nature portfolio

Peer Review File

Edaravone dexborneol for ischemic stroke with sufficient recanalization after thrombectomy: a randomized phase II trial

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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)
Chen et al. report on the findings of a randomized, double-blind, placebo-controlled clinical trial that investigated the effects of Edaravone Dexborneol on 90-day functional outcome in patients with acute ischemic stroke due to large-vessel occlusion and that underwent successful recanalization by endovascular treatment. The authors report no significant differences for primary and secondary outcomes but a numerically higher percentage of favorable outcome in patients treated with ED compared with placebo and numerically beneficial lower infarct volume in ED-treated patients compared with placebo. Ed-treated patients further show a lower number of sICH, while not being signficantly different.
The results of this RCT are of great interest to the community considering that its design targets a major unmet need - clinically ineffective recanalization. The trial results are very important in light of an anticipated revival of investigation on neuroprotective strategies in setting of successful recanalization, which is a core characteristic of one of the experimental stroke models that led to positive animal data but never validated in clinical trials before the EVT era.
I would have the following recommendations for the authors that could potentially help to further improve the manuscript:
- Abstract: I would suggest to add background on the drug in the background section of the abstract.
- Abstract: considering that sICH was neither a primary nor secondary outcome but rather a safety outcome, I would suggest to phrase the last sentence of the background differently.
- Introduction: I would suggest to particularly focus the description of the pathophysiology (lines 75-77) to reperfusion injury and its mechanisms as an explanation for clinically ineffective recanalization.
- Introduction: Please also give additional background on why the long duration of treatment was necessary. 12 days is a long time (potentially too long) for being widely applicable as most LVO-stroke patients are dischargegd earlier.
- Introduction: I would suggest to rework this sentence (lines 81-84) considering that multiple mechanisms are named twice (anti-oxidative, anti-inflammatory) and to back ED up with more references. Because of the lack of a reference list at the end of the paper I cannot judge the quality of the references.
- Introduction: Please see comment for abstract on "reduction of sICH"
- Results: please state the reasons for "unplanned discharge". 12 days is a long time even for LVO-stroke patients to stay in the hospital. Were these patients doing too well?
- Results: safety outcomes: considering that morphological ECASS categories were not predefined as safety outcomes in the protocol or SAP, I would suggest to either add "in post hoc analyses" or "in exploratory analyses".
- Results: In case the authors see biological meaning (e.g. see Esposito et al. Nature 2020), I would suggest to add an exploratory analysis on the relation of the time of day of first treatment / time of day of recanalization with treatment benefit. This analysis could be done categorical (building time groups) or continuous (sinusoidal regression) and investigate an interaction with treatment or the simple association with favorable outcome.
- Minor: some sentences (e.g. second sentence of the discussion) might benefit from slight re-wording.
- Discussion: This sentence (lines 237-239) would potentially benefit from using the term "neurovascular unit". Same sentence: I would suggest to change to "...the neuronal, glial, and vascular compartment". Or "protecting neurons as well as glial and vascular cells". This also applies to later sentences.
- Discussion: line 246: please adjust: it was a bit less than 7 %.
- Discussion: line 249: Discussion: from the number given in the results, it is 7.49. Is there a population of patients with MRI on admission/24h and at 7 days, to calculate infarct progression between treated and untreated patients? How many patients received MRI overall?

- Limitations: Please also discuss the duration of the treatment (see comments above).
- References: Only 2 are displayed.
- The manuscript and protocol are very well aligned with regards to wording, the SAP somewhat differs at some instances: e.g. The protocol is different from the SAP in that the protocol defines the change in infarct volume at 1 week as a secondary outcome (or does change [as also stated in the manuscript] refer to the difference between groups) while the SAP the absolute level of infarct volume at 1 week. Similarly, the protocol has 2 safety outcomes, the SAP three.
- Outcomes defined as safety outcomes in the SAP are defined as secondary outcomes in the protocol. There are also differences between the abstract of the protocol and the main protocol text on these items.
- SAP vs. Protocol and Manuscript: Was the treatment given 14 days (SAP) or 12 days (protocol)?
- SAP vs. Protocol and Manuscript: While the SAP states block randomization with a block size of 4, the protocol doesn't mention on this.
- The SAP under 5.1 lists "treated with uncompleted argatroban treatment" as a criterion for the PP population. This might have been a copying mistake.

Reviewer #2

(Remarks to the Author)
The authors conducted a pre-planned randomized controlled trial to assess the effectiveness of edaravone dexborneol (ED). A predetermined number of patients were enrolled, and a predefined statistical analysis was conducted, even though the efficacy of ED could not be demonstrated. There are several concerns in the study methodology.
1. The most critical issue pertains to the determination of the sample size. The failure to detect a difference in the primary outcome between the two groups, despite a numerically higher proportion of favorable outcomes in the ED group, can be attributed to the absence of sample size calculation. The author claimed that there were no formal sample size calculation because the authors believed there was no prior reference to guide them in the text. However, they assumed some data to estimate the sample size in the protocol.
2. The exclusion criteria were just opposite of inclusion criteria.
3. Please provide the details of minimization algorithm to conduct randomization.
4. The analytic populations (FAS, PPS) were well described in the protocol but unclear in the text.
5. Rationale to conduct the multivariate analyses with key prognostic covariates should be provided when assessing primary and secondary outcomes. In this randomized controlled trial (RCT), patients were randomly assigned to two groups, and theoretically, there should be no difference in patient characteristics. If these key prognostic covariates were relevant, they should consider to conduct the stratification at randomization.
6. Please assess the proportional hazard assumption for Cox models.
7. While changes in NIHSS score or infarct volume between the two groups were compared using a general linear model, these data were generally distributed normally so that rationale to construct the general linear models should be provided.
8. They reported the analyses with LOCF, worst-case/best-case scenario for primary outcome. The protocol or SAP did not provide the details and the primary outcome is binary at one timepoint (90 days). The reviewer could not understand what the author did in these analyses.
9. The text mentions 18 references, but only two are listed. The lack of sufficient references makes it difficult to assess the credibility of the content. Furthermore, there was no data for tables and figures in appendix.

Reviewer #3

(Remarks to the Author)
What are the noteworthy results?

There were no significant differences in any of the primary or secondary efficacy outcomes but some indication of better safety outcomes favoring the intervention group in PH-1 and HI-2.

Will the work be of significance to the field and related fields? How does it compare to the established literature? If the work is not original, please provide relevant references.

The authors could have given a better description of the contribution of this trial to the field. Given the published TASTE trial "Edaravone Dexborneol Versus Edaravone Alone for the Treatment of Acute Ischemic Stroke" (Xu et al , 2021), which the authors themselves referred to, a phase 3 trial with similar intervention, similar outcomes and a largely similar cohort of patients, the authors have to clarify more the added value of their work. Granted that the TASTE trial compared Edaravone dexborneol to an active control Edaravone, some explanation is needed for clarifying how a phase 2 study with placebo control is required as well. Are there any subtle differences between the patient cohorts justifying the need of the current work?

Does the work support the conclusions and claims, or is additional evidence needed?
There was no major discordance between the claims and the evidence provided.

Are there any flaws in the data analysis, interpretation and conclusions? Do these prohibit publication or require revision?
There was a significant difference in time to groin puncture favoring the intervention group at baseline but this factor has not been involved in the covariate adjustment in the subsequent analysis. Please explain why this factor was not required.
As safety outcome is the most noteworthy result of this work, I would expect some more details about the SAEs. Are they in

anyway related to the intervention?

Is the methodology sound? Does the work meet the expected standards in your field?
The methodology is largely sound. However, there was a lack of details about the factors for minimisation.
Medians and IQR were reported in the analysis for change in NIHSS score from baseline with GMR which I assume to be Geometric mean Ratio as the Treatment Effect Metric (Table 2). Please clarify whether GMR is indeed Geometric mean Ratio. If that is the case, the geometric mean will not always equal the median, only in cases where there is an exact consistent multiplicative relationship between all numbers. It is unclear whether this relationship holds or this dataset and so I am not entirely sure about using GMR as the treatment effect metric.

Is there enough detail provided in the methods for the work to be reproduced?
Largely yes, when the manuscript was being read together with the protocol and the statistical analysis plan.

Version 2:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The authors have addressed all of my comments. I have one remaining comment on the suggested 'time-of-day'-analysis:
- I suggest to rephrase the analysis to 'time-of-day' of recanalization rather than circadian rhythm time.
- I suggest to emphasize this potentially important finding in the discussion and discuss potential explanations as well as implications.
- In the results, I suggest to expand the sentence to indicate during which time of day of recanalization treatment is favored.

Reviewer #2

(Remarks to the Author)
Thank you for the revised manuscript and for your responses to each of the concerns. However, there were still several concerns.
1. The author added the discussion about the failure to detect a difference in the primary outcome, however, the rational for the missing sample size calculation was not presented.
Furthermore, the author revised SAP without changing it to a new version. This is a serious violation in good clinical practice for clinical research.
Why don't you use the description below for the sample size estimation which was deleted from revised SAP (4.5. Sample Size):
According to our recent unpublished data, the proportion of expected favorable functional outcome (mRS 0-2) at 90 days in control group is estimated to be about 50%. Using power = 80% and $\alpha = 0.05$ to carry out the two-side test, the 200 size will test the superiority hypothesis with about 19% difference, namely the proportion of expected favorable functional outcome at 90 days is about 69%.
2. The author added the references to clarify the rationale for selecting the key prognostic covariates, not for conducting the multivariate analyses. The author should provide the rationale to conduct the multivariate analyses in this RCT.
3. Please describe the proportional hazard assumption for Cox models and the analyses with LOCF in the statistical analysis section.
4. Please change "general linear model" to "geometric mean ratio" in the revised manuscript (line 182) and footnote d and f in Table 2.

Reviewer #3

(Remarks to the Author)
Will the work be of significance to the field and related fields?
The authors had better explained the relationship of INSIST-ED with other trials in the same field through a more detailed background/introduction and an additional reference.

Reporting of safety outcome
The authors had provided more details about adverse events in the trial but they did not follow the CONSORT Harm 2022 Statement in the reporting of these events. This made definition of adverse events, distribution of the events between treatment arms and evaluation of the balance between benefits and harms of the intervention less transparent.

Ref
Junqueira D R, Zorzela L, Golder S, Loke Y, Gagnier J J, Julious S A et al. CONSORT Harms 2022 statement, explanation, and elaboration: updated guideline for the reporting of harms in randomised trials BMJ 2023; 381 :e073725 doi:10.1136/bmj-2022-073725

Consistence in the description and the analysis of NIHSS score
The authors used Geometric Mean Ratio to analyse NIHSS score and yet used median (IQR) to summarise the scores. This made confirming the accuracy of the Geometric Mean Ratio difficult. Why not use geometric means and the 95% CI of the

geometric mean to summarise NINSS score in each treatment arm?
It is only in cases where there is an exact consistent multiplicative relationship between all numbers in a dataset that the median is equal to the geometric mean. It is unclear whether this relationship holds for this dataset.

Version 4:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
All comments have been fully adressed.

Reviewer #2

(Remarks to the Author)
Thank you for your responses to each of the concerns. I have a few comments on the revised manuscript.
1. In the revised SAP (version 2.0), the sample size was estimated by author's unpublished data (below). Why don't you use this description for sample size calculation in the manuscript?
"According to our recent unpublished data, the proportion of expected favorable functional outcome (mRS 0-2) at 90 days in control group is estimated to be about 50%. Using power = 80% and $\alpha = 0.05$ to carry out the two-side test, the 200 size will test the superiority hypothesis with about 19% difference, namely the proportion of expected favorable functional outcome at 90 days is about 69%."
2. I would like to repeat that, the patients are randomly assigned to two groups in RCT, then theoretically, it is normal to deem patient characteristics to be no difference between two groups. Please describe the rationale to conduct the multivariate analyses in the manuscript, not only in the SAP.
3. The author presented the details of imputation method for mRS score, however, it was not described in the SAP.

Reviewer #3

(Remarks to the Author)
The authors have addressed all of my comments.

Reviewer #4

(Remarks to the Author)
Thanks for having this opportunity to review this work. In this phase 2 trial, Chen et al aimed to explore the potential of ED as a cereborprotective drug among anterior circulation LVO-AIS patients with successful recanalization after EVT.

Abstract
1, there are 6 patients difference between the randomised groups. Any explanation? It seems variations in the enrolment may exist? How to assess its impact??
2, from the SAP, it shows unadjusted OR would be the primary result. It would be better to have unadjusted results.
3, 14 patients missed the mRS. it seems the different approaches for the missing outcome would affect the result. Has the authors considered another method, such as Tipping point analysis to assess when the result would be altered?
4, since there is a trend to indicate the benefit of ED, it would be good to have weak tone in the conclusion,…. Did not improve….. to be did not statically …..
Introduction
Clear
Method:
1,Threshold for the NIHSS score at baseline is not specified in the subgroup analysis.
Result:
1, when did the trial finish? What is the date for data lock? It seem the trial should be stopped no later than Oct 2022. However, The latest SAP dated 28/05/2024, which is unexpected late. Potential bias may exist as the team may see the data.
2, Figure 1, Can we assum the data is missing at ranom due to the clinical deterioration or economics reasons?
3 Figure 2, what do you mean adjusted P value?
4, Table 1, no need to do the group test between groups as per guidelines.
5, Some variables in the Table 1 may be clinically imbalance, such as mTICI score, hypertension. Can the author discuss more about the potential impact?
6, ,how to define the safety population? it seems some subject did not receive the drugs from Table 3. Has this being considered in the definition of the ITT population?
Discussion,
The trial was launched based on the hypothesis that Dexborneol was reported to exert neuroprotective effects through inhibiting nitric oxide (NO) and NO synthase pathways,

reducing ROS generation, restraining inflammatory process and caspase-related apoptosis. however, no data was collected to support this hypothesis.

Version 5:

Reviewer comments:

Reviewer #2

(Remarks to the Author)
Thank you for your responses to each of the concerns.
However, this study was randomized clinical trial so that all analyses should be described in SAP in advance. Any analyses which were not described in SAP should be clearly presented in the manuscript as a post-hoc with strong rationales.
This concerns include the multivariable models and imputation of missing of mRS score.
The lack of these information has great risk of biased results.

Reviewer #4

(Remarks to the Author)
Thanks for sending back your revision. I have two additional comments.
1, since some of randomised were excluded from the ITT population. To avoid confusion, it would be better to use modified ITT.
2, Tipping point analysis is not just to recalcuate the OR. It is mainly to check when and how the missing data would change the study results. A graph in the appendix would be great.
3, Thanks for providing the additional analysis, which can be placed in the appendix if possible.

Re: NCOMMS-23-33900 entitled "Improving Neuroprotective Strategy for Ischemic Stroke with sufficient recanalization after Thrombectomy by Edaravone Dexborneol (INSIST-ED): a prospective, randomized, double blinded, multi-centre study"

REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

Chen et al. report on the findings of a randomized, double-blind, placebo-controlled clinical trial that investigated the effects of Edaravone Dexborneol on 90-day functional outcome in patients with acute ischemic stroke due to large-vessel occlusion and that underwent successful recanalization by endovascular treatment. The authors report no significant differences for primary and secondary outcomes but a numerically higher percentage of favorable outcome in patients treated with ED compared with placebo and numerically beneficial lower infarct volume in ED-treated patients compared with placebo. Ed-treated patients further show a lower number of sICH, while not being signficantly different.

The results of this RCT are of great interest to the community considering that its design targets a major unmet need - clinically ineffective recanalization. The trial results are very important in light of an anticipated revival of investigation on neuroprotective strategies in setting of successful recanalization, which is a core characteristic of one of the experimental stroke models that led to positive animal data but never validated in clinical trials before the EVT era.

I would have the following recommendations for the authors that could potentially help to further improve the manuscript:

- Abstract: I would suggest to add background on the drug in the background section of the abstract.

Response: *Thank you very much for your suggestion. We have added "a compound with*

both antioxidative and anti-inflammatory properties," as the background on the drug as suggested (Page 3, line 42).

- Abstract: considering that sICH was neither a primary nor secondary outcome but rather a safety outcome, I would suggest to phrase the last sentence of the background differently.

Response: *Thank you very much for your suggestion. We have removed the contents of safety outcome from the last sentence of the background in our revised manuscript.*

- Introduction: I would suggest to particularly focus the description of the pathophysiology (lines 75-77) to reperfusion injury and its mechanisms as an explanation for clinically ineffective recanalization.

Response: *Thank you very much for your valuable suggestion. Reperfusion injury may result in clinically ineffective reperfusion after EVT by generation of harmful free radicals such as the superoxide, hydroxyl, and peroxynitrite radicals [1], which may lead to apoptosis or programmed cell death [2], disruption of neurovascular unit [3], endothelial injury [4], and aggravation of inflammation and immune dysregulation [5, 6]. We have added the description in the revised manuscript as suggested (Pages 5-6, lines 82-99).*

References

1. Savitz SI, Baron JC, Yenari MA, et al. Reconsidering Neuroprotection in the Reperfusion Era. Stroke. D2017;48(12):3413-3419.

2. Radak D, Katsiki N, Resanovic I, et al. Apoptosis and Acute Brain Ischemia in Ischemic Stroke. Current vascular pharmacology. 2017;15(2):115-122.

3. Schaeffer S, Iadecola C. Revisiting the neurovascular unit. Nature neuroscience. 2021;24(9):1198-1209.

4. Kuhn AL, Vardar Z, Kraitem A, et al. Biomechanics and hemodynamics of stent-retrievers. Journal of cerebral blood flow and metabolism: official journal of the International Society of Cerebral Blood Flow and Metabolism. 2020;40(12):2350-2365.

5. Mizuma A, You JS, Yenari MA. Targeting Reperfusion Injury in the Age of Mechanical

Thrombectomy. Stroke. 2018;49(7):1796-1802.

6. Nie X, Leng X, Miao Z, Fisher M, Liu L. Clinically Ineffective Reperfusion After Endovascular Therapy in Acute Ischemic Stroke. Stroke. 2023;54(3):873-881.

- Introduction: Please also give additional background on why the long duration of treatment was necessary. 12 days is a long time (potentially too long) for being widely applicable as most LVO-stroke patients are discharged earlier.

Response: *Thank you very much for your suggestion. According to your suggestion, we have added "14 days infusion of ED" as a background of 12 days treatment length in this study (Page 6, line 97). The "12+/-2 days" treatment length in this study was chosen based on two considerations: (1) the practical length of hospital stay for patients treated by endovascular treatment was usually 10-14 days in China [1-2]; (2) TASTE trial suggested that 14 days infusion of edaravone dexborneol improved clinical outcome in acute ischemic stroke [3]. In addition, the long length treatment time has been discussed as a limitation in the revised manuscript (Page 15, lines 290-295).*

References

1. Tu WJ, Wang LD, Special Writing Group of China Stroke Surveillance R. China stroke surveillance report 2021. Military Medical Research. Jul 19 2023;10(1):33.

2. Wang A, Jia B, Zhang X, et al. Efficacy and Safety of Butylphthalide in Patients With Acute Ischemic Stroke: A Randomized Clinical Trial. JAMA neurology. Aug 1 2023;80(8):851-859.

3. Xu J, et al. Edaravone Dexborneol Versus Edaravone Alone for the Treatment of Acute Ischemic Stroke: A Phase III, Randomized, Double-Blind, Comparative Trial. Stroke 52, 772-780 (2021).

- Introduction: I would suggest to rework this sentence (lines 81-84) considering that multiple mechanisms are named twice (anti-oxidative, anti-inflammatory) and to back ED up with more references. Because of the lack of a reference list at the end of the paper I cannot judge the quality of the references.

Response: *Sorry for the missing reference. We have reworked the sentence and backed*

ED up with more references in the introduction section as suggested (Page 5-6, lines 87-99). Full list of references could be fined in the revised manuscript (Pages 18-19).

- Introduction: Please see comment for abstract on "reduction of sICH"

Response: *We have removed the contents of safety outcome from the last paragraph of the introduction section as suggested.*

- Results: please state the reasons for "unplanned discharge". 12 days is a long time even for LVO-stroke patients to stay in the hospital. Were these patients doing too well?

Response: *Thank you very much for your suggestions. The detailed reasons for unplanned discharge have been added as foot note a and b. As replied above, the "12+/-2 days" treatment length in this study was chosen based on two considerations: (1) the practical length of hospital stay for patients treated by endovascular treatment was usually 10-14 days in China [1-2]; (2) TASTE trial suggested that 14 days infusion of edaravone dexborneol improved clinical outcome in acute ischemic stroke [3], which has been discussed as a limitation in the revised manuscript (Page 15, lines 290-295). In this study, most patients complied with the treatment.*

References

1. Tu WJ, Wang LD, Special Writing Group of China Stroke Surveillance R. China stroke surveillance report 2021. Military Medical Research. Jul 19 2023;10(1):33.

2. Wang A, Jia B, Zhang X, et al. Efficacy and Safety of Butylphthalide in Patients With Acute Ischemic Stroke: A Randomized Clinical Trial. JAMA neurology. Aug 1 2023;80(8):851-859.

3. Xu J, Wang A, Meng X, et al. Edaravone Dexborneol Versus Edaravone Alone for the Treatment of Acute Ischemic Stroke: A Phase III, Randomized, Double-Blind, Comparative Trial. Stroke. Mar 2021;52(3):772-780.

- Results: safety outcomes: considering that morphological ECASS categories were not predefined as safety outcomes in the protocol or SAP, I would suggest to either add "in post hoc analyses" or "in exploratory analyses".

Response: *Thank you very much for your valuable suggestion. We reported these safety outcomes as exploratory analyses in the revised manuscript as suggested (Page 13, line 236).*

- Results: In case the authors see biological meaning (e.g. see Esposito et al. Nature 2020), I would suggest to add an exploratory analysis on the relation of the time of day of first treatment / time of day of recanalization with treatment benefit. This analysis could be done categorical (building time groups) or continuous (sinusoidal regression) and investigate an interaction with treatment or the simple association with favorable outcome.

Response: *Thank you very much for your valuable suggestion. I agree that circadian rhythm affects mechanisms of disease and response to therapies. In this study, there was no significant difference in the day/night time during EVT between ED group and control group (P=0.8). As shown in the Table below, a significant interaction between the time of recanalization and treatment with regard to the primary outcome was observed (P=0.004). We added the baseline circadian rhythm information (Table 1) and interaction analysis (Figure 3) in the revised manuscript as suggested.*

Interaction Analysis for Primary Outcome with Circadian Rhythms

Subgroup	No. of patients	ED group (n=97)	Control group (n=103)	Odds Ratio (95% CI)	P value for interaction
Night-time	48	9/26	14/22	0.30 (0.09-0.99)	0.004
Day-time	138	45/66	35/72	2.27 (1.13-4.54)	

- Minor: some sentences (e.g. second sentence of the discussion) might benefit from slight re-wording.

Response: *Thank you very much for your suggestions. We have revised the second sentence of the discussion section as suggested (Pages 13, lines 249-251).*

- Discussion: This sentence (lines 237-239) would potentially benefit from using the

term "neurovascular unit". Same sentence: I would suggest to change to "...the neuronal, glial, and vascular compartment". Or "protecting neurons as well as glial and vascular cells". This also applies to later sentences.

Response: *Thank you very much for your suggestion. We have revised these sentences in the discussion section as suggested (Page 14, lines 259, 267).*

- Discussion: line 246: please adjust: it was a bit less than 7 %.

Response: *Sorry for this error. We have revised the data (Page 14, line 270).*

- Discussion: line 249: Discussion: from the number given in the results, it is 7.49. Is there a population of patients with MRI on admission/24h and at 7 days, to calculate infarct progression between treated and untreated patients? How many patients received MRI overall?

Response: *Sorry for the mistake! We have revised the number as suggested (Page 14, line 273). MRI was not performed routinely in this study. Only 17 patients received MRI on admission/24h.*

- Limitations: Please also discuss the duration of the treatment (see comments above).

Response: *We have discussed this limitation in the discussion section in our revised manuscript. (Page 15, lines 290-295)*

- References: Only 2 are displayed.

Response: *Sorry for the reference missing. All references have been displayed in the revised manuscript.*

- The manuscript and protocol are very well aligned with regards to wording, the SAP somewhat differs at some instances: e.g. The protocol is different from the SAP in that the protocol defines the change in infarct volume at 1 week as a secondary outcome (or does change [as also stated in the manuscript] refer to the difference between groups) while the SAP the absolute level of infarct volume at 1 week. Similarly, the protocol

has 2 safety outcomes, the SAP three.

Response: *Sorry for the mistake. This secondary outcome was infarct volume at 1 week. We have revised the protocol (Appendix 2, page 4, 8, 16).*

- Outcomes defined as safety outcomes in the SAP are defined as secondary outcomes in the protocol. There are also differences between the abstract of the protocol and the main protocol text on these items.

Response: Sorry for the mistake. *We have revised the mistakes in the protocol (Appendix 2, page 8).*

- SAP vs. Protocol and Manuscript: Was the treatment given 14 days (SAP) or 12 days (protocol)?

Response: *The duration of treatment was 12±2 days. We have revised the contents in SAP of the revised manuscript.*

- SAP vs. Protocol and Manuscript: While the SAP states block randomization with a block size of 4, the protocol doesn't mention on this.

Response: *In protocol, we wrongly used "minimization algorithm". Sorry for the missing information in the protocol. We have added this related information about block randomization in the revised protocol (Appendix 2, page 11).*

- The SAP under 5.1 lists "treated with uncompleted argatroban treatment" as a criterion for the PP population. This might have been a copying mistake.

Response: *Sorry for the mistake. We have amended the mistake in the revised SAP (Appendix 2, page 9).*

Reviewer #2 (Remarks to the Author):

The authors conducted a pre-planned randomized controlled trial to assess the

effectiveness of edaravone dexborneol (ED). A predetermined number of patients were enrolled, and a predefined statistical analysis was conducted, even though the efficacy of ED could not be demonstrated. There are several concerns in the study methodology.

1. The most critical issue pertains to the determination of the sample size. The failure to detect a difference in the primary outcome between the two groups, despite a numerically higher proportion of favorable outcomes in the ED group, can be attributed to the absence of sample size calculation. The author claimed that there were no formal sample size calculation because the authors believed there was no prior reference to guide them in the text. However, they assumed some data to estimate the sample size in the protocol.

Response: *Thank you very much for your valuable suggestion.*

(1) As correctly pointed out by the reviewer, as a phase II study without formal size calculation, the small sample size renders our findings inconclusive. We have added the discussion in the revised Limitation section (Page 15, lines 282-283).

(2) As replied to editor, the treatment effect size was inversely extrapolated based on 200 sample sizes. To avoid the confusion, effect size calculation has been deleted in the revised SAP and protocol. The deletion had no effect on the statistical analysis, which was in strict compliance with SAP.

2. The exclusion criteria were just opposite of inclusion criteria.

Response: *We have refined the exclusion criteria in the revised manuscript (Page 8, lines 123-128) according to the protocol and have indicated the full list of inclusion and exclusion criteria is available in Appendix 2.*

3. Please provide the details of minimization algorithm to conduct randomization.

Response: *In fact, we used block randomization in this trial, which was correct in SAP (Appendix 3). Sorry for the mistake. We have revised the information in the revised main text (Page 8, line 132) and protocol (Appendix 2, page 11).*

4. The analytic populations (FAS, PPS) were well described in the protocol but unclear

in the text.

Response: *We have added the description for PPS in the revised manuscript as suggested (Page 10, lines 183-187).*

5. Rationale to conduct the multivariate analyses with key prognostic covariates should be provided when assessing primary and secondary outcomes. In this randomized controlled trial (RCT), patients were randomly assigned to two groups, and theoretically, there should be no difference in patient characteristics. If these key prognostic covariates were relevant, they should consider to conduct the stratification at randomization.

Response: *Thank you very much for your suggestion. (1) We agree with the reviewer that two groups should be balanced in terms of patient characteristics if the sample size is sufficiently large. (2) In this study, we performed an adjusted analysis for age, sex, NIHSS score at randomization, premorbid function (mRS score), history of ischemic stroke, bridge therapy, time from the onset of symptom to vessel opening, level of collateral circulation, and number of thrombectomy, which were set as key prognostic covariates based on previous studies suggesting their association with clinical outcome [1-8]. Adjusted analysis is a sensitivity analysis required by the CONSORT guidelines. (3) We did not do a stratified randomization by those covariates because the sample size was small: too many strata mean very small number of patients in each stratum. (4) According to your suggestion, we have added "According to previous studies [1, 6-12]," to clarify the rational in the revised SAP (Page 10, lines 209-210).*

References

1. van Horn N, Kniep H, Leischner H, et al. Predictors of poor clinical outcome despite complete reperfusion in acute ischemic stroke patients. Journal of neurointerventional surgery. 2021;13(1):14-18.

2. Khatri P, Abruzzo T, Yeatts SD, et al. Good clinical outcome after ischemic stroke with successful revascularization is time-dependent. Neurology. 2009;73(13):1066-1072.

3. Goyal M, Menon BK, van Zwam WH, et al. Endovascular thrombectomy after large-vessel ischaemic stroke: a meta-analysis of individual patient data from five randomised trials. Lancet. 2016;387(10029):1723-1731.

4. Lemmens R, Hamilton SA, Liebeskind DS, et al. Effect of endovascular reperfusion in relation to site of arterial occlusion. Neurology. 2016;86(8):762-770.

5. Marto JP, Strambo D, Hajdu SD, et al. Twenty-Four-Hour Reocclusion After Successful Mechanical Thrombectomy: Associated Factors and Long-Term Prognosis. Stroke. 2019;50(10):2960-2963.

6. Liebeskind DS, Saber H, Xiang B, et al. Collateral Circulation in Thrombectomy for Stroke After 6 to 24 Hours in the DAWN Trial. Stroke. 2022;53(3):742-748.

7. Brugnara G, Neuberger U, Mahmutoglu MA, et al. Multimodal Predictive Modeling of Endovascular Treatment Outcome for Acute Ischemic Stroke Using Machine-Learning. Stroke. 2020;51(12):3541-3551.

8. Yoon W, Kim SK, Park MS, et al. Predictive Factors for Good Outcome and Mortality After Stent-Retriever Thrombectomy in Patients With Acute Anterior Circulation Stroke. Journal of stroke. 2017;19(1):97-103.

6. Please assess the proportional hazard assumption for Cox models.

Response: *We did an interaction test between treatment and the time variation in the Cox model and did not find significant interaction in the model (P=0.66), suggesting no violation of the hazard proportionality assumption.*

7. While changes in NIHSS score or infarct volume between the two groups were compared using a general linear model, these data were generally distributed normally so that rationale to construct the general linear models should be provided.

Response: *Thank you very much for your suggestion. For the two outcomes, normality assumption was assessed graphically and residual histogram showed serious violation of normality assumption, so GMR was used in this analysis. The explanation has been added as footnote e in Table 2.*

8. They reported the analyses with LOCF, worst-case/best-case scenario for primary outcome. The protocol or SAP did not provide the details and the primary outcome is binary at one timepoint (90 days). The reviewer could not understand what the author did in these analyses.

Response: *Given that there were missing data in the primary outcome due to the lost to follow-up, we conducted the supportive analyses using LOCF, worst-case scenario, and best-case scenario as sensitive analyses to assess the robustness of primary endpoint analysis in the ITT population. In the LOCF analysis, we used the mRS score in the last visit of patients lost to follow-up to interpret the 90-day mRS score; In the worst-case scenario analysis, we used the "6" as the 90-day mRS score; In the best-case scenario analysis, we used the "0" as the 90-day mRS score. The three analyses showed the similar results with ITT analysis, demonstrating that the results of ITT analysis of primary outcome were robust.*

9. The text mentions 18 references, but only two are listed. The lack of sufficient references makes it difficult to assess the credibility of the content. Furthermore, there was no data for tables and figures in appendix.

Response: *Sorry for missing reference and tables and figures in appendix. All references have been displayed in the revised manuscript. We have added the supplementary tables and figures in Appendix 1.*

Reviewer #3 (Remarks to the Author):

What are the noteworthy results?

There were no significant differences in any of the primary or secondary efficacy outcomes but some indication of better safety outcomes favoring the intervention group in PH-1 and HI-2.

Will the work be of significance to the field and related fields? How does it compare to the established literature? If the work is not original, please provide relevant references.

The authors could have given a better description of the contribution of this trial to the field. Given the published TASTE trial "Edaravone Dexborneol Versus Edaravone Alone for the Treatment of Acute Ischemic Stroke" (Xu et al , 2021), which the authors themselves referred to, a phase 3 trial with similar intervention, similar outcomes and a largely similar cohort of patients, the authors have to clarify more the added value of their work. Granted that the TASTE trial compared Edaravone dexborneol to an active control Edaravone, some explanation is needed for clarifying how a phase 2 study with placebo control is required as well. Are there any subtle differences between the patient cohorts justifying the need of the current work?

Response: *The TASTE trial investigated the effects of edaravone dexborneol (ED) versus edaravone among acute ischemic stroke (AIS) patients within 48 hours of stroke onset who did not receive intravenous thrombolysis or endovascular treatment (EVT), and the results showed that 90-day good functional outcomes favored the ED group. In the present study, intravenous ED was given to AIS-LVO patients with successful reperfusion after EVT, which was totally different from patients in the TASTE trial, given that reperfusion injury may lead to clinically ineffective reperfusion after EVT due to multiple mechanisms including generation of harmful free radicals, aggravation of inflammation and immune dysregulation. A neuroprotectant with multi-target on reperfusion injury may improve outcome of patients with LVO stroke. According to your suggestion, we have added the detailed background introduction (Pages 5-6, lines 82-99) and related discussion (Page 14, lines 262-264) in the revised manuscript.*

Does the work support the conclusions and claims, or is additional evidence needed?

There was no major discordance between the claims and the evidence provided.

Are there any flaws in the data analysis, interpretation and conclusions? Do these prohibit publication or require revision?

There was a significant difference in time to groin puncture favoring the intervention group at baseline but this factor has not been involved in the covariate adjustment in the subsequent analysis. Please explain why this factor was not required.

Response: *Thank you very much for your suggestion. As you mentioned, there was a*

significant difference in time to groin puncture favoring the intervention group at baseline. Given more important value of onset to recanalization time than groin punction to recanalization time in clinical outcome, as well as their collinearity, the covariate adjustment involved time from onset to recanalization and number of thrombectomy device passes as indicated in pre-established SAP. Based on your suggestion, we have added as a limitation discussion in the revised manuscript (Page 15, lines 286-290).

As safety outcome is the most noteworthy result of this work, I would expect some more details about the SAEs. Are they in anyway related to the intervention?

Response: *In this study, the SAEs included parenchymal hemorrhage, subarachnoid hemorrhage, cerebral hernia, respiratory failure, acute ischemic stroke, cerebral edema, sudden cardiac death, gastrointestinal hemorrhage, liver dysfunction, etc, which has been added as footnote c in Table 3. Of these SAEs, two patients occurred liver dysfunction possibly related to edaravone dexborneol.*

Is the methodology sound? Does the work meet the expected standards in your field?

The methodology is largely sound. However, there was a lack of details about the factors for minimisation.

Response: *As replied to Reviewer #2, instead of minimum randomization, we used block randomization in this trial, which was correct in SAP (Appendix 3). Sorry for the mistake. We have revised the information in the revised manuscript (Page 8, line 132) and protocol (Appendix 2, page 11).*

Medians and IQR were reported in the analysis for change in NIHSS score from baseline with GMR which I assume to be Geometric mean Ratio as the Treatment Effect Metric (Table 2). Please clarify whether GMR is indeed Geometric mean Ratio. If that is the case, the geometric mean will not always equal the median, only in cases where there is an exact consistent multiplicative relationship between all numbers. It is unclear whether this relationship holds or this dataset and so I am not entirely sure about using

GMR as the treatment effect metric.

Response: *Thank you very much for your suggestion. GMR stands for indeed Geometric Mean Ratio. We have added the abbreviation in the footnote of the related tables. Change in NIHSS score was transformed using log (change in NIHSS score +1) due to non-normal distribution of NIHSS. The resulting treatment effect metric from general linear model can be expressed as GMR.*

Is there enough detail provided in the methods for the work to be reproduced?

Largely yes, when the manuscript was being read together with the protocol and the statistical analysis plan.

Response: *Thank you very much for positive comments.*

Re: NCOMMS-23-33900 entitled "Improving Neuroprotective Strategy for Ischemic Stroke with sufficient recanalization after Thrombectomy by Edaravone Dexborneol (INSIST-ED): a prospective, randomized, double blinded, multi-centre study"

REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

The authors have addressed all of my comments. I have one remaining comment on the suggested 'time-of-day'-analysis:

- I suggest to rephrase the analysis to 'time-of-day' of recanalization rather than circadian rhythm time.

Response: *Thank you very much for suggestions. 'circadian rhythm time' has been rephrased as 'time-of-day' in the revised manuscript as suggested (Page 11, line 208; Page 12, line 239).*

- I suggest to emphasize this potentially important finding in the discussion and discuss potential explanations as well as implications.

Response: *Thank you very much for suggestions. We have added the related discussion in the revised manuscript as suggested (Page 15, lines 294-296).*

- In the results, I suggest to expand the sentence to indicate during which time of day of recanalization treatment is favored.

Response: *Thank you very much for suggestions. We have added 'suggesting the benefit of ED treatment during day-time of recanalization, compared with night-time of recanalization.' in the Results section as suggested (Page 13, lines 240-242).*

Reviewer #2 (Remarks to the Author):

Thank you for the revised manuscript and for your responses to each of the concerns. However, there were still several concerns.

1. The author added the discussion about the failure to detect a difference in the primary outcome, however, the rational for the missing sample size calculation was not presented.

Response: *Thank you very much for your suggestions. We have added the rational for the missing sample size calculation in the revised manuscript as suggested (Page 15-16, lines 302-303).*

Furthermore, the author revised SAP without changing it to a new version. This is a serious violation in good clinical practice for clinical research.

Response: *Thank you very much for your suggestions. To response 'some inconsistency between SAP vs Manuscript and Protocol' mentioned by Reviewer #1 in last review comments, we revised SAP in some places, for example '14 days' revised to '12±2 days', '2 weeks' revised to '12±2 days', '90 days' revised to '90±7 days', 'argatroban' revised to 'ED', and added some references, which had no effect on the statistical analysis. In last revision, we did not confer a new version due to our carelessness. In this review, we submitted revised SAP (version 2.0) as suggested and the detailed information about the difference between two versions.*

Why don't you use the description below for the sample size estimation which was deleted from revised SAP (4.5. Sample Size):

According to our recent unpublished data, the proportion of expected favorable functional outcome (mRS 0-2) at 90 days in control group is estimated to be about 50%. Using power = 80% and $\alpha = 0.05$ to carry out the two-side test, the 200 size will test the superiority hypothesis with about 19% difference, namely the proportion of

expected favorable functional outcome at 90 days is about 69%.

Response: *Thank you very much for your suggestions. I'm sorry we misinterpreted the last comments from you and the editor. In the last revision, the description about the sample size estimation was deleted in the revised SAP, given that the treatment effect size was extrapolated based on a sample size of 200. We re-added this description in the SAP.*

2. The author added the references to clarify the rationale for selecting the key prognostic covariates, not for conducting the multivariate analyses. The author should provide the rationale to conduct the multivariate analyses in this RCT.

Response: *Thank you very much for your suggestions. The 6.1.3 section of SAP has stated that "Adjusted analyses will also be carried out on the analysis of the primary endpoint to determine whether the treatment effect estimate is affected with the inclusion of covariables.". Thus, we conducted the multivariate analyses to detect the difference of primary endpoint between treatments after controlling the balance of prognostic covariates between treatments. We have added "According to previous studies [1, 6-12], these covariates were associated with outcomes after endovascular therapy in acute ischemic stroke. Thus, it is necessary to conduct adjusted (multivariate) analyses to control the imbalances in those covariates at baseline between treatment groups" in the revised SAP (version 2.0) to further state the rationale to conduct the analyses.*

3. Please describe the proportional hazard assumption for Cox models and the analyses with LOCF in the statistical analysis section.

Response: *Thank you very much for your suggestions. We have added "Proportional hazard assumption was tested by including an interaction between time and trial group in the model. The missing data in mRS score will be imputed in sensitivity analyses using the last observation carried forward method, worst-case scenario, and best-case scenario. The last observation carried forward method was defined as that the missing*

mRS score at 90 days will be imputed using the value of NIHSS measured at 24 hours-12 days using the following relationship: NIHSS 0-3 at 24 hours, or NIHSS 0-5 at 7-12 days will correspond to mRS 0-1 at 90 days, while others correspond to mRS 2-6 at 90 days." in the revised manuscript (Page 10, Lines 188-195).

4. Please change "general linear model" to "geometric mean ratio" in the revised manuscript (Page 10, Line 181), and footnote d and f in Table 2.

Response: *Thank you very much for your suggestions. "general linear model" has been revised to "geometric mean ratio" as suggested (Page 10, Line 186; Table 2).*

Reviewer #3 (Remarks to the Author):

Will the work be of significance to the field and related fields?

The authors had better explained the relationship of INSIST-ED with other trials in the same field through a more detailed background/introduction and an additional reference.

Response: *Thank you very much for your suggestions. We revised the Introduction section to explain the relationship of INSIST-ED with other trials in the same field as suggested (Page 5, lines 84-89).*

Reporting of safety outcome

The authors had provided more details about adverse events in the trial but they did not follow the CONSORT Harm 2022 Statement in the reporting of these events. This made definition of adverse events, distribution of the events between treatment arms and evaluation of the balance between benefits and harms of the intervention less transparent. Ref (Junqueira D R, Zorzela L, Golder S, Loke Y, Gagnier J J, Julious S A et al. CONSORT Harms 2022 statement, explanation, and elaboration: updated guideline for the reporting of harms in randomised trials BMJ 2023; 381 :e073725 doi:10.1136/bmj-2022-073725).

Response: *Thank you very much for your suggestions. Given that the study mainly explored the different efficacy between ED and control groups, the objective (hypotheses) and conclusion were stated about the efficacy. As you suggested, we have added the report about harms in the methods and results of abstract followed the CONSORT Harm 2022 Statement in the revised manuscript. The other sections, such as methods and results in the main text, have reported the information about harms followed the Statement.*

Consistence in the description and the analysis of NIHSS score

The authors used Geometric Mean Ratio to analyse NIHSS score and yet used median (IQR) to summarise the scores. This made confirming the accuracy of the Geometric Mean Ratio difficult. Why not use geometric means and the 95% CI of the geometric mean to summarise NINSS score in each treatment arm? It is only in cases where there is an exact consistent multiplicative relationship between all numbers in a dataset that the median is equal to the geometric mean. It is unclear whether this relationship holds for this dataset.

Response: *Thank you very much for positive comments. We have changed the description of "Change in NIHSS score from baseline" from median (IQR) to GM (GMSD) in the revised Table 2 of manuscript.*

Re: NCOMMS-23-33900D entitled "Improving Neuroprotective Strategy for Ischemic Stroke with sufficient recanalization after Thrombectomy by Edaravone Dexborneol (INSIST-ED): a prospective, randomized, double blinded, multi-centre study"

We very much appreciate your review of our manuscript and the reviewers' valuable comments. We have carefully considered the reviewers' comments and revised our manuscript as suggested. Revisions are tracked in the revised manuscript. The reviewers' comments (in bold) and our point-by-point responses are below.

We hope the revision of the manuscript have fully addressed all concerns by the reviewers.

REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

All comments have been fully addressed.
Response: Thank you very much for your positive comments.

Reviewer #2 (Remarks to the Author):

Thank you for your responses to each of the concerns. I have a few comments on the revised manuscript.
1. In the revised SAP (version 2.0), the sample size was estimated by author's unpublished data (below). Why don't you use this description for sample size calculation in the manuscript?
"According to our recent unpublished data, the proportion of expected favorable functional outcome (mRS 0-2) at 90 days in control group is estimated to be about 50%. Using power = 80% and α = 0.05 to carry out the two-side test, the 200 sizes will test the superiority hypothesis with about 19% difference, namely the proportion of expected favorable functional outcome at 90 days is about 69%."
Response: Thank you very much for your suggestion. We have added this description in the revised manuscript as suggested (Pages 9-10, lines 176-180).

2. I would like to repeat that, the patients are randomly assigned to two groups in RCT, then theoretically, it is normal to deem patient characteristics to be no difference between two groups. Please describe the rationale to conduct the multivariate analyses in the manuscript, not only in the SAP.
Response: Thank you very much for your suggestion. Given the potential bias due to relatively small sample size in this phase 2 trial, we performed the multivariate analyses as a sensitivity analysis. We have added the rationale to conduct the multivariate analyses in the revised manuscript as suggested (Page 10, lines 192-193).

3. The author presented the details of imputation method for mRS score, however, it was not described in the SAP.

Response: Thank you very much for your suggestion. Sorry for missing the details of imputation method for mRS score in the SAP which was routinely performed in our previous studies. Thank you very much for your reminding us this important issue and will include this information in the SAP of our further studies.

Reviewer #3 (Remarks to the Author):

The authors have addressed all of my comments.

Response: Thank you very much for your positive comments.

Reviewer #4 (Remarks to the Author):

Thanks for having this opportunity to review this work. In this phase 2 trial, Chen et al aimed to explore the potential of ED as a cereborprotective drug among anterior circulation LVO-AIS patients with successful recanalization after EVT.

Abstract

1, there are 6 patients difference between the randomised groups. Any explanation? It seems variations in the enrolment may exist? How to assess its impact??

Response: Thank you very much for your professional question. After randomization, 101 patients were assigned into ED group and 103 patients were assigned into Control group. In ED group, 4 patients were further excluded after randomization due to decline to participate (n=1), and error enrollment after randomization (n=3). Finally, 97 patients were assigned into ED group. For clarify this difference, we have added a footnote (footnote a) in the revised Figure 1.

2, from the SAP, it shows unadjusted OR would be the primary result. It would be better to have unadjusted results.

Response: Thank you very much for this suggestion. We have added the unadjusted results in the revised Abstract (Page 3, line 57; page 4, line 59).

3, 14 patients missed the mRS. it seems the different approaches for the missing outcome would affect the result. Has the authors considered another method, such as Tipping point analysis to assess when the result would be altered?

Response: We have performed Tipping point analysis as suggested. The proportion of patients with mRS (0–2) at 90 days was 60.8% (54/97) in the ED group and 56.3% (58/103) in the control group (unadjusted OR 0.97, 95% CI 0.56-1.7; P = 0.93; adjusted OR 0.88 95% CI 0.48-1.61; P = 0.68). We have added the results in the revised manuscript as suggested. (Page 11, lines 205-207; Page 13, lines 257-260).

4, since there is a trend to indicate the benefit of ED, it would be good to have weak tone in the conclusion,…. Did not improve….. to be did not statically …..

Response: Thank you very much for your suggestion. We have revised the manuscript as suggested.

(Page 4, line 65)

Introduction

Clear

Method:

1,Threshold for the NIHSS score at baseline is not specified in the subgroup analysis.

Response: Sorry for missing this information. We have added this data in the revised manuscript as suggested (Page 11, line 219).

Result:

1, when did the trial finish? What is the date for data lock? It seem the trial should be stopped no later than Oct 2022. However, The latest SAP dated 28/05/2024, which is unexpected late. Potential bias may exist as the team may see the data.

Response: Sorry for the confusion. Based on other reviewers' comments from the previous round of reviews, the SAP was revised at 28/05/2024, but the statistical methods were not changed in this SAP dated 28/05/2024. The data from your trial was analyzed in accordance with the original SAP. We added this statement in the revised manuscript (Page 11, lines 208-210).

2, Figure 1, Can we assum the data is missing at ranom due to the clinical deterioration or economics reasons?

Response: Thank you very much for your question. After randomization, we have collected the basic characteristics in all patients. 12 patients gave up the treatment due to clinical deterioration (n=6) and economic reasons (n=6), but some follow-up data were also collected according to the time points of withdrawn.

3 Figure 2, what do you mean adjusted P value?

Response: Sorry for the mistake. We have deleted 'adjusted' in the revised manuscript (Page 22, line 480).

4, Table 1, no need to do the group test between groups as per guidelines.

Response: Thank you very much for your suggestion. We have revised Table 1 as suggested.

5, Some variables in the Table 1 may be clinically imbalance, such as mTICI score, hypertension. Can the author discuss more about the potential impact?

Response: Thank you very much for your invaluable suggestion. We further adjusted the two variables and the results were consistent (see the following Table). We have added the related discussion as one limitation in the revised manuscript as suggested (Page 17, lines 328-331).

Outcome	ED Group (n=97)	Control Group (n=103)	Treatment Effect Metric	Unadjusted		Adjusted [a]	
				Treatment Difference (95% CI)	P Value	Treatment Difference (95% CI)	P Value
Primary outcome							
mRS score 0-2 at 90±7 days, No. (%) [b]	54/92 (58.7)	49/94 (52.1)	OR	1.37 (0.76-2.44)	0.29	1.37 (0.70-2.67)	0.36

Secondary outcomes							
mRS score 0-1 at 90±7 days, No. (%) [b]	41/92 (44.6)	38/94 (40.4)	OR	1.19 (0.66-2.12)	0.57	1.07 (0.57-2.03)	0.83
mRS score distribution at 90±7 days [b]			OR [c]	0.74 (0.44-1.22)	0.24	0.83 (0.49-1.40)	0.48
Change in NIHSS score from baseline, GM (GMSD) [d]							
at 24 hours	0.651 (1.85)	0.709 (1.81)	GMR [e]	-0.02 (-0.11 to 0.08)	0.70	0.01 (-0.08 to 0.10)	0.98
at 48 hours	0.508 (2.05)	0.598 (1.89)	GMR [e]	-0.07 (-0.16 to 0.01)	0.09	-0.04 (-0.13 to 0.04)	0.31
at 12±2 days	0.297 (2.56)	0.384 (2.24)	GMR [e]	-0.11 (-0.23 to 0.00)	0.06	-0.07 (-0.18 to 0.05)	0.26
Infarct volume at 1 week, GM (GMSD) [f]	1.170 (2.00)	1.291 (1.58)	GMR [e]	0.12 (-0.07 to 0.31)	0.22	0.08 (-0.10 to 0.26)	0.38
All-cause mortality within 90±7 days, No. (%)	14 (14.4)	17 (16.5)	HR [g]	0.84 (0.41-1.70)	0.62	0.96 (0.45-2.03)	0.90

Abbreviation: ED, edaravone dexborneol; CI, confidence interval; mRS, modified Rankin Scale; OR, odds ratio; NIHSS, National Institutes of Health Stroke Scale; GM, geometric mean; GMSD, geometric mean standard deviation; GMR, geometric mean ratio; HR, hazard ratio.

[a] Adjusted for key prognostic covariates (age, sex, pre-stroke score on the modified Rankin Scale, NIHSS score at baseline, previous ischemic stroke, time from onset to recanalization, cerebral collateral circulation grade, number of thrombectomy device passes, intravenous thrombolysis, mTICI score, and hypertension).

[b] mRS scores range from 0 to 6: 0, no symptoms, 1 = symptoms without clinically significant disability, 2 = slight disability, 3 = moderate disability, 4 = moderately severe disability, 5 = severe disability; and 6 = death. Data missing for 14 participants for mRS score at 90±7 days.

[c] Calculated with ordinal regression analysis.

[d] NIHSS scores range 0–42, with higher scores indicating greater stroke severity. The log (NIHSS+1) was analyzed using a geometric mean ratio by generalized linear model. Data missing for 10 participants for NIHSS score at 24 hours, 23 participants at 48 hours and 34 participants at 12±2 days.

[e] GMR was used because normality assumption was assessed graphically and residual histogram showed serious violation of normality assumption.

[f] Determined by brain CT or MRI. The log (Infarct volume +1) was analyzed using a geometric mean by generalized linear model. Data missing for 11 participants for infarct volume at 1 week. Data eliminating for 1 participant due to excessive deviation.

[g] Calculated with Cox regression model.

6, ,how to define the safety population? it seems some subject did not receive the drugs from Table 3. Has this being considered in the definition of the ITT population?

Response: Thank you very much for your professional suggestion. As shown in SAP, the safety population consists of all randomised subjects who receive at least one dose of study drug. In this trial, there was no participant without study drug. Because the safety outcome was defined as symptomatic intracranial hemorrhage (sICH) within 48 hours, parenchymal hemorrhage at 48

hours, and serious adverse events (SAE), 5 patients lacked safety outcomes due to discharging within 48 hours after receiving the treatment (3 in ED group and 2 in Control group). To clarify this issue, we have added the footnote in the revised Table 3.

Discussion,

The trial was launched based on the hypothesis that Dexborneol was reported to exert neuroprotective effects through inhibiting nitric oxide (NO) and NO synthase pathways, reducing ROS generation, restraining inflammatory process and caspase-related apoptosis. however, no data was collected to support this hypothesis.

Response: Thank you very much for your invaluable suggestion. We further refined this limitation in the revised manuscript (Page 16, lines 322-323).

Re: NCOMMS-23-33900D entitled "Improving Neuroprotective Strategy for Ischemic Stroke with sufficient recanalization after Thrombectomy by Edaravone Dexborneol (INSIST-ED): a prospective, randomized, double blinded, multi-centre study"

We greatly appreciate the time and effort you have dedicated to ensuring the quality of our work and the reviewers' thoughtful and constructive comments on our manuscript. We have carefully considered the reviewers' comments and revised our manuscript as suggested. Revisions are tracked in the revised manuscript. The reviewers' comments (in bold) and our point-by-point responses are below.

REVIEWER COMMENTS

Reviewer 2's comments: Thank you for your responses to each of the concerns.
However, this study was randomized clinical trial so that all analyses should be described in SAP in advance. Any analyses which were not described in SAP should be clearly presented in the manuscript as a post-hoc with strong rationales.
This concerns include the multivariable models and imputation of missing of mRS score.
The lack of these information has great risk of biased results.
Response: Thank you very much for your professional suggestion. We have added the description of the multivariable models and imputation of missing of mRS score in the revised manuscript. (Page 16, lines 305-306)

Reviewer 3's comments: Thanks for sending back your revision. I have two additional comments.
1, since some of randomised were excluded from the ITT population. To avoid confusion, it would be better to use modified ITT.
Response: Thank you very much for your suggestion. We used modified ITT in the revised manuscript as suggested.

2, Tipping point analysis is not just to recalcuate the OR. It is mainly to check when and how the missing data would change the study results. A graph in the appendix would be great.
Response: Thank you very much for your suggestion. We have added a graph in the appendix as suggested (eFigure 3 in Appendix 1).

3, Thanks for providing the additional analysis, which can be placed in the appendix if possible.
Response: As replied to last comment, we have added a graph in the appendix as suggested (eFigure 3 in Appendix 1).