in manuscript:
Archival tumor tissue blocks (FFPE formalin-fixed, paraffin-embedded tumor tissue) at baseline were collected to examine Topo I expression by IHC assays (Topo-I antibody, SC-32736, SANTA, U.S.)……(Line 324-326, Page 11)

Regarding clinical efficacy and safety:
- Among patients who received dose reductions, what were the dose reduction levels?
Response: According to the protocol, two dose reductions was allowed, and the following was the recommended dose reduction for HE072.
Table 1. Recommended dose reduction for HE072

Initial dose	50 mg/m^2	70 mg/m^2	90 mg/m^2
First reduction	43 mg/m^2	50 mg/m^2	70 mg/m^2
Second reduction	Treatment discontinuation	43 mg/m^2	50 mg/m^2

No changes in manuscript.

- In the efficacy section and in Table 4, compared to Figure 1 and the subgroup analysis in Figure S2, the denominators differ (89 in the efficacy section and in Table 4, 95 in Figure 1, and 101 in Figure S2). Please clarify.

Response: We apologize for any confusion caused. In the efficacy section, we claimed that 89 patients and 14 patients with HER2 negative BMBC were evaluable for efficacy. Following your suggestion, we exclude two patients with ER>10% and present the efficacy of 87 patients with TNBC and 14 HER2 negative BMBC in the revised manuscript.

In Figure 1, 87 patients with mTNBC and 14 patients with HER2 negative BCBM were evaluable for efficacy, therefore, EAS included 101 patients.

In Figure S2, the subgroup analysis was conducted in all patients with mTNBC (*n*=101).

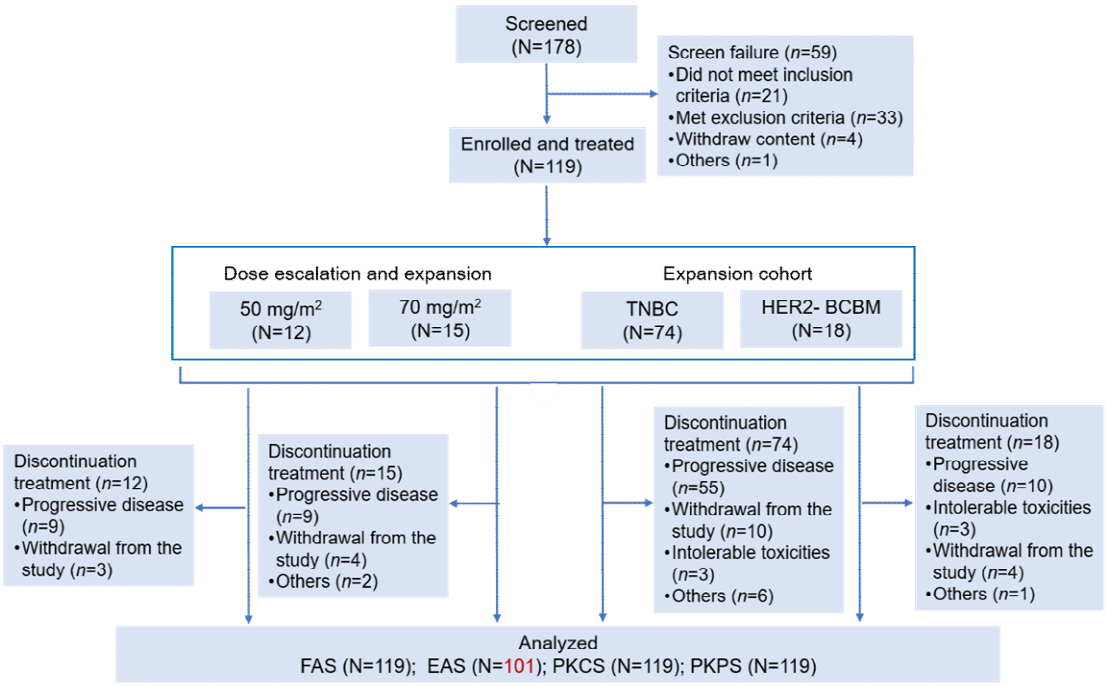
Change in manuscript:

As of June 5 2023, 12 patients were not evaluable for response due to adverse events (*n*=6) or severe adverse events (*n*=3) or withdrawal of consent (*n*=3). Two patients with ER>10% had been excluded from efficacy evaluable set. Therefore, 87 (88.1%) of the 101 patients with TNBC were included in efficacy evaluable set. After a median follow-up of 10.1 months (range 8.8-10.8)……(Line 147-151, Page 6)

Table 4. Efficacy of HE072 in patients with TNBC and ~~Triple~~ HER2 negative BCBM (EAS)

	TNBC			~~Triple~~ HER2 negative BCBM	
	50 mg/m²	70 mg/m²	Total	70 mg/m² (RECIST v1.1)	70 mg/m² (RANO-BM)
	n=11	*n*=~~78~~76*	*n*=~~89~~87*	*n*=~~12~~14	*n*=~~12~~14
Best overall response, *n* (%)					
Complete response	0	0	0	0	0
Partial response	4 (36.4)	18 (~~23.1~~23.7)	22 (~~24.7~~25.3)	1 (~~8.3~~7.1)	0
Stable disease	3 (27.3)	34 (~~43.6~~44.7)	37 (~~41.6~~42.5)	~~5~~8 (~~41.7~~57.1)	~~4~~6 (~~33.3~~42.9)
Progressive disease	4 (36.4)	~~25~~24 (~~32.1~~31.6)	~~29~~28 (~~32.6~~32.2)	~~2~~3 (~~16.7~~21.4)	4 (~~33,3~~28.6)
Not evaluable	0	~~1 (1.3)~~0	~~1 (1.1)~~0	~~4~~2 (~~33.3~~14.3)	4 (~~33.3~~28.6)
ORR/CNS ORR, *n* (%)	4 (36.4)	18 (~~23.1~~23.7)	22 (~~24.7~~25.3)	1 (~~8.3~~7.1)	0
95%CI	(10.9, 69.2)	(~~14.3~~14.7, ~~34.0~~34.8)	(~~16.2~~16.6, ~~35.0~~35.8)	(0.2, ~~38.5~~33.9)	(0, ~~26.5~~23.2)
DCR/CNS CBR, *n* (%)	7 (63.6)	52 (~~66.7~~68.4)	59 (~~66.3~~67.8)	~~6~~9 (~~50.0~~64.3)	~~4~~6 (~~33.3~~42.9)

(Table 4, Page 23)



(Figure 1, separate file)

- The text in Results/safety should focus on AEs in the RP2D (70 mg/m^2) dose level, not the combined 50 mg/m^2 + 70 mg/m^2 cohorts.

Response: We deeply appreciate the insightful suggestion, and have revised the safety results accordingly.

Change in manuscript:

At 70 mg/m^2 dose level (*n*=107), a total of 104 patients (97.2%) had one or more treatment emergent adverse event (TEAE) of any grade, and 50 patients (46.7%) had ≥ grade 3 TEAEs. Treatment related adverse events (TRAEs) occurred in 103 patients (96.3%, Table 2). The most common TRAEs included diarrhea (72/107, 67.3%), leukopenia (64/107, 59.8%), nausea (61/107, 57.0%), anemia (59/107, 55.1%), neutropenia (57/107, 53.3%), vomiting (54/107, 50.5%), hypokalemia (37/107, 34.6%), fatigue (35/107, 32.7%),weight loss (30/107, 28.0%), lymphocytopenia (27/107, 25.2%), γ-glutamyl transpeptidase elevation (26/107, 24.3%), decreased appetite (25/107, 23.4%), hyponatremia (23/107, 21.5%), increased ALT (22/107, 20.6%), and abdominal pain (22/107, 20.6%)(Table 2).

Serious TEAEs were reported in 25 patients (23.4%), of whom 20 patients (18.7%) experienced serious TEAEs related to HE072 (Table S2). TRAEs leading to permanent treatment discontinuation were reported in five patients (4.7%), including diarrhea (*n*=3), upper abdominal pain (*n*=1) and coma (*n*=1). (Line 97-113, Page 4)

- Regarding the determination of RP2D, the explanation for why the 3rd dose level of 90 mg/m^2 was not pursued remains opaque. Please clarify.

Response: 90 mg/m^2 was not explored in this trial based on the following considerations:

1. Preliminary efficacy (one PR at 50 mg/m^2, two PRs and one SD at 70 mg/m^2) were observed at 50 mg/m^2 and 70 mg/m^2 dose levels, the safety profile of HE072 was consistent with previous study.

2. Bioequivalence between HE072 and Onivyde has been established for total irinotecan, free irinotecan and SN-38 (NCT04482257). Exposure-response analysis of Onivyde (PMID: 28445610) showed that efficacy was associated with longer duration of SN-38 AUC, and there was significant association between an increased risk of AEs and total irinotecan and SN-38 C_{max}.
3. According to pharmacokinetics profile of HE072, C_{max} of SN-38 would increase significantly at a dose level of 90 mg/m^2, thus leading to an increased risk of safety. While the increment of SN-38 AUC was not greater than that of C_{max}, thus, the efficacy might not be improved with the increasing dose to 90 mg/m^2.

No changes in manuscript.

- Efficacy, bottom page 5, reports early closure of the BCBM cohort because only 1/18 had ORR, but Figure S2 reports 2 responders out of 8. 1) Where did the other responder come from? 2) Who are the 8 patients if the evaluable subgroup included 12 TNBC with BCBM?
Response: We apologize for any confusion caused. In this trial, subgroup analysis was conducted in patients with mTNBC, and there were 8 patients with mTNBC enrolled in the dose escalation and expansion part had CNS metastasis. As shown in Figure S2, of these 8 patients with mTNBC and CNS metastasis, two patients achieved partial response after HE072 treatment. While in cohort expansion part, 18 patients with HER2 negative BCBM were enrolled, and one patient achieved partial response. In Table 4, 12 patients with TNBC with BCBM were from HER2 negative BCBM cohort. To avoid any confusion, we have added the statement on the subgroup analysis population in the method section, and present the efficacy of HE072 in patients HER2 negative BCBM instead of TNBC with BCBM in result section and Table 4.

Change in manuscript:
Prespecified subgroup analyses (age [< 65 years, ≥ 65 years], number of previous systemic therapies [< 5, ≥ 5], ECOG PS [0, 1, 2], metastatic organs [< 4, ≥ 4], previous treatment with PD-1/PD-L1 inhibitor [yes, no], CNS metastasis [yes, no]) of best overall response rate in patients with metastatic TNBC was planned. (Line 358-361, Page 12)
Among 18 HER2 negative BCBMs, ~~12 were triple negative BCBMs.~~ the median PFS ~~of triple negative BCBMs~~ was 5.6 months (95% CI 1.4-NA), the median OS was not reached (95% CI 4.5-NA). (Line 44-46, Page 2)
~~Among 18 HER2 negative BCBMs, 12 were triple negative BCBMs.~~ As of June 5 2023, of 18 patients with HER2 negative BCBM. 1 patient (7.1%, 95% CI 0.2-33.9) achieved PR and 9 patients (64.3%, 95% CI 35.1-87.2) achieved disease control. The median PFS was 5.6 months (95% CI 1.4-NA), median OS was not reached (95% CI 4.5-NA) and 12-month OS rate was 55.5% (95% CI 27.4-76.5). (Line 167-171, Page 6)

Table 4. Efficacy of HE072 in patients with TNBC and ~~Triple~~ HER2 negative BCBM (EAS)

	TNBC			~~Triple~~ HER2 negative BCBM	
	50 mg/m²	70 mg/m²	Total	70 mg/m² (RECIST v1.1)	70 mg/m² (RANO-BM)
	n=11	*n*=~~78~~76*	*n*=~~89~~87*	*n*=~~12~~14	*n*=~~12~~14
Best overall response, *n* (%)					
Complete response	0	0	0	0	0
Partial response	4 (36.4)	18 (~~23.1~~23.7)	22 (~~24.7~~25.3)	1 (~~8.3~~7.1)	0
Stable disease	3 (27.3)	34 (~~43.6~~44.7)	37 (~~41.6~~42.5)	~~5.8~~ (~~41.7~~57.1)	~~4.6~~ (~~33.3~~42.9)
Progressive disease	4 (36.4)	~~25.~~24 (~~32.1~~31.6)	~~29.~~28 (~~32.6~~32.2)	~~2.~~3 (~~16.7~~21.4)	4 (~~33,~~28.6)
Not evaluable	0	~~1 (1.3)~~0	~~1 (1.1)~~0	~~4.~~2 (~~33.3~~14.3)	4 (~~33.3~~28.6)
ORR/CNS ORR, *n* (%)	4 (36.4)	18 (~~23.1~~23.7)	22 (~~24.7~~25.3)	1 (~~8.3~~7.1)	0
95%CI	(10.9, 69.2)	(~~14.3~~14.7, ~~34.0~~34.8)	(~~16.2~~16.6, ~~35.0~~35.8)	(0.2, ~~38.5~~33.9)	(0, ~~26.5~~23.2)
DCR/CNS CBR, *n* (%)	7 (63.6)	52 (~~66.7~~68.4)	59 (~~66.3~~67.8)	~~6.~~9 (~~50.0~~64.3)	~~4.~~6 (~~33.3~~42.9)

(Table 4, Page 23)

Response to Reviewer #3:

The author presented an open-label Phase 1b trial to evaluate the safety, tolerability, and preliminary anti-tumour activity of irinotecan liposome in patients with advanced breast cancer. They reported that HE072 showed acceptable safety profile and promising antitumor activity in heavily pre-treated patients with mTNBC was observed, which warrants further validation.

1) There are however several methodological concerns due to insufficient information being provided for the statistical methods/analysis:

• The authors specified in their protocol that the study used a 3+3 design and incorporated 3 dose levels, with up to 36 patients being needed in the Dose Escalation study component (Part 1) and up to 100 patients in the Dose Expansion cohort (Part 2).

They reported: “Once a dose level has been determined to have not exceeded the MTD, up to six to nine additional subjects may be enrolled for dose expansion to provide additional safety information on that dose level.” The total numbers at 50 mg/m^2 and 70 mg/m^2 dose levels show contradiction with what they have reported in the protocol – “at the same time, 50 mg/m^2 and/or 70 mg/m^2 will be selected to enroll a certain number of subjects in each group (in addition to the subjects in the dose escalation period, 12 to 15 subjects in each group or no more than 30 subjects in total)”, and also deviates from their planned design (which we would expect to be 3 or 6 at the initial doses). Please explain the deviation from the planned 3+3 design and the contradiction.

Response: We apologize for the word-to-word awkward translation. In the protocol, it should be “(including the subjects in the dose escalation period, 12 to 15 subjects in each dose group or no more than 30 subjects in total)”. Therefore, 3 or 6 subjects per dose would expect to be enrolled in the dose escalation part, another 6-9 subjects per dose would expect to be enrolled in the dose expansion part, a total of 12-15 subjects per dose level would be enrolled in the dose escalation and expansion part. In this trial, 12 patients in 50 mg/m^2 group and 15 patients in 70 mg/m^2 group were enrolled in the dose escalation and expansion part, which is consistent with the protocol.

In this trial, no DLT occurred at 50 mg/m^2 dose level, one DLT occurred at 70 mg/m^2 dose level. Therefore, the total number of patients enrolled in dose escalation was 9. One partial response was observed at 50 mg/m^2 dose level. Based on the safety and efficacy result of HE072 in the dose escalation period, the investigator and sponsor decided that a dose level of 90 mg/m^2 was not explored, and another 18 patients were enrolled in 50 mg/m^2 and 70 mg/m^2 for dose expansion.

Change in protocol:

If 70 mg/m^2 is tolerated, the investigator, in consultant with the sponsor, will decide whether to increase the dose to 90 mg/m^2 or a dose between 70 mg/m^2 and/or 90 mg/m^2; at the same time, 50 mg/m^2 and/or 70 mg/m^2 will be selected to enroll a certain number of subjects in each group (including the subjects in the dose escalation period, 12 to 15 subjects in each group or no more than 30 subjects in total). (Page 16, Page 74)

• They mentioned that the total sample size was expected to be up to approximately 136 treated patients with 36 for the dose escalation cohort and 100 for the dose expansion cohort. They specified that the sample size was not determined based on statistical assumption, so how was the sample size of 100 determined? Please clarify.

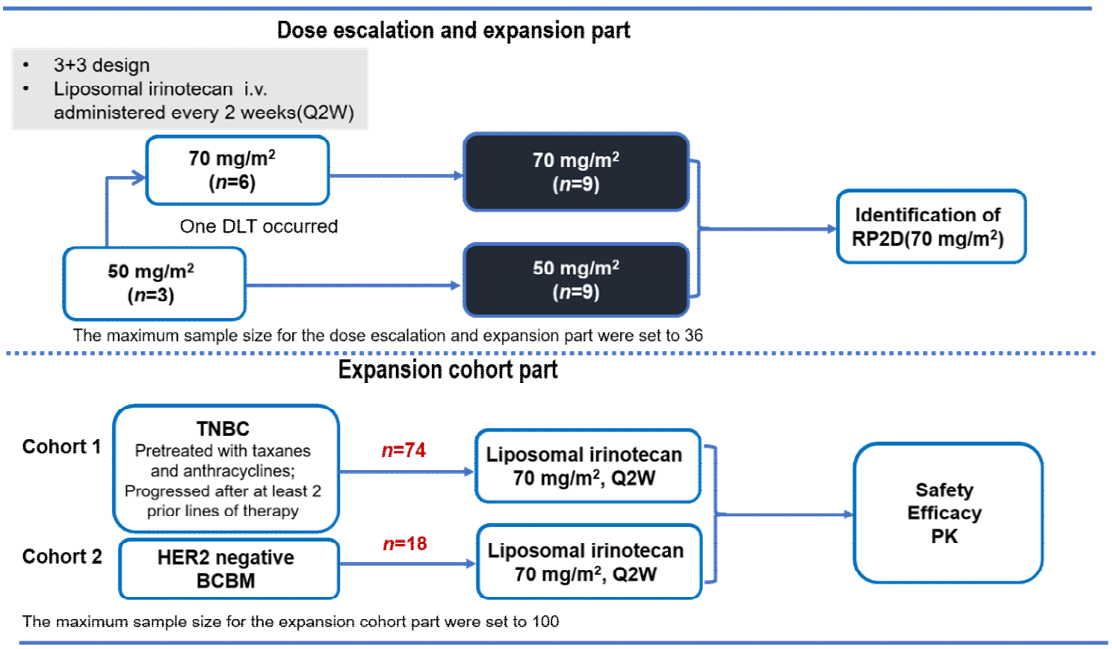
Response: In this trial, the sample size was not determined based on statistical assumption, 100 patients (50 per cohort) were enrolled in the cohort expansion part. 50 patients per each cohort expected to detect the preliminary efficacy of HE072. In this trial, 18 patients with HER2 negative BCBM were enrolled, due to lower ORR the enrollment of this cohort was terminated prematurely. While the efficacy of metastatic TNBC seemed to be promising, thus additional 24 patients with metastatic TNBC were enrolled.

No changes in manuscript.

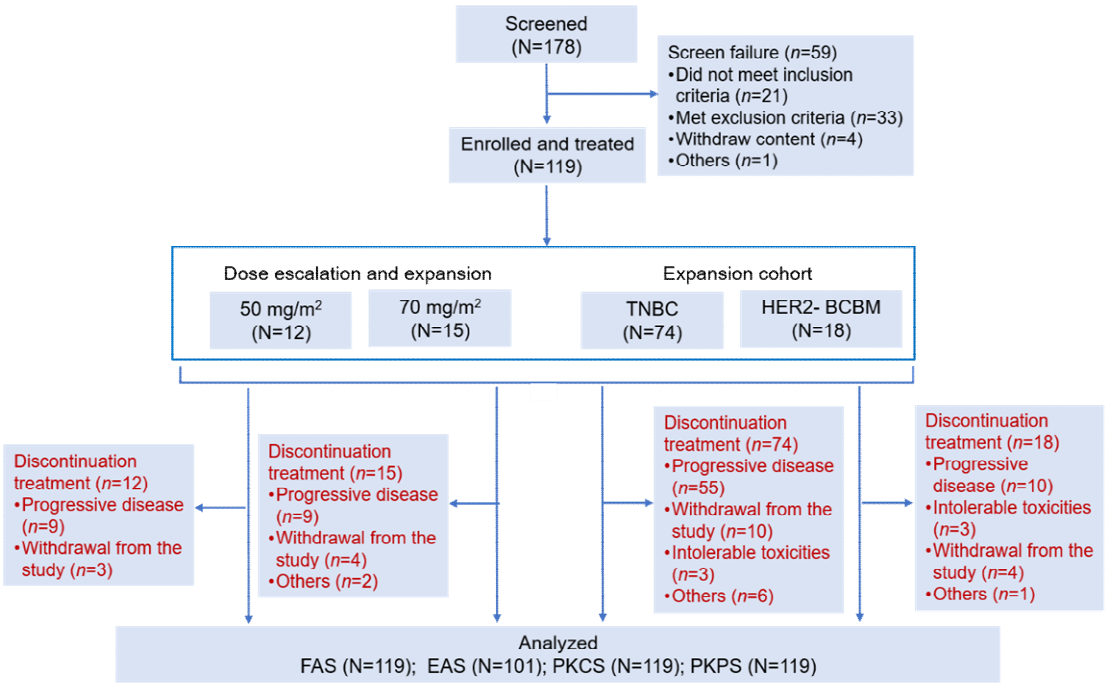
• Please specify the number of patients in each cohort of expansion part in the CONSORT flow diagram (Figure S1). The losses or exclusions (with reasons) after allocation to each dose level should also be clearly presented.

Response: We appreciate your valuable suggestion, and have added the number of patients in each cohort in Figure S1 and the treatment discontinuation reasons of each dose levels in Figure 1.

Change in manuscript and appendix:



(Figure S1, Appendix Page 6)



(Figure 1, Separate file)

• The author reported: "In the dose escalation and expansion part, 3+3 design was used to investigate three dose levels of HE072 (50 mg/m^2, 70 mg/m^2 and 90 mg/m^2)". Please provide the rationale of planned dose levels.

Response: Bioequivalence between HE072 and Onivyde has been established for total irinotecan, free irinotecan and SN-38 (NCT04482257). The starting dose (50 mg/m^2) was selected based on a combination of pharmaceutical/preclinical data of HE072 and dose in clinical study of Onivyde (NCT03328884).

Dose increment	Dose levels
/	50 mg/m^2
40%	70 mg/m^2
30%	90 mg/m^2

In this trial, dose increments were set as 40% and 30% rather than Fibonacci or half-log increments for the following reasons:

1. HE072 had shown similar safety profiles as Onivyde in the preclinical studies.
2. Bioequivalence study had been conducted to evaluate the efficacy and safety of HE072 and Onivyde, and demonstrated that there was no significant difference in safety profiles. No new safety signal was observed.
3. Phase I and II study had been performed to explore the safety and efficacy of Onivyde 50 mg/m^2, 70 mg/m^2 in patients with metastatic breast cancer, including triple negative breast cancer, and showed preliminary anti-tumor activity and consistent safety profiles.

No changes in manuscript.

• Definitions of analysis population sets for their key objectives, e.g., for safety, dose-escalation, and other key endpoints should be reported (in the main paper or appendix).

- Please provide the definition of DLT evaluable patients in the Method part.

Response: We appreciate your valuable suggestion, and have added the definition of DLT evaluable set accordingly.

Change in manuscript:

DLT was assessed in DLT evaluable set, which included patients enrolled in the DLT evaluation period and completed the DLT evaluation or withdrawn the study due to AEs in the DLT evaluation period. (Line 345-347, Page 12)

- Please provide definition of analysis population sets (if any) in the main context of the paper. The definition of Safety analysis set and DLT evaluable set are currently missing in the main paper.

Response: We appreciate your valuable suggestion, and have added the definition of safety analysis and DLT evaluable set accordingly.

Change in manuscript:

DLT was assessed in DLT evaluable set, which included patients enrolled in the DLT evaluation period and completed the DLT evaluation or withdrawn the study due to AEs in the DLT evaluation period. Safety was assessed in safety analysis set, which included all patients who received at least one dose of HE072 and have safety assessment result. (Line 345-348, Page 12)

- Please specify the primary/secondary/exploratory endpoints in the Outcome part. The author didn't mention Duration of response (DoR) and time to response (TTR) in the Outcome part but they're in the table. Are they secondary endpoints?

Response: Duration of response is the secondary endpoint of the study, while time to response is not. In the manuscript (Line 148, Page 6), we reported that "The median duration of response was 5.5 months (95%CI 4.3-9.4)."

Time to response is not the secondary endpoint of the study, we remove the result of TTR from the Table 4.

Change in manuscript:

	n=12	*n*=89	*n*=101	*n*=12
~~Median TTR, month (IQR)~~	~~1.4 (1.4~2.1)~~	~~1.5 (1.4~2.3)~~	~~1.5 (1.4~2.3)~~	~~1.5~~
Median DoR, month (95%CI)	2.6 (2.6~NA)	5.5 (4.4~9.4)	5.5 (4.3~9.4)	11.2

(Table 4, Page 23)

2) Statistical methods for additional analyses are inadequate. The authors mentioned in the protocol (provided as a supplementary document) that statistical analysis plan (SAP) would be a separate document and would include a more technical and detailed information on the subgroup analysis. However, the SAP was not provided with the submitted article. Hence, information on the implemented statistical approaches for subgroup analysis is missing and remain unclear in both the report and protocol.

Response: According to the SAP, the ORR and DCR subgroup analyses will be performed based on FAS. The ORR will be calculated using the number of subjects with confirmed CR and PR assessed by the investigator according to RECIST v1.1. The DCR will be calculated using the number of

subjects with confirmed CR, PR and SD. The 95% CI will be calculated using the Clopper-Pearson exact method. Subjects with missing postbaseline tumor assessments will be considered as non-responders and will be calculated in the denominator. If BOR is determined to be SD, SD is required to continue for at least 42 days after the first dose. Confirmed ORR and DCR are defined as confirmation of CR and PR as required per the protocol (i.e., CR or PR lasts for at least 28 days). We also provide the SAP together with the revised manuscript.

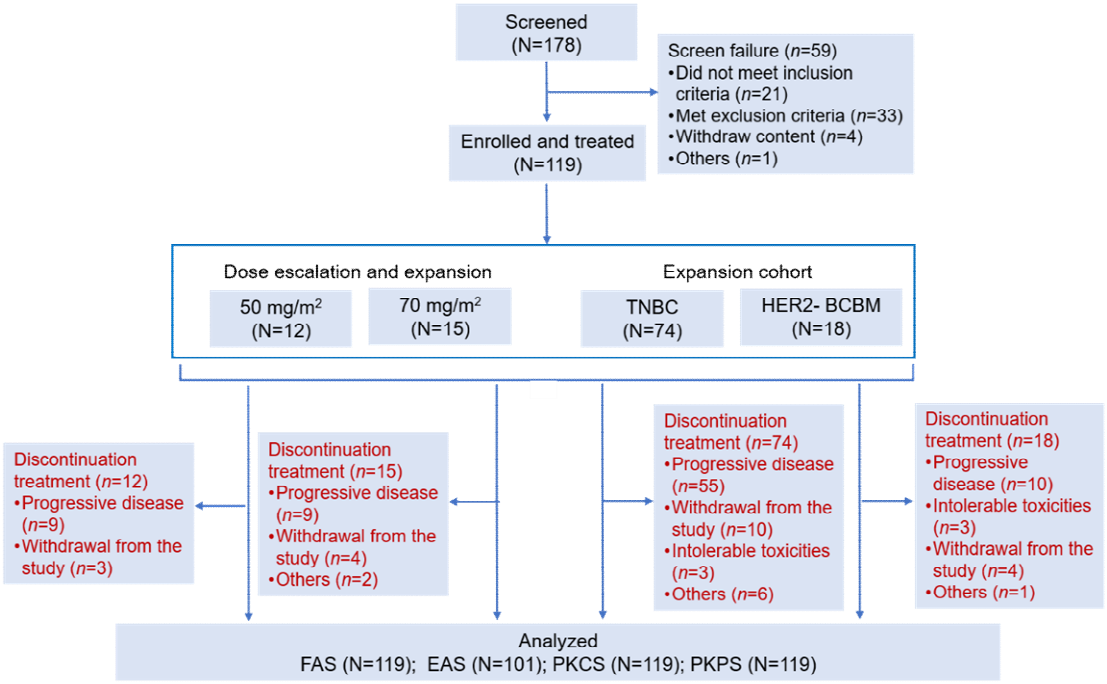
No changes in manuscript.

3) The authors are requested to provide information on the following points:

• For each group, losses and exclusions after allocation to each dose level, together with reasons

Response: We have added the treatment discontinuation reasons of each dose levels in Figure 1.

Change in manuscript:



• Composition of any decision making or safety review committee or group; summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details can be found (such as in a charter or protocol)

Response: The decision-making group in this trial is comprised of investigators, physicians, project managers, data managers, statisticians from sponsor.

The group's responsibilities are to:

1. Review the safety data, efficacy data, and/or pharmacokinetic (PK) data obtained from different dose and different cohort.
2. Discuss on the study progress, safety, efficacy, PK, individual medical data (if applicable).
3. Conduct preliminary and final evaluation of the data, including safety (such as adverse events and serious adverse events, laboratory data, ECG, concomitant medications), efficacy (such as objective response, disease progression-free survival), PK data, recruitment and accrual, and

other information with an impact on study outcome (such as scientific or therapeutic advancement).

4. Make recommendations relating to the escalation to the next dose level (such as 70 mg/m^2 Q2W), the addition of intermediate dose level, the selection of proper dose regimen, the expansion at a certain dose.
5. Make recommendations concerning continuation, termination, or modifications of a certain study cohort or the whole study based on the observed results.
6. Make recommendations regarding the modifications of the study protocol and statistical analysis plan.

The decision-making group would develop consensus on recommendations and decisions in regular meetings.

For conflict of interest, investigators are independent from the sponsor. Other members including physicians, project managers, data managers, statisticians are employees of the sponsor.

No special section on the decision-making group was included in the protocol.

No changes in manuscript.

• Description of who had access to interim results and made the interim and final decision to terminate the trial (or part(s) of the trial, eg, end of dose-escalation), and measures to safeguard the confidentiality of interim information

Response: The decision-making group had access to the results and made decision on the trial. No data integrity protection plan was used in this trial.

No changes in manuscript.

Additionally, it is highly encouraged that the authors complete the CONSORT-DEFINE checklist (https://www.bmj.com/content/383/bmj-2023-076387/related) to ensure all essential contents required for the reporting of phase I trials are included. Completing this CONSORT checklist will ensure comprehensive reporting, enhance the manuscript quality, and facilitate peer review and reproducibility.

Response: We appreciate your valuable suggestion, and have provided the CONSORT-DEFINE checklist as a separate file.

No changes in manuscript.

4) The abstract could be made more informative to include the dose escalation design. The introduction part shall also mention specific objectives (eg, relating to safety, activity, pharmacokinetics, pharmacodynamics, recommended dose(s)) of the trial.

Response: Given the word limit for abstract in *Nature communications*, we have attempted to keep our abstract concise within 200 words. However, if the editor deems it appropriate to include additional sentences, we would gladly adhere to the suggestion. Thank you for your valuable suggestion, and we adjust the abstract accordingly.

Change in manuscript:

This study consisted of two parts. In part 1, the 3+3 design was used to investigate three dose levels of HE072 (50, 70 and 90 mg/m^2). In part 2, patients were enrolled in two cohorts (mTNBC and HER2 negative breast cancer brain metastasis [BCBM]), and received an intravenous infusion of HE072 70 mg/m^2 every two weeks (Q2W). The primary endpoints were maximum tolerated dose (MTD) and RP2D, and treatment emergent adverse events (TEAEs)……(Line 33-37, Page 2)

5) The author reported: "As of June 5 2023, 89 (88.1%) of the 101 patients with TNBC were evaluable for response". However, according to Figure 1, TNBC (N=74) are included in the expansion cohort and EAS(N=95) are analysed, according to Figure 4 (Efficacy of HE072 in patients with TNBC and Triple negative BCBM), TNBC (N=78) are with MTD. please explain the deviations.

Response: We apologize for any confusion caused. In this trial, 119 patients were enrolled, including 101 mTNBC and 18 HER2 negative BCBM. Among 101 patients with mTNBC, 12 patients were not evaluable for response due to adverse events (*n*=6) or severe adverse events (*n*=3) or withdrawal of consent (*n*=3). Two patients with ER>10% was incorrectly enrolled, and excluded from efficacy evaluable set. Therefore, 87 of 101 patients with TNBC were evaluable.

In Figure 1, 87 patients with mTNBC and 14 patients with HER2 negative BCBM were evaluable for efficacy, therefore, EAS included 101 patients.

For the number of patients who received HE072 at 70 mg/m^2 dose level, 107 patients were at 70 mg/m^2 dose level, including 89 patients with mTNBC (74 in expansion cohort part and 15 in dose expansion part) and 18 patients with HER2 negative BCBM, which was shown in Figure 1.

In Table 4, 13 patients with mTNBC at 70 mg/m^2 dose level were not included in efficacy evaluable set, we could only present the efficacy of 76 patients with mTNBC at 70 mg/m^2 dose level in Table 4.

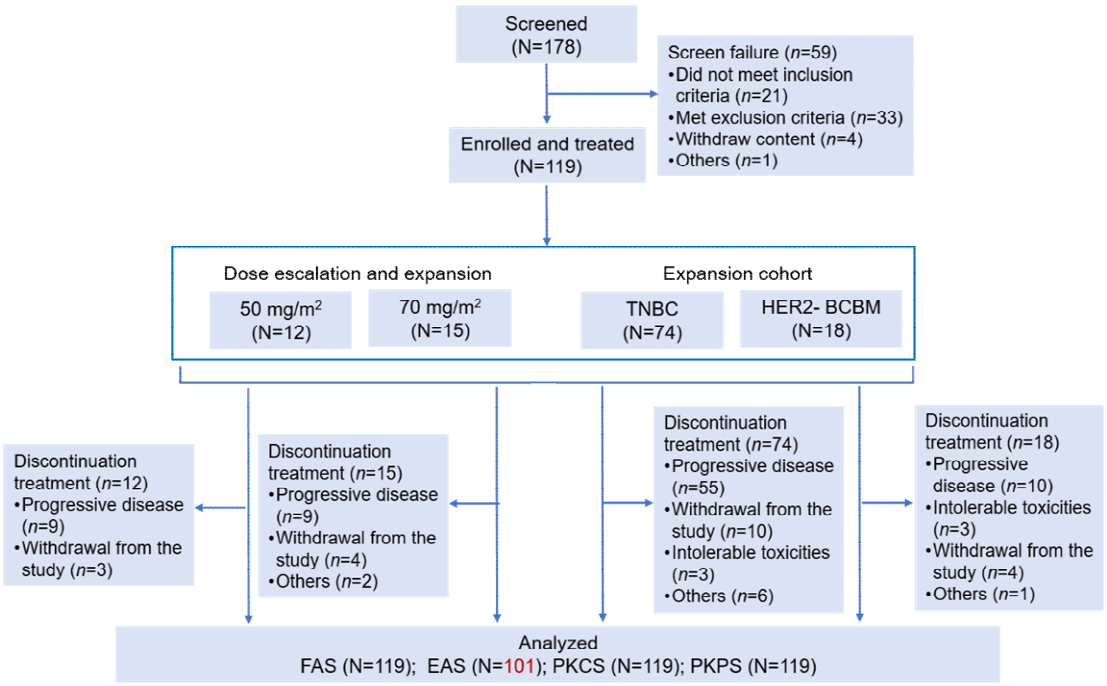
Change in manuscript:

As of June 5 2023, 12 patients were not evaluable for response due to adverse events (*n*=6) or severe adverse events (*n*=3) or withdrawal of consent (*n*=3). Two patients with ER>10% had been excluded from efficacy evaluable set. Therefore, 87 (88.1%) of the 101 patients with TNBC were included in efficacy evaluable set. After a median follow-up of 10.1 months (range 8.8-10.8)……(Line 147-151, Page 6)

Table 4. Efficacy of HE072 in patients with TNBC and Triple negative BCBM (EAS)

	TNBC			Triple negative BCBM	
	50 mg/m^2	70 mg/m^2	Total	70 mg/m^2 (RECIST v1.1)	70 mg/m^2 (RANO-BM)
	n=11	*n*=~~78~~76*	*n*=~~89~~87*	*n*=12	*n*=12
Best overall response, *n* (%)					
Complete response	0	0	0	0	0
Partial response	4 (36.4)	18 (~~23.1~~23.7)	22 (~~24.7~~25.3)	1 (8.3)	0
Stable disease	3 (27.3)	34 (~~43.6~~44.7)	37 (~~41.6~~42.5)	5 (41.7)	4 (33.3)
Progressive disease	4 (36.4)	~~25.~~24 (~~32.1~~31.6)	~~29.~~28 (~~32.6~~32.2)	2 (16.7)	4 (33.3)
Not evaluable	0	~~1 (1.3)~~0	~~1 (1.1)~~0	4 (33.3)	4 (33.3)
ORR/CNS ORR, *n* (%)	4 (36.4)	18 (~~23.1~~23.7)	22 (~~24.7~~25.3)	1 (8.3)	0
95%CI	(10.9, 69.2)	(~~14.3~~14.7, ~~34.0~~34.8)	(~~16.2~~16.6, ~~35.0~~35.8)	(0.2, 38.5)	(0, 26.5)
DCR/CNS CBR, *n* (%)	7 (63.6)	52 (~~66.7~~68.4)	59 (~~66.3~~67.8)	6 (50.0)	4 (33.3)
95%CI	(30.8, 89.1)	(~~55.1~~56.8, ~~76.9~~78.6)	(~~55.5~~56.9, ~~76.0~~77.4)	(21.1, 78.9)	(9.9, 65.1)

(Table 4, Page 23)



(Figure 1, separate file)

6) I believe it's a typo in the footnote of Table 4: TOR-time to response, which shall be changed to TTR.

Response: We apologize for the carelessness, and have made correction accordingly.

Change in manuscript:

TTR-time to response. (Table 4 footnote, Page 23)

Response to Reviewer #4:

The authors report their findings from a phase I study exploring the safety and preliminary efficacy of liposomal irinotecan (HE072) in patients with metastatic triple-negative breast cancer (mTNBC) after ≥ 2 prior lines of chemotherapy.

The authors report some activity of this agent in these patients in terms of ORR, duration of response and also include PFS and OS data. Further investigation is suggested based on the response rates seen here in a heavily pretreated population. The authors also justify the rationale for MTD.

While the title of the manuscript clearly focuses of patients with metastatic triple negative breast cancer, the authors have also included patients with brain metastases, which possibly takes the focus away from the primary results and may not actually be needed. Suggest removing these patients from the study or else include one section with a sub analysis and reporting these findings, although as mentioned I am not sure this is needed. Consider also adding the term "Intravenous" into the title to provide further clarification.

Response: We appreciate your valuable suggestion, and have added the term "Intravenous" into the title.

We can't remove the patients with brain metastases as the request of the editor that all patients including HER2 negative BCBM must be presented in the revised manuscript.

Change in manuscript:

Safety and efficacy of intravenous liposomal irinotecan (HE072) in metastatic triple-negative breast cancer after ≥ 2 prior lines of chemotherapy: a phase Ib study (Line 2-4, Page 1)

Consider adding more about the methods within the abstract, including dosing schedules, routes of administration and primary endpoint.

Response: Given the word limit for abstract in *Nature communications*, we have attempted to keep our abstract concise within 200 words. However, if the editor deems it appropriate to include additional sentences, we would gladly adhere to the suggestion. Thank you for your valuable suggestion, and we adjust the abstract accordingly.

Change in manuscript:

This study consisted of two parts. In part 1, the 3+3 design was used to investigate three dose levels of HE072 (50, 70 and 90 mg/m^2). In part 2, patients were enrolled in two cohorts (mTNBC and HER2 negative breast cancer brain metastasis [BCBM]), and received an intravenous infusion of HE072 70 mg/m^2 every two weeks (Q2W). The primary endpoints were maximum tolerated dose (MTD) and RP2D, and treatment emergent adverse events (TEAEs)……(Line 33-37, Page 2)

Consider revision of some language within the Introduction (Compared to other type of breast cancer, TNBC can't benefit from endocrine therapy and human epidermal growth factor receptor (HER) 2 targeting therapy and have limited treatment options.) and other sections ("The waterfall plot also showed very nice tumor shrinkage").

Response: We appreciate your suggestion, and have added the statement accordingly. Chemotherapy alone or in combination with other therapy(therapies) are recommended for later line treatment of TNBC, had have a limited efficacy. There are unmet medical needs for metastatic TNBC, hence several trials have been conducted to explore the efficacy and safety of novel treatment regimens. Sacituzumab govitecan (SG), an antibody-drug conjugate significantly improved PFS and OS in metastatic TNBC patients, with median PFS increased from 1.7 month to 5.6 months and median OS from 6.7 months to 12.1 months, and has been approved in metastatic TNBC patients treated with ≥ 2 lines of chemotherapy.

Change in manuscript:

Compared to other type of breast cancer, TNBC can't benefit from endocrine therapy and human epidermal growth factor receptor (HER) 2 targeting therapy and have limited treatment options. Sacituzumab govitecan (SG) has been approved for treatment of unresectable locally advanced or metastatic TNBC who have received at least two systemic therapies in China in June 2022…… (Line 57-59, Page 3)

The Methods section should also be moved up, placed after the introduction and preceding the Results section. Also Line 276 when describing dose reductions, were they permanent or temporary?

Response: The dose reduction was temporary. According to the protocol, the patients would be given escalated dose of HE072 if they could gain benefit at the discretion of investigator. In this trial, 26 patients experienced dose reduction, of which only one patient had dose reduction after 4th dosing of HE072 from 70 mg/m^2 to 50 mg/m^2, and re-escalated to 70 mg/m^2 at 9th dosing.

No changes in manuscript.

Some additional clarification is required in the Results section. Line 78 refers to 18 pts with HER2 negative BCBM, and the accompanying tables state that some of these may have low ER/PR expression. Table 1 also reports only 87% were ER/PR<1%. Did any of these patients receive endocrine therapy? If not this should be stated within the manuscript.
Response: 12 (66.7%) of 18 patients with HER2 negative BCBM were ER/PR<1%, 3 patients had ER<1% and PR 1-9% positive, ER 1-9% positive and PR<1%, or ER 1-9% positive and PR 1-9% positive, other 3 patients had ER or PR>10%. 5 of 18 patients had previously received endocrine therapy.

Change in manuscript:
Five of 18 patients with HER2 negative BCBM had previously received endocrine therapy. (Line 93-94, Page 4)

The authors have provided a detailed description of adverse events and safety, reporting 45% incidence of Grade 3 or higher AEs, however the abstract reports an acceptable safety profile. Were patient reported outcomes collected?
Response: We apologize for the careless expression, and have removed the statement accordingly. In this trial, no patient-reported outcomes were collected.

Change in manuscript:
This is the first study of a liposomal irinotecan, HE072 tested specifically in mTNBC. ~~HE072 showed acceptable safety profile.~~ (Line 46-48, Page 2)

Additional information regarding missed doses, dose reductions, dose delays, dose interruptions and dose discontinuation should be included. Consider adding an additional table with this information.
Response: We appreciate your valuable suggestion. The information regarding dose adjustment (dose reduction, dose delay and dose interruption) were shown in Table S3.

No changes in manuscript.

Response to Reviewer #5:
I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.
Response: We deeply appreciate your constructive suggestions, that's very helpful to improve our manuscript.

Response to Reviewer #6:
I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Response: We deeply appreciate your constructive suggestions, that’s very helpful to improve our manuscript.

REVIEWERS' COMMENTS

Reviewer #1 (Remarks to the Author):

This study offers safety data for new treatment option for patients with mTNBC, area of great unmet need, with liposomal irinotecan (HE072) after ≥ 2 prior lines of chemo. Around 25% patients had overall response- the disease control rate was 67.8%, median PFS and OS were 4.8 months and 14.1 months which are unfortunately on-par with average single-agent chemotherapy (maybe a bit better than single-agent gemcitabine etc. which is around 3 months mPFS).

Response: We deeply appreciate your constructive suggestions, that's very helpful to improve our manuscript.

Reviewer #2 (Remarks to the Author):

Thank you for your extensive revisions to this phase 1b study of the liposomal irinotecan agent HE072 in heavily pretreated triple-negative breast cancer (TNBC) as well as patients with HER2- breast cancer and CNS metastasis. The manuscript is well-written, and we appreciate the changes and responses you made to feedback from the reviewers. A few minor questions and comments are below:

- Regarding comparison to SG, we appreciate your explanation in your rebuttal regarding how the timing of approval in SG in China played a role in why no patients on this study received prior SG. However, you would do well to state this explicitly in the discussion of the paper as well.

Response: We appreciate your valuable suggestion, and have added the statement accordingly.

Change in Manuscript:

Secondly, few patients (20.8%) had previously received treatment with checkpoint inhibitors and no patient received treatment with SG (the reason was that all the patients of this study were enrolled between May 2021 and December 2022, while SG was approved in China for treatment of locally advanced and metastatic TNBC in June 2022), thus further investigations are needed to explore the efficacy of HE072 in these patients. (Line 245-247, Page 9)

- In Line 172, reference 7 is fine as a reference to a preclinical trial of liposomal vs non-liposomal irinotecan, but if there is preclinical work comparing HE072 to Onivyde as you state in the manuscript, it needs to be referenced here.

Response: We are grateful for your valuable suggestion. The results of preclinical trial comparing HE072 to Onivyde cannot be referenced because they have not been submitted or published yet.

No change in manuscript

The English grammar overall is much improved, but there still remain minor mistakes and omissions. Suggestions include:

1. Line 68 – "…thus increasing…and allowing more irinotecan to accumulate in the tumor zone.
2. Line 80 - Results should be plural

3. Line 88 – …inhibitors, and 23.8%...
4. Line 123 – “of the R2PD” (missing space)
5. Line 175 – “…of an immune checkpoint inhibitor.”...For patients after first-line therapy (the patient did not fail the therapy).
6. Line 176 – “…first-line therapy, few treatment options are limited…”
7. Line 183-184 – “In the ASCENT trial13, the antibody-drug conjugate SG significantly improved…”
8. Line 194 – “In total, nine patients with…”

Response: We apologize for the carelessness, and have made correction accordingly.

Change in Manuscript:

HE072 used pegylated liposome encapsulating an irinotecan sucroseoctasulfate salt, with vesicle size of 110 nm, which could prevent rapid elimination by mononuclear phagocyte system, thus ~~increase~~increasing the circulation longevity of the drug and ~~allow~~allowing more irinotecan accumulating into tumor zone. (Line 70-73, Page 3)

Results (Line 84, Page 4)

Of all TNBC patients, 20.8% had received PD-1/PD-L1 inhibitors, and 23.8% had more than four metastatic sites. (Line 91-92, Page 4)

Determination of ~~theRP2D~~ the RP2D (Line 129, Page 5)

For the first-line treatment of metastatic TNBC, the median PFS is only 9-10 months even with addition of an immune checkpoint inhibitor. For ~~the~~ patients ~~who failed~~ after first-line therapy, few treatment options are limited and generally have disappointing efficacy. (Line 180-182, Page 7)

In ASCENT trial, ~~SG, an~~the antibody-drug conjugate SG significantly improved PFS and OS in metastatic TNBC patients previously treated with at least 2 lines of chemotherapy compared with conventional chemotherapy, with median PFS and OS increased from 1.7 month to 5.6 months (HR 0.41, $p < 0.001$) and from 6.7 months to 12.1 months (HR 0.48, $p < 0.001$), respectively. (Line 189-193, Page 7)

In total, ~~Totally~~nine patients with metastatic TNBC were evaluable for efficacy, and three (33.3%) achieved partial response. (Line 200-201, Page 7)

Reviewer #3 (Remarks to the Author):

By addressing our concerns, the author has significantly improved the methodology standards of this article. No further comments then.

Response: We deeply appreciate your constructive suggestions, that’s very helpful to improve our manuscript.

Reviewer #6 (Remarks to the Author):

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Response: We deeply appreciate your constructive suggestions, that’s very helpful to improve our manuscript.

Reviewer #7 (Remarks to the Author):
I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.
Response: We deeply appreciate your constructive suggestions, that’s very helpful to improve our manuscript.