PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Repurposing bromocriptine for Aβ metabolism in Alzheimer's disease (REBRAnD) study: Randomised Placebo-Controlled Double-Blind Comparative Trial and Open-Label Extension Trial to Investigate the Safety and Efficacy of Bromocriptine in Alzheimer's Disease with Presenilin 1 (PSEN1) Mutations
AUTHORS	Kondo, Takayuki; Banno, Haruhiko; Okunomiya, Taro; Amino, Yoko; Endo, Kayoko; Nakakura, Akiyoshi; Uozumi, Ryuji; Kinoshita, Akemi; Tada, Harue; Morita, Satoshi; Ishikawa, Hidehiro; Shindo, Akihiro; Yasuda, Ken; Taruno, Yosuke; Maki, Takakuni; Suehiro, Takashi; Mori, Kohji; Ikeda, Manabu; Fujita, Koji; Izumi, Yuishin; Kanemaru, Kazutomi; Ishii, Kenji; Shigenobu, Kazue; Kutoku, Yumiko; Sunada, Yoshihide; Kawakatsu, Shinobu; Shiota, Shunji; Watanabe, Toshifumi; Uchikawa, Osamu; Takahashi, Ryosuke; Tomimoto, Hidekazu; Inoue, Haruhisa

VERSION 1 – REVIEW

REVIEWER	Besser, Lilah Florida Atlantic University
REVIEW RETURNED	16-Nov-2020

GENERAL COMMENTS	The authors provide a clearly described protocol, but there are a number of items that need further details or changes to clarify to the reader and to make it acceptable for publication. 1. Primary outcome reporting: Please provide the range of scores for the SIB-J and NPI, the exact time points you will be administering the test (ex: week 1, week 20, week 36), and any available data/references that indicate that this is an acceptable test for measuring longitudinal change in cognition/psychiatric symptoms. You indicate you will measure changes in scores over specific time frames but not when the SIB-J and NPI will be administered specifically. Describe how learning effects may influence scores because the test is

	repeated multiple times over one year. Under the safety endpoint, it would be helpful to describe the most commonly expected AEs (as you provided in the discussion – nausea, etc.). 2. Sample size: You mention you are recruiting at a 2 to 1 ratio but then your sample size is 10, which is an odd target. Shouldn't the target be 6, 9 or 11 then? Why was this target chosen? Although the expected eligibility pool is low in Japan, I believe a phase 2 trial would require a larger sample size to establish efficacy and power / sample size calculations should be provided. In addition, you need to add a discussion of loss to follow-up and how you will accommodate that to ensure you have enough participants completing the trial. 3. Recruitment: Please provide the recruitment procedure in more detail. Although you provide inclusion and exclusion criteria and recruitment setting (7 hospitals), you did not state who will be involved in recruiting (e.g., neurologists, nurses, study coordinators) and whether they will be blinded. 4. Allocation implementation: Please specify who will enroll and assign participants to interventions. 5. Data collection methods: Please provide who will be collecting data for the primary outcome, the validation/reliability (if available) and a citation for all of the instruments/tests that will be used including secondary and reference endpoints (e.g., MMSE-J, plasma AB, etc.). 6. Retention methods: what methods will you use to help retain participants until the end of the study (e.g., phone call reminders?) 7. Statistical methods: please provide more specific details on planned statistical analyses. Are you calculating mean differences? What significance level will you be using, especially because you have multiple endpoints (multiple comparisons). 8. Population analyzed: You will need to specify how you plan to analyze the data if data are missing or if participants are non-compliant with protocol. 9. Access to data: Who will have access to the full dataset after the trial? Additional suggestions that would help improve organization/clarity: 1. Please be sure to add to the Box 1 exclusions: CYP3A4 inhibitors/inducers and dopamine antagonists, which are listed in the discussion but I don't think we included in Box 1. 2. Please provide in the introduction or methods the dosage that has been shown to be safe in Parkinson's disease patients. 3. Under randomization, you mention you will be stratifying based on MMSE. However, it is unclear how/why you are

	doing that and how this affects your statistical analyses. Please provide more details. 4. Under secondary and reference endpoints, please provide more details on when these will be conducted/measured. 5. In two places in your protocol, you state procedures “were” assessed/done instead of “will be” assessed/done. Please change to the correct verb tense. If you have not yet conducted any of the steps outlined in the protocol, you need to change to “will be” assessed/done. Another example in Discussion was “this enrichment made it difficult” instead of “this enrichment will make it difficult”. 6. Under patient and public involvement, you state “No patient involved”. I think you meant no public will be involved in this study? 7. In the discussion, you discuss details of the efficacy of study using iPSC-based repurposing. Please add these details to the introduction instead. 8. The last paragraph of the discussion, you need to add cognitive and neuropsychiatric endpoints for efficacy in addition to your mention of biomarker-based efficacy detection. 9. Figure 1 for the placebo group, you need to change all mention of TW-012R to “placebo”.

REVIEWER	Mehan, Sidharth ISF College of Pharmacy, Pharmacology
REVIEW RETURNED	22-Nov-2020

GENERAL COMMENTS	Dear Author, Thanks for submitting your research manuscript entitled “Repurposing bromocriptine for Aβ metabolism in Alzheimer's disease (REBRAnD) study: Randomised Placebo-Controlled Double-Blind Comparative Trial and Open-Label Extension Trial to Investigate the Safety and Efficacy of Bromocriptine in Alzheimer’s Disease with Presenilin 1 (PSEN1) Mutations” Please find out the following comments. Abstract 1. In the introduction, discussion, and the concluding section, the logic behind this study is not well explained. 2. The abstract provides no clear understanding of the schedules of the study, along with the rationale and causes confusion. 3. The author didn’t provide the clear conclusion of study. Introduction: 1. In p2 the authors could more elaborate the word ‘(PSEN1-AD)’ that how the mutation in this gene is responsible for disease could be little more explainable. 2. In p3 the rationale behind the use of bromocriptine is poorly understood and needs more explanation. 3. Referencing is poor, in p4 there is no referencing in the entire paragraph.

	4. “As a result, we found that bromocriptine reduces the production of Aβ and the Aβ42/40 ratio.” This line should again be revised once more. 5. “Pluripotent stem cells (iPSCs)” could be explained more. 6. Authors must also focus on how the mutation in PSEN-1 gene contributes to the AD and what conformational changes they produce in the human that affect the healthy life. 7. “We also compared the effectiveness of bromocriptine among different AD types.” This line should be made more transparent for a better review Methods and analysis: 1. The legend of the table is poor. Mention and revise it properly. 2. Authors should give the justifications about from where the patients taken in the study and how the patients are confirmed for the presence of PSEN-1 mutants. 3. Study design is very poor and there is no clinical efficacy of the study. 4. In study design the rationale behind the use of screening period, double-blind phase, extension phase needs more justification. 5. “Patients taking oral donepezil hydrochloride will be carefully monitored because the blood concentration may be increased by the additional oral administration of the trial drug” explain more how this drug effects the trial. 6. The methods and the procedures used in the study should be revised once again for the mistakes, and at this level, some points are not clear especially in the study plan, which should be made more justified by the authors. Outcomes: 1. The outcomes of the study failed to clearly mentioned the results after trial and very poor explanation is given in the outcome section. 2. Again referencing is lacking and no justification is given for the points written. 3. Author should mention the clear results of the study with appropriate data. 4. Author should mention the p value with data points. Discussion: 1. The discussion part should be to the point, straight forward, no canvassing, only the pure result discussion along with the future perspectives, lacunas and recent advancements. 2. Overall the whole manuscript must be again thoroughly revised with the corrections. The authors should use small bites of sentences to tell their views or literature review in a comprehensive way, rather than using complicated and hectic lines and sentences. 3. Add more figures and give clear explanation of outcomes of the results with justification. 4. Proper pattern of the approval of ethical committee should be mentioned here.

REVIEWER	Yap, Kai Zhen

	National University of Singapore, Department of Pharmacy
REVIEW RETURNED	26-Nov-2020

GENERAL COMMENTS	I'm grateful for the opportunity to review this study protocol. This is an important area of research in dementia treatment. However, many of the details required under the SPIRIT checklist are missing. The following are some examples to be addressed by the authors. Extensive English editing should also be considered. Introduction: Include more details about the postulated mechanism of action of how bromocriptine "improves Aß phenotype especially in PSEN1-AD". Objectives: The information stated in point form should be incorporated in the Methods and Analysis section under the various appropriate subheading. Specific hypotheses in terms of the outcomes should also be specified. Study design: This should be stated explicitly under method section Trial population and rationale for selecting participants: The rationale for excluding patient who have changed AD drug treatment within 2 months was not explicitly provided. It would be good to provide the reference for use of MMSE-J cut-off equal or less than 25. Also, details of how and where patients would be approached and screened (esp for PSEN 1 mutation) should be included for clarity. Sample size: What is the duration for recruitment and would it be feasible to recruit the sample size proposed given the incidence rate? Also, why is the ratio of 2:1 selected over 1:1 allocation to intervention and control? Also, details on blinding and sequence generation should be provided. Study design (page 9): The details contained under this section should be more appropriately titled as "intervention and control". Details of the screening done during the 8-week screening period is not provided. In the extension phase, what determines increasing the dose to beyond 4 tablets? Page 10: It is not clear what is meant by "not increasing the dose or decreasing the dose is allowed at the discretion of the investigator from the viewpoint of safety of the participants". Does this not contradict what is going to be done in the extension phase? Also, description and comparison between the intervention and control tablets are not provided. Steps for monitoring of adherence should also be provided. Primary, secondary and reference endpoints: What are the key pharmacokinetic parameters referred to and what does it mean "for blood bromocriptine concentration on Day 1"? Also, it would be good to provide the purpose and rationale for including the measurement of (1) to (11). Lastly, all items listed are outcome measures, not "endpoints", further details

	on when and how they these measures would be made and how reported and analysed would also be needed for clarity. Statistical analysis: What other stats tests would be used if assumptions of t-test is violated? Any subgroup analysis - since allocation is stratified according to MMSE-J score?

VERSION 1 – AUTHOR RESPONSE

Response to Reviewer #1

The authors provide a clearly described protocol, but there are a number of items that need further details or changes to clarify to the reader and to make it acceptable for publication.

(Reply to Reviewer #1)

> We really appreciate the constructive comments from Reviewer #1.

1. Primary outcome reporting: Please provide the range of scores for the SIB-J and NPI, the exact time points you will be administering the test (ex: week 1, week 20, week 36), and any available data/references that indicate that this is an acceptable test for measuring longitudinal change in cognition/psychiatric symptoms. You indicate you will measure changes in scores over specific time frames but not when the SIB-J and NPI will be administered specifically. Describe how learning effects may influence scores because the test is repeated multiple times over one year. Under the safety endpoint, it would be helpful to describe the most commonly expected AEs (as you provided in the discussion – nausea, etc.).

(Reply to Reviewer #1)

> We appreciate Reviewer #1's comment. We added a new table to describe the trial evaluation schedule in the revised manuscript. We also added a description of the details of SIB-J, NPI and the learning effects in the Outcomes section in the revised manuscript, together with references, as follows.

(New table in the highlighted revised manuscript pp.19–21)

Table 1. Trial schedule

		Screening period		Double-blind phase																
				Escalation to 4 tablets/day									Escalation to 9 tablets/day							Taper
Timing		Before enrollment	Before start of trial treatment	At start of administration	Week 1	Week 2	Week 3	Week 4	Week 8	Week 12	Week 16	Week 20	Week 21	Week 22	Week 23	Week 24	Week 28	Week 32	Week 36	Week 37
Visit/medical examination		○	○	○	○	Phone	Phone	○	○	○	○	○	○	Phone	Phone	○	○	○	○	○
Informed consent		○																		
Neuropsychological/Motor assessment	ADAS-J cog	○																		
	SIB-J		○	○								○							○	
	NPI		○	○				○	○	○	○	○				○	○	○	○	
	MENFIS		○	○				○	○	○	○	○				○	○	○	○	
	MMSE-J	○	○	○								○							○	
	DAD		○	○								○							○	
	UPDRS part III		○	○								○							○	
	Apathy Scale		○	○								○							○	
	UMNB		○	○								○							○	
Laboratory biomarkers	Plasma biomarkers		○	○						○		○					○		○	
	CSF biomarkers			○															○	
Digital biomarkers	Wearable physical activity		→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	
	Finger tapping		○	○				○	○	○	○	○				○	○	○	○	
PET	Brain amyloid PET		○															○		
	Brain tau PET		○															○		
Safety assessment	Cardiac ultrasound	○										○							○	
	Chest X-ray	○						○				○				○			○	
	ECG	○			▲			○	▲	▲	▲	○	▲			○	▲	▲	○	▲

	Head MRI	○																	○	
	Blood bromocriptine concentration			○ *						○	○	○					○	○	○	
	Laboratory tests	○						○				○				○			○	
	AEs		→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→

		Extension phase										
		Escalation to 4–9 tablets/day †										End of trial
Timing		Week 37	Week 38	Week 39	Week 40	Week 41	Week 42	Week 43	Week 44	Week 45	Week 48	Week 50
Visit/medical examination		○	Phone	Phone	○	Phone	Phone	Phone	○	Phone	○	○
Informed consent												
Neuropsychological/motor assessment	ADAS-J cog											
	SIB-J											
	NPI											
	MENFIS											
	MMSE-J											
	DAD											
	UPDRS part III											
	Apathy Scale											
	UMNB											
Laboratory biomarkers	Plasma biomarkers											
	CSF biomarkers											
Digital biomarkers	Wearable physical activity											
	Finger tapping											
PET	Brain amyloid PET											
	Brain tau PET											
Safety assessment	Cardiac ultrasound											○
	Chest X-ray											○
	ECG	▲			▲				▲		▲	○
	Head MRI											
	Blood bromocriptine concentration											

	Laboratory tests				○							○
	AEs	→	→	→	→	→	→	→	→	→	→	→

○: To be performed

▲: To be performed if donepezil hydrochloride is co-administered.

• After the final visit of the last participant at Week 37, the blind will be broken following data lock to start analyses.

*Schedule for measuring blood bromocriptine concentration on Day 1

Time after first administration of trial drug (h)	Before administration	1	2	3	4	6
Time window (in principle)	−2 hours	± 5 minutes	± 5 minutes	± 5 minutes	± 5 minutes	± 5 minutes
Plasma bromocriptine	○	○	○	○	○	○

†Standard dose is 4 tablets/day, up to 9 tablets/day at maximum.

ADAS-J cog, Alzheimer's Disease Assessment Scale-cognitive subscale Japanese version; AEs, adverse events; DAD, Disability Assessment for Dementia; MENFIS, Mental Function Impairment Scale; MMSE-J, Mini Mental State Examination-Japanese; NPI, Neuropsychiatric Inventory; SIB-J, Severe Impairment Battery-Japanese; UMNB, Upper Motor Neuron Burden Score; UPDRS part III, Movement Disorder Society-sponsored revision of the Unified Parkinson’s Disease Rating Scale Part III.

(In the Outcomes section in the highlighted revised manuscript p.10, lines 1–20; p.11, lines 16–18)

Primary endpoint 1) safety

As the primary analysis, safety will be evaluated by collecting incidence information and severity of adverse events (AEs) or adverse reactions from the starting time of the administration of the trial drug to the end of the follow-up period. Known frequent adverse effects of bromocriptine include nausea and vomiting (8.3%), anorexia (2.5%), and gastric discomfort, based on 5212 cases with Parkinson's syndrome [Interview Form Parlodel Tablets 2.5 mg, Sun Pharma Japan Ltd. https://medical.mt-pharma.co.jp/di/file/dc/plo.pdf (accessed on 1st March, 2021)].

Primary endpoint 2) Severe Impairment Battery-Japanese (SIB-J)

SIB-J is validated in the Japanese version and will be used to evaluate cognitive function [Psychol Assessment 1990;2:298–303; Severe Impairment Battery (SIB). London, UK. Pearson Assessment 1993; Jpn J Geriatr Psychiatry 2005;16:683–691.]. Higher scores indicate better cognitive function (range 0–100). Individual time courses of observed values and changes from baseline in SIB-J score up to Week 20 and Week 36 after trial drug administration will be shown.

Primary endpoint 3) Neuropsychiatric Inventory (NPI)

NPI (Japanese version) is a validated clinical instrument for evaluating psychopathology in dementia [Neurology 1994;44:2308–14; Neurology 1997;48:10S–16S; No To Shinkei 1997;49:266–71]. Higher scores indicate more severe neuropsychiatric derangements (range 10–120). Individual time courses of observed values and changes from baseline in NPI score up to Week 20 and Week 36 after trial drug administration will be shown.

Learning effect cannot be negligible, but this trial has a placebo arm, and previous trials using SIB-J, NPI, MENFIS and DAD adopted similar measuring time frames and no obvious learning effects were reported [Alzheimers Dement (N Y) 2019;5:398-408; Sci Rep 2020;10:18627].

References

SIB:

Saxton J, McGonigle-Gibson KL, Swihart AA, et al. Assessment of the severely impaired patient: Description and validation of a new neuropsychological test battery. Psychol Assessment 1990;2:298–303.

Saxton J, McGonigle K, Swihart A, et al. Severe Impairment Battery (SIB). London, UK. Pearson Assessment 1993.

Niina R, Homma A, Sugai Y, et al. Reliability, validity and clinical availability of a Japanese version of Severe Impairment Battery (SIB) and a Japanese version of modified Alzheimer's Disease Cooperative Study-Activities of Daily Living Inventory (ADCS-ADL). Jpn J Geriatr Psychiatry 2005;16:683–691.

NPI:

Cummings JL, Mega M, Gray K, et al. The Neuropsychiatric Inventory. Comprehensive assessment of psychopathology in dementia. Neurology 1994;44:2308–14.

Cummings JL. The Neuropsychiatric Inventory. Assessing psychopathology in dementia patients. Neurology 1997;48:10S–16S.

Hirono N, Mori E, Ikejiri Y, et al. Japanese version of the neuropsychiatric inventory a scoring system for neuropsychiatric disturbances in dementia patients. No To Shinkei 1997;49:266–71.

Learning effect:

Watanabe M, Nakamura Y, Yoshiyama Y, et al. Analyses of natural courses of Japanese patients with Alzheimer's disease using placebo data from placebo-controlled, randomized clinical trials: Japanese Study on the Estimation of Clinical course of Alzheimer's disease. Alzheimers Dement (N Y) 2019;5:398-408.

Noguchi-Shinohara M, Ono K, Hamaguchi T, et al. Safety and efficacy of Melissa officinalis extract containing rosmarinic acid in the prevention of Alzheimer's disease progression. Sci Rep 2020;10:18627.

2. Sample size: You mention you are recruiting at a 2 to 1 ratio but then your sample size is 10, which is an odd target. Shouldn’t the target be 6, 9 or 11 then? Why was this target

chosen? Although the expected eligibility pool is low in Japan, I believe a phase 2 trial would require a larger sample size to establish efficacy and power / sample size calculations should be provided. In addition, you need to add a discussion of loss to follow-up and how you will accommodate that to ensure you have enough participants completing the trial.
(Reply to Reviewer #1)
> We appreciate Reviewer #1's comment. We revised the manuscript as follows:

(In the Sample size section in the original manuscript)
Given such rarity, the patient accumulation is extremely low and the targeted sample size for this randomised trial is 10 (≥ 4 patients in TW-012R and ≥ 2 patients in placebo) based on feasibility and the aforementioned validity.

(In the Sample size section in the revised manuscript p.8, lines 2–8)
Given such rarity, the patient accumulation is extremely low and the targeted sample size for this randomised trial is ≥ 4 patients in TW-012R and ≥ 2 patients in placebo based on feasibility and the aforementioned validity. Unequal randomisation will be employed because of ethical considerations. There is no formal hypothesis or power sample size calculation due to the exploratory nature of this study. Thus, we did not perform sample size calculation. To ensure that enough participants complete the trial, clinical research coordinators will communicate well and frequently both with the central coordinating centre and the participants/care givers.

3. Recruitment: Please provide the recruitment procedure in more detail. Although you provide inclusion and exclusion criteria and recruitment setting (7 hospitals), you did not state who will be involved in recruiting (e.g., neurologists, nurses, study coordinators) and whether they will be blinded.
(Reply to Reviewer #1)
> We appreciate Reviewer #1's comment. We added a sentence to clarify the recruiting procedure in the revised manuscript as follows.

(In the Trial population and rationale for selecting participants section in the revised manuscript p.7, line 17–18)
Recruiting will be done by neurologists who treat PSEN1-AD patients and are blinded to their allocation.

4. Allocation implementation: Please specify who will enroll and assign participants to interventions.
(Reply to Reviewer #1)
> We appreciate Reviewer #1's comment. We added details of the registration and enrolment in the revised manuscript as follows.

(In the Randomisation, allocation, and blinding section in the original manuscript)
The allocation sequence will be prepared by an external contract research organization, and trial participants, care givers, outcome assessors and investigators will be blinded.

(In the Randomisation, allocation, and blinding section in the revised manuscript p.9, lines 25–28)
The allocation sequence will be prepared by an external contract research organization, and trial participants, care givers, outcome assessors and investigators will be blinded. Registration will be done by investigators etc. using the web enrolment system. When a patient is determined to be eligible by the web enrolment system, the allocated drug number will be displayed as the enrolment result.

5. Data collection methods: Please provide who will be collecting data for the primary outcome, the validation/reliability (if available) and a citation for all of the instruments/tests that will be used including secondary and reference endpoints (e.g., MMSE-J, plasma AB, etc.).
(Reply to Reviewer #1)
> We appreciate Reviewer #1's comment. We added details of data collection for the primary outcomes and 16 references in regard to SIB, NPI, MMSE, Apathy Scale, plasma Aβ, NfL, total-

tau, p-tau, NfL, CSF Aβ, total-tau, p-tau, and plasma Aβ-related peptides in the revised manuscript as follows.

(In the Outcomes section in the revised manuscript p.10, lines 9–10, 16-17)
(SIB-J) Participants will be questioned and assessed by the investigator, sub-investigator, clinical psychologist or speech therapist.
(NPI) Caregivers will be interviewed and assessed by the investigator, sub-investigator, clinical psychologist or speech therapist.

References

SIB:
Saxton J., McGonigle-Gibson KL., Swihart AA., Miller VJ, & Boller F. Assessment of the severely impaired patient: Description and validation of a new neuropsychological test battery. Psychological Assessment: A Journal of Consulting and Clinical Psychology 1990;2:298–303.
Saxton J, McGonigle K, Swihart A, et al. Severe Impairment Battery (SIB). London, UK. Pearson Assessment 1993.

NPI:
Cummings JL, Mega M, Gray K, et al. The Neuropsychiatric Inventory. Comprehensive assessment of psychopathology in dementia. Neurology 1994;44:2308–14.
Cummings JL. The Neuropsychiatric Inventory. Assessing psychopathology in dementia patients. Neurology 1997;48:10S–16S.

MMSE:
Folstein MF, Folstein SE, McHugh PR. “Mini-mental state”: A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975;12:189–98.
Sugishita M, Hemmi I, Takeuchi T. Reexamination of the Validity and Reliability of the Japanese Version of the Mini-Mental State Examination (MMSE-J). Japanese Journal of Cognitive Neuroscience 2016;18:168–83.

Sugishita M, Koshizuka Y, Sudou S, et al. The validity and reliability of the Japanese version of the Mini- Mental State Examination (MMSE-J) with the original procedure of the attention and calculation task(2001). Japanese Journal of Cognitive Neuroscience 2018;20:91–110.

Apathy Scale (AES-I-J):
Marin RS, Biedrzycki RC, Firinciogullari S. Reliability and validity of the apathy evaluation scale. Psychiat Res 1991;38:143–62.

Plasma Aβ1-40, 1-42, total-tau, p-tau:
Palmqvist S, Janelidze S, Stomrud E, et al. Performance of Fully Automated Plasma Assays as Screening Tests for Alzheimer Disease–Related β-Amyloid Status. JAMA Neurol 2019;76:1060–69.

Plasma NfL:
Schultz SA, Strain JF, Adedokun A, et al. Serum neurofilament light chain levels are associated with white matter integrity in autosomal dominant Alzheimer's disease. Neurobiol Dis 2020;142:104960.

Plasma total-tau:
Mielke MM, Hagen CE, Wennberg AMV, et al. Association of Plasma Total Tau Level With Cognitive Decline and Risk of Mild Cognitive Impairment or Dementia in the Mayo Clinic Study on Aging. JAMA Neurol 2017;74:1073–80.

Plasma p-tau:
Karikari TK, Pascoal TA, Ashton NJ, et al. Blood phosphorylated tau 181 as a biomarker for Alzheimer's disease: a diagnostic performance and prediction modelling study using data from four prospective cohorts. Lancet Neurol 2020;19:422–33.
Tatebe H, Kasai T, Ohmichi T, et al. Quantification of plasma phosphorylated tau to use as a biomarker for brain Alzheimer pathology: pilot case-control studies including patients with Alzheimer's disease and down syndrome. Mol Neurodegener 2017;12:63.

CSF Aβ1-40, Aβ1-42, total-tau, p-tau:

Bateman RJ, Xiong C, Benzinger TLS, et al. Clinical and Biomarker Changes in Dominantly Inherited Alzheimer's Disease. N Engl J Med 2012;367:795–804.

Dubois B, Feldman HH, Jacova C, et al. Advancing research diagnostic criteria for Alzheimer's disease: the IWG-2 criteria. Lancet Neurol 2014;13:614–29.

Plasma Aβ-related peptides:

Nakamura A, Kaneko N, Villemagne VL, et al. High performance plasma amyloid-β biomarkers for Alzheimer’s disease. Nature 2018;554:249–54.

6. Retention methods: what methods will you use to help retain participants until the end of the study (e.g., phone call reminders?)

(Reply to Reviewer #1)

> We appreciate Reviewer #1’s comment. We provided more details on the retention methods including our new protocol amendment to make it possible to deliver trial drugs during the COVID-19 pandemic in the revised manuscript as follows.

(In the Intervention and control section in the revised manuscript p.9, lines 8–11)

Due to the COVID-19 pandemic in 2020, we amended our protocol to make it possible to deliver study drugs if a participant cannot visit a medical facility. For participant retention and safety reasons, a participant will have 10 phone-call-visits in addition to monthly onsite-visits.

7. Statistical methods: please provide more specific details on planned statistical analyses. Are you calculating mean differences? What significance level will you be using, especially because you have multiple endpoints (multiple comparisons).

(Reply to Reviewer #1)

> We appreciate Reviewer #1’s comment. We added more details for the planned statistical analysis. We have clarified that no adjustments will be made for multiple testing in the revised manuscript as follows.

(In the Sample size section in the revised manuscript p.8, lines 5–6)
There is no formal hypothesis or power sample size calculation due to the exploratory nature of this study. Thus, we did not perform sample size calculation.

(In the Statistical analysis section in the revised manuscript p.12, lines 8–10)
Owing to the exploratory nature of this study, no adjustments will be made for multiple testing. To support the robustness of the results, a sensitivity analysis with and without non-compliant patients will be conducted.

According to the recommendations of the American Statistical Association (see the following references), in this setting with such a small sample size, setting a significance level should be avoided when interpreting p-values. Thus, we will provide confidence intervals and p-values for descriptive purposes.

References:
Wasserstein RL, Lazar NA. The ASA's Statement on p-Values: Context, Process, and Purpose. Am Stat 2016;70:129–33.
Wasserstein RL, Schirm AL, Lazar NA. Moving to a world beyond "P<0.05." Am Stat 2019;73:1–19.
Amrhein V, Greenland S, McShane B. Scientists rise up against statistical significance. Nature 2019;567:305–307.
Pike H. Statistical significance should be abandoned, say scientists. BMJ 2019;364:l1374.

8. Population analyzed: You will need to specify how you plan to analyze the data if data are missing or if participants are non-compliant with protocol.
(Reply to Reviewer #1)
> We appreciate Reviewer #1's comment. We added a sensitivity analysis both with and without non-compliant patients in the Statistical Analysis section. We stated that there will be no

imputation for missing data in the Statistical Analysis section in the revised manuscript as follows.

(In the Statistical analysis section in the revised manuscript p.12, lines 8–10)
Owing to the exploratory nature of this study, no adjustments will be made for multiple testing. To support the robustness of the results, a sensitivity analysis with and without non-compliant patients will be conducted.

9. Access to data: Who will have access to the full dataset after the trial?

(Reply to Reviewer #1)

> We appreciate Reviewer #1's comment. We explained who will have access to the full dataset in the revised manuscript as follows.

(In the Data management, monitoring, and auditing section in the revised manuscript p.11, lines 23–25)
The full dataset without personal identifiable information will be accessed by data managers, statisticians and investigators.

Additional suggestions that would help improve organization/clarity:

1. Please be sure to add to the Box 1 exclusions: CYP3A4 inhibitors/inducers and dopamine antagonists, which are listed in the discussion but I don't think we included in Box 1.

(Reply to Reviewer #1)

> We appreciate Reviewer #1's comment. We added explanations of the CYP3A4 inhibitors/inducers and dopamine antagonists in Box 1 in the revised manuscript as follows.

(In Box 1 in the highlighted revised manuscript p.23, lines 30–31)

CYP3A4 inhibitors/inducers and dopamine antagonists are contraindicated during the trial period except for domperidone and quetiapine in emergent settings.

2. Please provide in the introduction or methods the dosage that has been shown to be safe in Parkinson's disease patients.

(Reply to Reviewer #1)

> We appreciate Reviewer #1's comment. We added the approved dosage for Parkinson's disease in the revised manuscript as follows.

(In the Introduction section in the revised manuscript p.6, lines 8–9)

Bromocriptine was approved for the treatment of Parkinson's disease (approved dosage 22.5mg/day).

3. Under randomization, you mention you will be stratifying based on MMSE. However, it is unclear how/why you are doing that and how this affects your statistical analyses. Please provide more details.

(Reply to Reviewer #1)

> We appreciate Reviewer #1's comment. We added information regarding how and why randomisation will be stratified according to the baseline MMSE-J score in addition to our planned subgroup analysis in the revised manuscript as follows. A subgroup analysis in accordance with the baseline MMSE-J score (< 13 or ≥ 13) will be performed to investigate the patient response to bromocriptine.

(In the Randomisation, allocation, and blinding section in the revised manuscript p.9, lines 23–24)

Randomisation will be performed using permuted blocks and stratified based on the important confounding variable of the baseline MMSE-J score (< 13 or ≥ 13).

4. Under secondary and reference endpoints, please provide more details on when these will be conducted/measured.

(Reply to Reviewer #1)

> We appreciate Reviewer #1's comment. We added a new table (shown previously) so that the readers can clearly understand the evaluation schedule.

5. In two places in your protocol, you state procedures "were" assessed/done instead of "will be" assessed/done. Please change to the correct verb tense. If you have not yet conducted any of the steps outlined in the protocol, you need to change to "will be" assessed/done. Another example in Discussion was "this enrichment made it difficult" instead of "this enrichment will make it difficult".

(Reply to Reviewer #1)

> We appreciate Reviewer #1's comment. We made the necessary changes for the correct verb tense in the revised manuscript as follows.

In the following sentence, we left "were" as it is, because patients and their family burdens WERE assessed IN the design of this study phase BEFORE the study start.

"However, in the design of this study, patients and their family burdens were assessed with the help of neurologists and clinical trial coordinators of Kyoto University Hospital."

(In the Outcomes section in the original manuscript)

For exploratory purposes, additional measurement with wearable physical activity meter (Silmee™W20, TDK Corporation, Tokyo, Japan), measurement with finger tapping device (UB-2, Maxell, Ltd. Tokyo, Japan), brain amyloid PET [22], brain tau PET [23], Upper Motor Neuron Burden Score (UMNB) [24], and plasma Aβ-related peptides were selected as reference endpoints.

(In the Outcomes section in the revised manuscript p.11, lines 7–11)

For exploratory purposes, additional measurement with wearable physical activity meter (Silmee™W20, TDK Corporation, Tokyo, Japan), measurement with finger tapping device (UB-

2, Maxell, Ltd. Tokyo, Japan), brain amyloid PET [22], brain tau PET [23], Upper Motor Neuron Burden Score (UMNB) [24], and plasma Aβ-related peptides will be assessed as reference endpoints.

(In the Discussion section in the original manuscript)
This enrichment made it difficult to recruit many patients for this study, considering the rarity of such patients in Japan.

(In the Discussion section in the revised manuscript p.14, lines 28–29)
This enrichment will make it difficult to recruit many patients for this study, considering the rarity of such patients in Japan.

6. Under patient and public involvement, you state “No patient involved”. I think you meant no public will be involved in this study?
(Reply to Reviewer #1)
> We appreciate Reviewer #1’s comment. We changed to “No patient or public were involved in this study design” in the revised manuscript p.12, line 16.

7. In the discussion, you discuss details of the efficacy of study using iPSC-based repurposing. Please add these details to the introduction instead.
(Reply to Reviewer #1)
> We appreciate Reviewer #1’s comment. We added details of the efficacy study using iPSC-based repurposing in the Introduction section in the revised manuscript as follows.

(In the Introduction section in the original manuscript)
As a result, we found that bromocriptine reduces the production of Aβ and the Aβ42/40 ratio. We also compared the effectiveness of bromocriptine among different AD types, and we were able to clarify that bromocriptine improves Aβ phenotypes especially in PSEN1-AD [6,7].

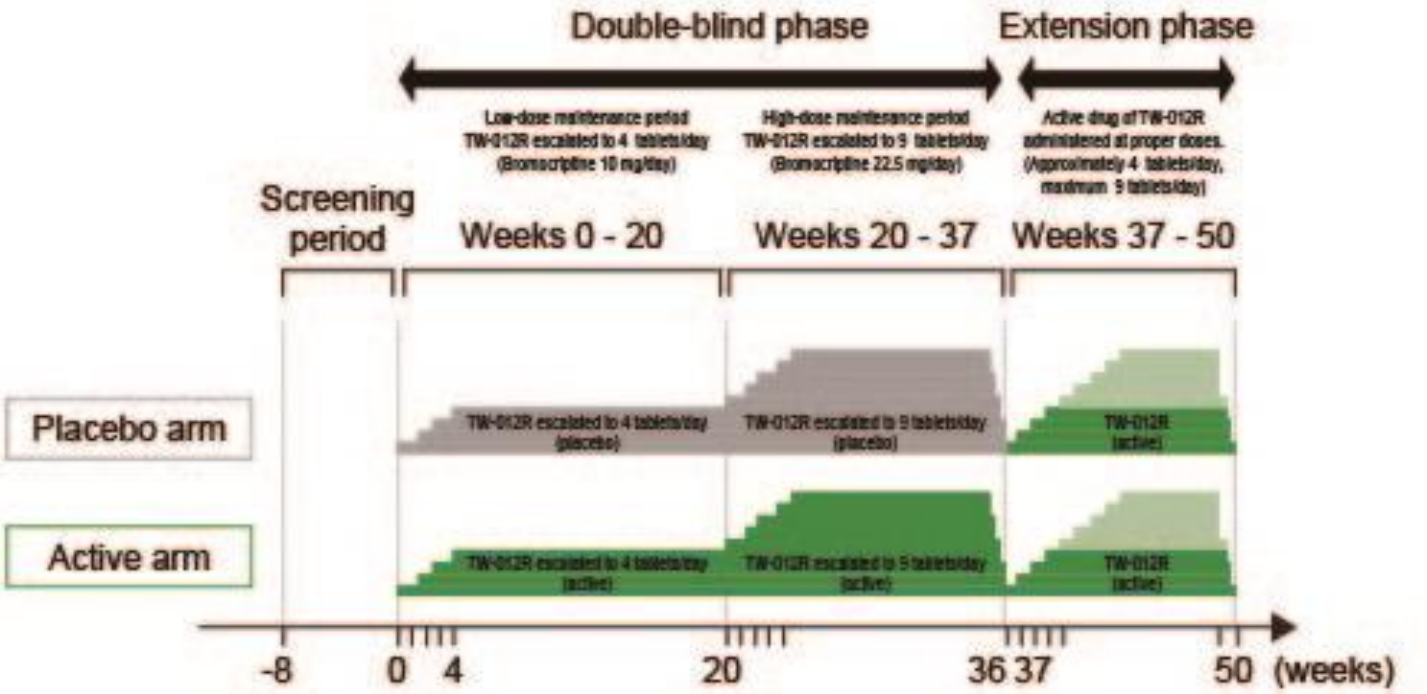
(In the Introduction section in the highlighted revised manuscript p.5, lines 29–33, p.6, lines 1–3)
After the screening, we found bromocriptine to be the most potent modifier of Aβ production for PSEN1-AD neurons among existing drugs. Dose-dependency assay showed that bromocriptine reduced the Aβ42 dose and Aβ42/40 ratio by up to ~50% and ~40%, respectively. Furthermore, we prepared cortical neurons of several patients with PSEN1-AD and sporadic AD to evaluate the specificity of bromocriptine for PSEN1-AD. Bromocriptine reduced the Aβ42 dose and Aβ42/40 ratio of PSEN1-AD neurons more strongly than those of sporadic AD neurons. From these results, we selected patients with PSEN1-AD as a bromocriptine-responsive subgroup in AD [11,12: same references as original 6,7].

8. The last paragraph of the discussion, you need to add cognitive and neuropsychiatric endpoints for efficacy in addition to your mention of biomarker-based efficacy detection.
(Reply to Reviewer #1)
> We appreciate Reviewer #1's comment. We added cognitive and neuropsychiatric aspects for efficacy in addition to the biomarker-based efficacy detection in the revised manuscript as follows.

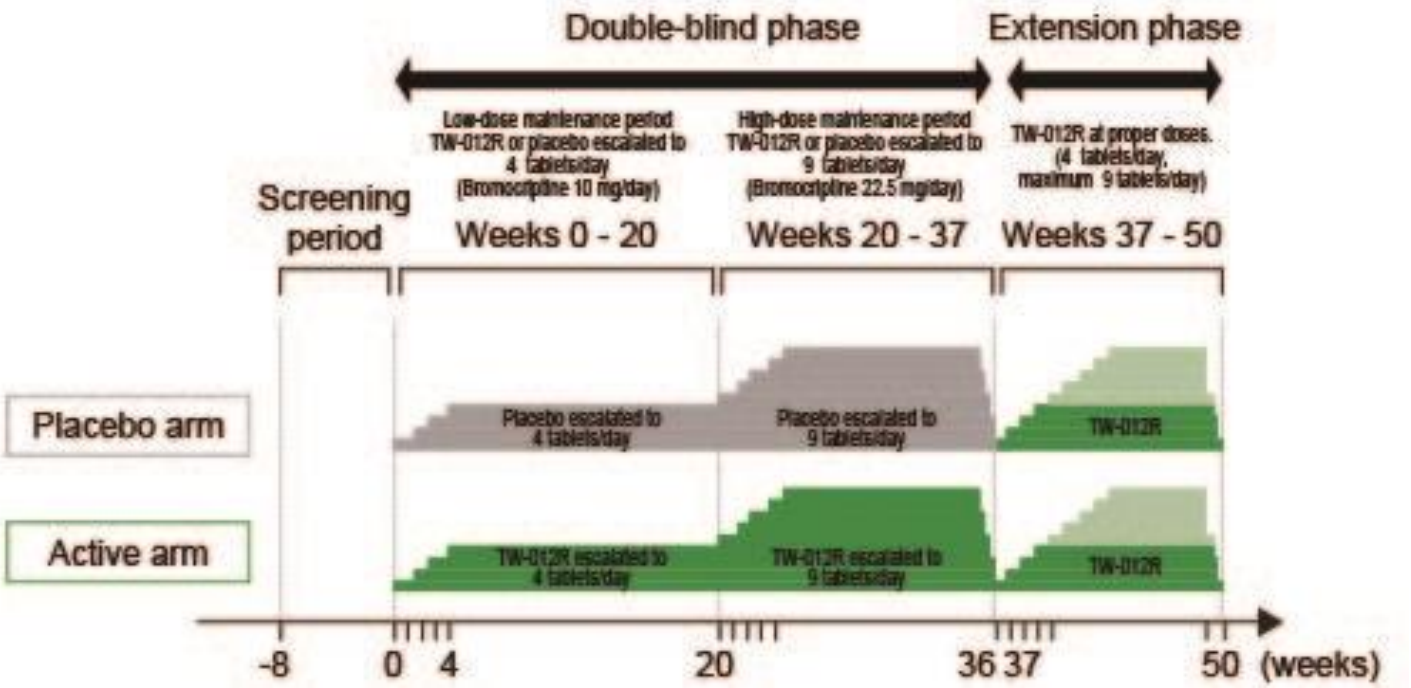
(In the Discussion section in the revised manuscript p.15, lines 7–9)
This study is the first to use bromocriptine for PSEN1-AD patients, and it presents both a low- and a high-dose safety verification, as well as cognitive, neuropsychiatric and biomarker-based efficacy detection.

9. Figure 1 for the placebo group, you need to change all mention of TW-012R to "placebo".
(Reply to Reviewer #1)
> We thank Reviewer #1 for this comment. We changed "TW-012R" in the placebo group to "placebo" in Figure 1.

(Original Figure 1)



(Revised Figure 1)



Response to Reviewer #2

Abstract

1. In the introduction, discussion, and the concluding section, the logic behind this study is not well explained.

(Reply to Reviewer #2)

> We are most grateful for Reviewer #2's comment. We emphasized the rationale of this trial design by explaining the meaning of trial phases (double-blind phase and extension phase). The revised version is described below after comment number 3.

2. The abstract provides no clear understanding of the schedules of the study, along with the rationale and causes confusion.

(Reply to Reviewer #2)

> We appreciate Reviewer #2's comment. We revised the explanation of our trial schedule to make it straight-forward for readers. The revised version is described below after comment number 3.

3. The author didn't provide the clear conclusion of study.

(Reply to Reviewer #2)

> We appreciate Reviewer #2's comment. We explained the meaning of our trial, that we used an enrichment strategy with iPSCs to select the study population, in the revised manuscript as follows.

(In Abstract in the original manuscript)

Introduction

Alzheimer's disease (AD) is one of the most common causes of dementia. Pathogenic variants in the PSEN1 gene are the most frequent cause of early-onset AD. Medications for patients with AD bearing PSEN1 mutation (PSEN1-AD) are limited to symptomatic therapies and no established radical treatments are available. Induced pluripotent stem cell (iPSC)-based drug repurposing identified bromocriptine as a therapeutic candidate for PSEN1-AD. In this study, we

will investigate the safety and efficacy of an orally administered dose of bromocriptine in patients with PSEN1-AD.

Methods and analysis

This is a multi-centre, randomised, placebo-controlled trial. Ten AD patients with PSEN1 mutations and Mini Mental State Examination-Japanese (MMSE-J) score of ≤ 25 will be randomly assigned, in a 2 : 1 ratio, to the trial drug or placebo group. This clinical trial consists of the screening period (8 weeks), double-blind phase (37 weeks), and extension phase (13 weeks). The double-blind phase is composed of the low-dose maintenance period (10 mg/day, up to week 20), high-dose maintenance period (22.5 mg/day, weeks 21–36), and tapering period of the trial drug (1 week). Additionally, there is an extension period (10–22.5 mg/day, weeks 37–50). Primary outcomes are safety and efficacy in cognitive and psychological function. Also, exploratory investigations for the efficacy of bromocriptine by neurological scores and biomarkers will be conducted.

(In Abstract in the highlighted revised manuscript p.3, lines 2–21)

Introduction

Alzheimer's disease (AD) is one of the most common causes of dementia. Pathogenic variants in the PSEN1 gene are the most frequent cause of early-onset AD. Medications for patients with AD bearing PSEN1 mutation (PSEN1-AD) are limited to symptomatic therapies and no established radical treatments are available. Induced pluripotent stem cell (iPSC)-based drug repurposing identified bromocriptine as a therapeutic candidate for PSEN1-AD. In this study, we used an enrichment strategy with iPSCs to select the study population, and we will investigate the safety and efficacy of an orally administered dose of bromocriptine in patients with PSEN1-AD.

Methods and analysis

This is a multi-centre, randomised, placebo-controlled trial. AD patients with PSEN1 mutations and Mini Mental State Examination-Japanese (MMSE-J) score of ≤ 25 will be randomly assigned, at a 2 : 1 ratio, to the trial drug or placebo group (≥ 4 patients in TW-012R and ≥ 2 patients in placebo). This clinical trial consists of the screening period, double-blind phase (9 months), and extension phase (3 months). The double-blind phase for evaluating the efficacy and safety is composed of the low-dose maintenance period (10 mg/day), high-dose

maintenance period (22.5 mg/day), and tapering period of the trial drug. Additionally, there is an open-labelled active drug extension period for evaluating long-term safety. Primary outcomes are safety and efficacy in cognitive and psychological function. Also, exploratory investigations for the efficacy of bromocriptine by neurological scores and biomarkers will be conducted.

Introduction:

1. In p2 the authors could more elaborate the word '(PSEN1-AD)' that how the mutation in this gene is responsible for disease could be little more explainable.

(Reply to Reviewer #2)

> We appreciate Reviewer #2's comment. We agree with the reviewer, and we added an explanation of the PSEN1 mutation in the Alzheimer pathology in the revised manuscript as shown below.

(In the Introduction section in the original manuscript)

Notably, AD patients with mutations in the PSEN1 gene (PSEN1-AD) experience onset in their 20s to 50s, and their cognitive function deteriorates rapidly, leading to death within several years as curative therapies are unavailable [4]. Thus, the development of new drugs for PSEN1-AD is eagerly awaited.

(In the Introduction section in the highlighted revised manuscript p.5, lines 14–20)

Notably, AD patients with mutations in the PSEN1 gene (PSEN1-AD) experience onset in their 20s to 50s, and their cognitive function deteriorates rapidly, leading to death within several years, as curative therapies are unavailable [4]. Thus, the development of new drugs for PSEN1-AD is eagerly awaited. Converging basic and clinical evidence suggests that mutant PSEN1 could affect the function of γ-secretases in neurons and increase the Aβ42 levels in plasma of FAD patients, transfected cells, and transgenic mice [Reference: Nature 1996;383:710–13, Neuron 1996;17:1005–13, Science 2002;297:353–56]. This abnormal production of Aβ was estimated to originate from the altered conformation of the γ-secretase complex and changes in the active site of the cleavage process [Reference: J Neurochem 2006;96:732–42, Cold Spring Harb Perspect Med 2012;2:a006304].

References:

Duff K, Eckman C, Zehr C, et al. Increased amyloid-beta42(43) in brains of mice expressing mutant presenilin 1. Nature 1996;383:710–13.

Borchelt DR, Thinakaran G, Eckman CB, et al. Familial Alzheimer's disease-linked presenilin 1 variants elevate Abeta1-42/1-40 ratio in vitro and in vivo. Neuron 1996;17:1005–13.

Hardy J, Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. Science 2002;297:353–56.

Bentahir M, Nyabi O, Verhamme J, et al. Presenilin clinical mutations can affect gamma-secretase activity by different mechanisms. J Neurochem 2006;96:732–42.

Strooper BD, Iwatsubo T, Wolfe MS, et al. Presenilins and γ-secretase: structure, function, and role in Alzheimer Disease. Cold Spring Harb Perspect Med 2012;2:a006304.

2. In p3 the rationale behind the use of bromocriptine is poorly understood and needs more explanation.

(Reply to Reviewer #2)

> We appreciate Reviewer #2's comment. We added an explanation for why we selected bromocriptine and PSEN1-AD patients as its therapeutic target in the revised manuscript as shown below.

(In the Introduction section in the original manuscript)

As a result, we found that bromocriptine reduces the production of Aβ and the Aβ42/40 ratio. We also compared the effectiveness of bromocriptine among different AD types, and we were able to clarify that bromocriptine improves Aβ phenotypes especially in PSEN1-AD [6,7].

(In the Introduction section in the highlighted revised manuscript p.5, lines 29–33, p.6, lines 1–3)

After the screening, we found bromocriptine to be the most potent modifier of Aβ production for PSEN1-AD neurons among existing drugs. Dose-dependency assay showed that bromocriptine reduced the Aβ42 dose and Aβ42/40 ratio by up to ~50% and ~40%, respectively. Furthermore, we prepared cortical neurons of several patients with PSEN1-AD and sporadic AD to evaluate

the specificity of bromocriptine for PSEN1-AD. Bromocriptine reduced the Aβ42 dose and Aβ42/40 ratio of PSEN1-AD neurons more strongly than those of sporadic AD neurons. From these results, we selected patients with PSEN1-AD as a bromocriptine-responsive subgroup in AD [11,12: same references as original 6,7].

3. Referencing is poor, in p4 there is no referencing in the entire paragraph.

(Reply to Reviewer #2)

> In response to Reviewer #2's comment, we have added 6 references in the introduction section, and 26 references throughout the manuscript. P4 is our article summary, and it does not contain references.

4. "As a result, we found that bromocriptine reduces the production of Aβ and the Aβ42/40 ratio." This line should again be revised once more.

(Reply to Reviewer #2)

> We appreciate Reviewer #2's comment. We added an explanation for why we selected bromocriptine and PSEN1-AD patients as the therapeutic target of bromocriptine in the revised manuscript as shown below.

(In the Introduction section in the original manuscript)

As a result, we found that bromocriptine reduces the production of Aβ and the Aβ42/40 ratio. We also compared the effectiveness of bromocriptine among different AD types, and we were able to clarify that bromocriptine improves Aβ phenotypes especially in PSEN1-AD [6,7].

(In the Introduction section in the highlighted revised manuscript p.5, lines 29–33, p.6, lines 1–3)

After the screening, we found bromocriptine to be the most potent modifier of Aβ production for PSEN1-AD neurons among existing drugs. Dose-dependency assay showed that bromocriptine reduced the Aβ42 dose and Aβ42/40 ratio by up to ~50% and ~40%, respectively. Furthermore, we prepared cortical neurons of several patients with PSEN1-AD and sporadic AD to evaluate the specificity of bromocriptine for PSEN1-AD. Bromocriptine reduced the Aβ42 dose and Aβ42/40 ratio of PSEN1-AD neurons more strongly than those of sporadic AD neurons. From

these results, we selected patients with PSEN1-AD as a bromocriptine-responsive subgroup in AD [11,12: same references as original 6,7].

5. “Pluripotent stem cells (iPSCs)” could be explained more.

(Reply to Reviewer #2)

> We appreciate Reviewer #2’s comment. We agree with the reviewer, and we added an explanation of induced pluripotent stem cells (iPSCs) in the revised manuscript as shown below.

(In the Introduction section in the original manuscript)

To develop therapeutic compounds for PSEN1-AD, we previously established induced pluripotent stem cells (iPSCs) from patients with PSEN1-AD, and conducted compound screening by using Aβ phenotypes and neuronal toxicity as a readout to identify hit compounds.

(In the Introduction section in the revised manuscript p.5, lines 21–26)

To develop therapeutic compounds for PSEN1-AD, we previously established induced pluripotent stem cells (iPSCs) from patients with PSEN1-AD. iPSCs were established by introducing a small number of genes into patients' cells. Established iPSCs can differentiate into any type of cell in the body and proliferate indefinitely. iPSC technology provided in vitro models of inaccessible human cell types and impacted investigations of disease mechanisms especially in brain disorders. We established an iPSC-based screening system by modelling Aβ phenotypes of PSEN-1AD [Reference: Kondo T, Imamura K, Funayama M, et al. iPSC-Based Compound Screening and In Vitro Trials Identify a Synergistic Anti-amyloid β Combination for Alzheimer’s Disease. Cell Rep 2017;21:2304–12.].

6. Authors must also focus on how the mutation in PSEN-1 gene contributes to the AD and what conformational changes they produce in the human that affect the healthy life.

(Reply to Reviewer #2)

> We appreciate Reviewer #2's comment. We agree with the reviewer and added some description concerning the conformational changes of mutant PSEN1 in the revised manuscript as shown below.

(In the Introduction section in the original manuscript)
Notably, AD patients with mutations in the PSEN1 gene (PSEN1-AD) experience onset in their 20s to 50s, and their cognitive function deteriorates rapidly, leading to death within several years as curative therapies are unavailable [4]. Thus, the development of new drugs for PSEN1-AD is eagerly awaited.

(In the Introduction section in the revised manuscript p.5, lines 14–20)
Notably, AD patients with mutations in the PSEN1 gene (PSEN1-AD) experience onset in their 20s to 50s, and their cognitive function deteriorates rapidly, leading to death within several years as curative therapies are unavailable [4]. Thus, the development of new drugs for PSEN1-AD is eagerly awaited. Converging basic and clinical evidence suggests that mutant PSEN1 could affect the function of γ-secretases in neurons and increase the Aβ42 levels in plasma of FAD patients, transfected cells, and transgenic mice [Reference: Nature 1996;383:710–13, Neuron 1996;17:1005–13, Science 2002;297:353–56]. This abnormal production of Aβ was estimated to originate from the altered conformation of the γ-secretase complex and changes in the active site of the cleavage process [Reference: J Neurochem 2006;96:732–42, Cold Spring Harb Perspect Med 2012;2:a006304].

Reference:
Duff K, Eckman C, Zehr C, et al. Increased amyloid-beta42(43) in brains of mice expressing mutant presenilin 1. Nature 1996;383:710–13.
Borchelt DR, Thinakaran G, Eckman CB, et al. Familial Alzheimer's disease-linked presenilin 1 variants elevate Abeta1-42/1-40 ratio in vitro and in vivo. Neuron 1996;17:1005–13.
Hardy J, Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. Science 2002;297:353–56.
Bentahir M, Nyabi O, Verhamme J, et al. Presenilin clinical mutations can affect gamma-secretase activity by different mechanisms. J Neurochem 2006;96:732–42.

Strooper BD, Iwatsubo T, Wolfe MS, et al. Presenilins and γ-secretase: structure, function, and role in Alzheimer Disease. Cold Spring Harb Perspect Med 2012;2:a006304.

7. “We also compared the effectiveness of bromocriptine among different AD types.” This line should be made more transparent for a better review

(Reply to Reviewer #2)

> We appreciate Reviewer #2’s comment. We added an explanation to clarify why we selected bromocriptine and PSEN1-AD patients as the therapeutic target of bromocriptine in the revised manuscript as shown below.

(In the Introduction section in the original manuscript)

As a result, we found that bromocriptine reduces the production of Aβ and the Aβ42/40 ratio. We also compared the effectiveness of bromocriptine among different AD types, and we were able to clarify that bromocriptine improves Aβ phenotypes especially in PSEN1-AD [6,7].

(In the Introduction section in the revised manuscript p.5, lines 29–33, p.6, lines 1–3)

After the screening, we found bromocriptine to be the most potent modifier of Aβ production for PSEN1-AD neurons among existing drugs. Dose-dependency assay showed that bromocriptine reduced the Aβ42 dose and Aβ42/40 ratio by up to ~50% and ~40%, respectively. Furthermore, we prepared cortical neurons of several patients with PSEN1-AD and sporadic AD to evaluate the specificity of bromocriptine for PSEN1-AD. Bromocriptine reduced the Aβ42 dose and Aβ42/40 ratio of PSEN1-AD neurons more strongly than those of sporadic AD neurons. From these results, we selected patients with PSEN1-AD as a bromocriptine-responsive subgroup in AD [11,12: same references as original 6,7].

Methods and analysis:

1. The legend of the table is poor. Mention and revise it properly.

(Reply to Reviewer #2)

> We appreciate Reviewer #2's comment. In response to Reviewer #2's comment, we added detailed explanation in the legend in the revised manuscript as shown below.

(In Figure legend in the revised manuscript p.18, lines 2–7)

Figure 1. Design of REBRAnD study

The study consists of the screening period (8 weeks), double-blind phase (37 weeks), and extension phase (13 weeks). The double-blind phase for evaluating the efficacy and safety is composed of the low-dose maintenance period (up to 10 mg/day), high-dose maintenance period (up to 22.5 mg/day), and tapering period of the trial drug. Additionally, there is an open-labelled active drug extension period (up to 10 or 22.5 mg/day) for evaluating long-term safety.

2. Authors should give the justifications about from where the patients taken in the study and how the patients are confirmed for the presence of PSEN-1 mutants.

(Reply to Reviewer #2)

> We appreciate Reviewer #2's comment. In response to this comment, we added a detailed explanation of the recruiting process in the revised manuscript as shown below.

(In the Methods and analysis section in the revised manuscript p.7, lines 17–19)

Recruiting will be done by neurologists who are treating PSEN1-AD patients and are blinded to the allocation. All participants in the study need to be confirmed for the presence of PSEN-1 mutation by genetic analyses.

3. Study design is very poor and there is no clinical efficacy of the study.

(Reply to Reviewer #2)

> We appreciate Reviewer #2's comment. In response to this comment, we added a detailed explanation of the study design. The revised version is described below after comment number 4.

4. In study design the rationale behind the use of screening period, double-blind phase, extension phase needs more justification.

(Reply to Reviewer #2)

> We appreciate Reviewer #2's comment. In response to this comment, we added the rationale for each of the study phases in the revised manuscript as shown below.

(In the Intervention and control (previously, the Study design) section in the original manuscript)
This clinical trial consists of the screening period (8 weeks), double-blind phase (37 weeks), and extension phase (13 weeks). The double-blind phase is composed of the low-dose maintenance period (20 weeks), high-dose maintenance period (16 weeks), and tapering period of the trial drug (1 week) (Figure 1).

(In the Intervention and control section in the revised manuscript p.8, lines 10–14)
This clinical trial consists of the screening period (8 weeks), double-blind phase (37 weeks), and extension phase (13 weeks). The double-blind phase for evaluating the efficacy and safety is composed of the low-dose maintenance period (20 weeks), high-dose maintenance period (16 weeks), and tapering period of the trial drug (1 week). Additionally, there is an open-labelled active drug extension period for evaluating long-term safety (Figure 1).

5. "Patients taking oral donepezil hydrochloride will be carefully monitored because the blood concentration may be increased by the additional oral administration of the trial drug" explain more how this drug effects the trial.

(Reply to Reviewer #2)

> We appreciate Reviewer #2's comment. In response to this comment, we explained the clinical significance of adding bromocriptine on top of donepezil in the revised manuscript as shown below.

(In the Trial population and rationale for selecting participants section in the original manuscript)

The concomitant use of existing drugs for the treatment of AD (donepezil, galantamine, rivastigmine, and memantine) is allowed, but patients who have changed the regimen within 2 months before providing informed consent are excluded.

(In the Trial population and rationale for selecting participants section in the revised manuscript p.7, lines 12–16)
The concomitant use of existing drugs for the treatment of AD (donepezil, galantamine, rivastigmine, and memantine) will be allowed to evaluate the safety and efficacy of future practical bromocriptine use upon approval, but patients who have changed the regimen within 2 months before providing informed consent will be excluded because the efficacy of bromocriptine alone will be difficult to evaluate.

6. The methods and the procedures used in the study should be revised once again for the mistakes, and at this level, some points are not clear especially in the study plan, which should be made more justified by the authors.
(Reply to Reviewer #2)
> We appreciate Reviewer #2's comment. In response to this comment, we revised the methods section to explain the rationale behind the study design, rationale of recruiting strategy, detailed statistical analyses, and randomisation methods in the revised manuscript as shown below.

(In the Intervention and control section in the revised manuscript p.8, lines 10–16)
This clinical trial consists of the screening period (8 weeks), double-blind phase (37 weeks), and extension phase (13 weeks). The double-blind phase for evaluating the efficacy and safety is composed of the low-dose maintenance period (20 weeks), high-dose maintenance period (16 weeks), and tapering period of the trial drug (1 week). Additionally, there is an open-labelled active drug extension period for evaluating long-term safety (Figure 1). Participants who are screened during the screening period and assessed as eligible will be randomly assigned to the bromocriptine and placebo groups.

(In the Trial population and rationale for selecting participants in the revised manuscript p.7, lines 8–11)

PSEN1-AD is the target disease. The MMSE-J score will be specified at 25 points or lower to enrol patients with dementia and mild cognitive impairment and to exclude those with normal cognitive function. We will exclude MCI participants with MMSE-J scores of 26 or 27, who are reported to have lower risk and longer duration of MCI conversion to AD [Tokuchi R, Hishikawa N, Kurata T, et al. Clinical and demographic predictors of mild cognitive impairment for converting to Alzheimer's disease and reverting to normal cognition. J Neurol Sci 2014;346:288–92.].

(In the Statistical analysis section in the revised manuscript p.12, lines 4–10)

For efficacy endpoints, changes from baseline will be summarised and compared at weeks 20 and 36 using descriptive statistics including mean differences with 95% confidence intervals and p values derived from Student’s t test with the assumption of normality. If the assumption is violated, nonparametric tests will be considered. No imputation will be performed for missing data. Owing to the exploratory nature of this study, no adjustments will be made for multiple testing. To support the robustness of the results, a sensitivity analysis with and without non-compliant patients will be conducted.

(In the Randomisation, allocation, and blinding section in the revised manuscript p.9, lines 20–28)

Patients meeting all the inclusion criteria and not meeting any of the exclusion criteria (Box 1) at the time of enrolment in the trial will be randomly assigned, at a 2 : 1 ratio, to receive either TW-012R or placebo. Owing to the ethical issue for patients assigned to the placebo group, unequal randomisation will be employed. Randomisation will be performed using permuted blocks and stratified based on the important confounding variable of the baseline MMSE-J score (< 13 or ≥ 13). The allocation sequence will be prepared by an external contract research organization, and trial participants, care givers, outcome assessors and investigators will be blinded. Registration will be done by investigators etc. using the web enrolment system. When the patient is determined to be eligible by the web enrolment system, the allocated drug number will be displayed as the enrolment result.

Outcomes:

1. The outcomes of the study failed to clearly mentioned the results after trial and very poor explanation is given in the outcome section.

(Reply to Reviewer #2)

> We appreciate Reviewer #2's comment, and we added detailed explanations and 16 references of the outcomes (details are shown after the next comment). Also, a new sentence was added to describe the impact on PSEN1-AD patients and the future direction using digital and exploratory biomarkers in AD clinical trials in the revised manuscript as follows.

(In the Discussion section in the revised manuscript p.15, lines 3–4)

Of note, digital biomarkers will potentially reduce participants' and caregivers' burden in future trials.

2. Again referencing is lacking and no justification is given for the points written.

(Reply to Reviewer #2)

> In response to Reviewer #2's comment, we added 16 references in regard to SIB, NPI, MMSE, Apathy Scale, plasma Aβ, NfL, total-tau, p-tau, NfL, CSF Aβ, total-tau, p-tau, and plasma Aβ-related peptides in the revised manuscript as shown below.

(In the Outcomes section in the original manuscript)

Primary endpoint 1) safety

As the primary analysis, safety will be evaluated by collecting incidence information and severity of adverse events (AEs) or adverse reactions from the starting time for administration of the trial drug to the end of the follow-up period.

Primary endpoint 2) Severe Impairment Battery-Japanese (SIB-J)

SIB-J is validated in Japanese version and will be used to evaluate cognitive function [16]. Individual time courses of observed values and changes from baseline in SIB-J score up to Week 20 and Week 36 after the trial drug administration will be shown.

Primary endpoint 3) Neuropsychiatric Inventory (NPI)

NPI (Japanese version) is a validated clinical instrument for evaluating psychopathology in dementia [17]. Individual time courses of observed values and changes from baseline in NPI score up to Week 20 and Week 36 after the trial drug administration will be shown.

(In the Outcomes section in the revised manuscript p.10, lines 1–20; p.11, lines 16–18)

Primary endpoint 1) safety

As the primary analysis, safety will be evaluated by collecting incidence information and severity of adverse events (AEs) or adverse reactions from the starting time of the administration of the trial drug to the end of the follow-up period (Table 1). Known frequent adverse effects of bromocriptine include nausea and vomiting (8.3%), anorexia (2.5%), and gastric discomfort, based on 5212 cases with Parkinson's syndrome [Interview Form Parlodel Tablets 2.5 mg, Sun Pharma Japan Ltd. https://medical.mt-pharma.co.jp/di/file/dc/plo.pdf (accessed on 1st March, 2021)].

Primary endpoint 2) Severe Impairment Battery-Japanese (SIB-J)

SIB-J is validated in the Japanese version and will be used to evaluate cognitive function [Psychol Assessment 1990;2:298–303; Severe Impairment Battery (SIB). London, UK. Pearson Assessment 1993; Jpn J Geriatr Psychiatry 2005;16:683–691.]. Higher scores indicate better cognitive function (range 0–100). Individual time courses of observed values and changes from baseline in SIB-J score up to Week 20 and Week 36 after trial drug administration will be shown.

Primary endpoint 3) Neuropsychiatric Inventory (NPI)

NPI (Japanese version) is a validated clinical instrument for evaluating psychopathology in dementia [Neurology 1994;44:2308–14; Neurology 1997;48:10S–16S; No To Shinkei 1997;49:266–71]. Higher scores indicate more severe neuropsychiatric derangements (range 10–120). Individual time courses of observed values and changes from baseline in NPI score up to Week 20 and Week 36 after trial drug administration will be shown.

Learning effect cannot be negligible, but this trial has a placebo arm, and previous trials using SIB-J, NPI, MENFIS and DAD adopted similar measuring time frames and no obvious learning effects were reported. [Watanabe M, Nakamura Y, Yoshiyama Y, et al. Alzheimers Dement (N Y) 2019;5:398-408. Noguchi-Shinohara M, Ono K, Hamaguchi T, et al. Sci Rep 2020;10:18627.]

References

SIB:

Saxton J., McGonigle-Gibson KL., Swihart AA., Miller VJ, & Boller F. Assessment of the severely impaired patient: Description and validation of a new neuropsychological test battery. Psychological Assessment: A Journal of Consulting and Clinical Psychology 1990;2:298–303.

Saxton J, McGonigle K, Swihart A, et al. Severe Impairment Battery (SIB). London, UK. Pearson Assessment 1993.

Niina R, Homma A, Sugai Y, et al. Reliability, validity and clinical availability of a Japanese version of Severe Impairment Battery (SIB) and a Japanese version of modified Alzheimer's Disease Cooperative Study-Activities of Daily Living Inventory (ADCS-ADL). Jpn J Geriatr Psychiatry 2005;16:683–691.

NPI:

Cummings JL, Mega M, Gray K, et al. The Neuropsychiatric Inventory. Comprehensive assessment of psychopathology in dementia. Neurology 1994;44:2308–14.

Cummings JL. The Neuropsychiatric Inventory. Assessing psychopathology in dementia patients. Neurology 1997;48:10S–16S.

Hirono N, Mori E, Ikejiri Y, et al. Japanese version of the neuropsychiatric inventory a scoring system for neuropsychiatric disturbances in dementia patients. No To Shinkei 1997;49:266–71.

MMSE:

Folstein MF, Folstein SE, McHugh PR. “Mini-mental state”: A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975;12:189–98.

Sugishita M, Hemmi I, Takeuchi T. Reexamination of the Validity and Reliability of the Japanese Version of the Mini-Mental State Examination (MMSE-J). Japanese Journal of Cognitive Neuroscience 2016;18:168–83.
Sugishita M, Koshizuka Y, Sudou S, et al. The validity and reliability of the Japanese version of the Mini- Mental State Examination (MMSE-J) with the original procedure of the attention and calculation task(2001). Japanese Journal of Cognitive Neuroscience 2018;20:91–110.

Apathy Scale (AES-I-J):
Marin RS, Biedrzycki RC, Firinciogullari S. Reliability and validity of the apathy evaluation scale. Psychiat Res 1991;38:143–62.

Plasma Aβ1-40, 1-42, total-tau, p-tau:
Palmqvist S, Janelidze S, Stomrud E, et al. Performance of Fully Automated Plasma Assays as Screening Tests for Alzheimer Disease–Related β-Amyloid Status. JAMA Neurol 2019;76:1060–69.

Plasma NfL:
Schultz SA, Strain JF, Adedokun A, et al. Serum neurofilament light chain levels are associated with white matter integrity in autosomal dominant Alzheimer's disease. Neurobiol Dis 2020;142:104960.

Plasma total-tau:
Mielke MM, Hagen CE, Wennberg AMV, et al. Association of Plasma Total Tau Level With Cognitive Decline and Risk of Mild Cognitive Impairment or Dementia in the Mayo Clinic Study on Aging. JAMA Neurol 2017;74:1073–80.

Plasma p-tau:
Karikari TK, Pascoal TA, Ashton NJ, et al. Blood phosphorylated tau 181 as a biomarker for Alzheimer's disease: a diagnostic performance and prediction modelling study using data from four prospective cohorts. Lancet Neurol 2020;19:422–33.

Tatebe H, Kasai T, Ohmichi T, et al. Quantification of plasma phosphorylated tau to use as a biomarker for brain Alzheimer pathology: pilot case-control studies including patients with Alzheimer's disease and down syndrome. Mol Neurodegener 2017;12:63.

CSF Aβ1-40, Aβ1-42, total-tau, p-tau:
Bateman RJ, Xiong C, Benzinger TLS, et al. Clinical and Biomarker Changes in Dominantly Inherited Alzheimer's Disease. N Engl J Med 2012;367:795–804.
Dubois B, Feldman HH, Jacova C, et al. Advancing research diagnostic criteria for Alzheimer's disease: the IWG-2 criteria. Lancet Neurol 2014;13:614–29.

Plasma Aβ-related peptides:
Nakamura A, Kaneko N, Villemagne VL, et al. High performance plasma amyloid-β biomarkers for Alzheimer’s disease. Nature 2018;554:249–54.

3. Author should mention the clear results of the study with appropriate data.
(Reply to Reviewer #2)
> We appreciate Reviewer #2’s comment. Since our manuscript focuses on the study protocol, results will include the statistical analysis plan and our drug development plan. According to the recommendations of the American Statistical Association, in this setting with its small sample size, a significance level should be avoided when interpreting p-values. Thus, we will provide confidence intervals and p-values for descriptive purposes. In addition, individual profile of each participant will be evaluated.

References:
Wasserstein RL, Lazar NA. The ASA’s Statement on p-Values: Context, Process, and Purpose. Am Stat 2016;70:129–33.
Wasserstein RL, Schirm AL, Lazar NA. Moving to a world beyond “P<0.05.” Am Stat 2019;73:1–19.
Amrhein V, Greenland S, McShane B. Scientists rise up against statistical significance. Nature 2019;567:305–307.

Pike H. Statistical significance should be abandoned, say scientists. BMJ 2019;364:l1374.

4. Author should mention the p value with data points.

(Reply to Reviewer #2)

> We appreciate Reviewer #2's comment. We stated the p-values that were computed for changes from baseline to weeks 20 and 36 in the revised manuscript as follows. Owing to the exploratory nature of the study, no adjustments for multiple testing across data points will be made. We also added a new table of the evaluation schedule.

(In the Statistical analysis section in the revised manuscript p.12, lines 4–7)

For efficacy endpoints, changes from baseline will be summarised and compared at weeks 20 and 36 using descriptive statistics including mean differences with 95% confidence intervals and p values derived from Student's t test with the assumption of normality.

(New table in the revised manuscript pp.19–21)

Table 1. Trial schedule

		Screening period		Double-blind phase																
				Escalation to 4 tablets/day									Escalation to 9 tablets/day							Taper
Timing		Before enrollment	Before start of trial treatment	At start of administration	Week 1	Week 2	Week 3	Week 4	Week 8	Week 12	Week 16	Week 20	Week 21	Week 22	Week 23	Week 24	Week 28	Week 32	Week 36	Week 37
Visit/medical examination		○	○	○	○	Phone	Phone	○	○	○	○	○	○	Phone	Phone	○	○	○	○	○
Informed consent		○																		
Neuropsychological/Motor assessment	ADAS-J cog	○																		
	SIB-J		○	○								○							○	
	NPI		○	○				○	○	○	○	○				○	○	○	○	
	MENFIS		○	○				○	○	○	○	○				○	○	○	○	
	MMSE-J	○	○	○								○							○	
	DAD		○	○								○							○	
	UPDRS part III		○	○								○							○	
	Apathy Scale		○	○								○							○	
	UMNB		○	○								○							○	
Laboratory biomarkers	Plasma biomarkers		○	○						○		○					○		○	
	CSF biomarkers			○															○	
Digital biomarkers	Wearable physical activity		→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	
	Finger tapping		○	○				○	○	○	○	○				○	○	○	○	
PET	Brain amyloid PET		○															○		
	Brain tau PET		○															○		
	Cardiac ultrasound	○										○							○	

Safety assessment	Chest X-ray	○						○				○				○			○	
	ECG	○			▲			○	▲	▲	▲	○	▲			○	▲	▲	○	▲
	Head MRI	○																	○	
	Blood bromocriptine concentration			○ *						○	○	○					○	○	○	
	Laboratory tests	○						○				○				○			○	
	AEs		→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→

		Extension phase										
		Escalation to 4–9 tablets/day †										End of trial
Timing		Week 37	Week 38	Week 39	Week 40	Week 41	Week 42	Week 43	Week 44	Week 45	Week 48	Week 50
Visit/medical examination		○	Phone	Phone	○	Phone	Phone	Phone	○	Phone	○	○
Informed consent												
Neuropsychological/motor assessment	ADAS-J cog											
	SIB-J											
	NPI											
	MENFIS											
	MMSE-J											
	DAD											
	UPDRS part III											
	Apathy Scale											
	UMNB											
Laboratory biomarkers	Plasma biomarkers											
	CSF biomarkers											
Digital biomarkers	Wearable physical activity											
	Finger tapping											
PET	Brain amyloid PET											
	Brain tau PET											
Safety assessment	Cardiac ultrasound											○
	Chest X-ray											○
	ECG	▲			▲				▲		▲	○
	Head MRI											
	Blood bromocriptine concentration											

	Laboratory tests				○							○
	AEs	→	→	→	→	→	→	→	→	→	→	→

◯: To be performed

▲: To be performed if donepezil hydrochloride is co-administered.

• After the final visit of the last participant at Week 37, the blind will be broken following data lock to start analyses.

*Schedule for measuring blood bromocriptine concentration on Day 1

Time after first administration of trial drug (h)	Before administration	1	2	3	4	6
Time window (in principle)	−2 hours	± 5 minutes	± 5 minutes	± 5 minutes	± 5 minutes	± 5 minutes
Plasma bromocriptine	◯	◯	◯	◯	◯	◯

†Standard dose is 4 tablets/day, up to 9 tablets/day at maximum.

ADAS-J cog, Alzheimer's Disease Assessment Scale-cognitive subscale Japanese version; AEs, adverse events; DAD, Disability Assessment for Dementia; MENFIS, Mental Function Impairment Scale; MMSE-J, Mini Mental State Examination-Japanese; NPI, Neuropsychiatric Inventory; SIB-J, Severe Impairment Battery-Japanese; UMNB, Upper Motor Neuron Burden Score; UPDRS part III, Movement Disorder Society-sponsored revision of the Unified Parkinson’s Disease Rating Scale Part III.

Discussion:

1. The discussion part should be to the point, straight forward, no canvassing, only the pure result discussion along with the future perspectives, lacunas and recent advancements.
(Reply to Reviewer #2)

> We appreciate Reviewer #2's comment. We added the strengths and limitations of our trial in the revised manuscript as follows.

(In the Discussion section in the revised manuscript p.15, lines 4–6)

Strengths of our study include participant enrichment using iPSC technology and exploratory biomarker investigation. A possible limitation of this study may be the difficulty in recruiting large numbers of patients due to the rarity of PSEN1-AD.

2. Overall the whole manuscript must be again thoroughly revised with the corrections. The authors should use small bites of sentences to tell their views or literature review in a comprehensive way, rather than using complicated and hectic lines and sentences.
(Reply to Reviewer #2)

> We appreciate Reviewer #2's comment. We moved methodological sentences to the Methods section and thoroughly revised the Discussion section to clarify the meanings of our clinical trial.

3. Add more figures and give clear explanation of outcomes of the results with justification.
(Reply to Reviewer #2)

> We appreciate Reviewer #2's comment. We added a new table (shown previously) so that the readers can clearly understand the evaluation schedule including the meanings of outcomes. We also explained future perspectives of the AD clinical trials in the revised manuscript as shown below.

(In the Discussion section in the revised manuscript p.14, lines 20–22)

Further, remote informed consent and cognitive/neuropsychiatric evaluation by tablet or digital devices can be used in future trials for AD to reduce the study burden for participants and their caregivers.

4. Proper pattern of the approval of ethical committee should be mentioned here.

(Reply to Reviewer #2)

> We appreciate Reviewer #2's comment. In response to this comment, we added the details of the IRB approvals (p.12, lines 24–27) and explained how the central trial office will handle the data and the results of the trial ethically under the Ethics and Dissemination subheading as follows.

(In the Ethics and Dissemination section in the revised manuscript p.13, lines 19–20)

The central trial office, data centre and statisticians will use only the participant identification codes and cannot access the participants' personal information.

Response to Reviewer #3

I'm grateful for the opportunity to review this study protocol. This is an important area of research in dementia treatment. However, many of the details required under the SPIRIT checklist are missing. The following are some examples to be addressed by the authors. Extensive English editing should also be considered.

(Reply to Reviewer #3)

> We are grateful for the constructive comments from Reviewer #3 to improve our manuscript. In terms of the SPIRIT checklist, we re-examined the checklist closely, revised it and added our model consent form. We used another English editing service to make our article more professional.

Introduction:

Include more details about the postulated mechanism of action of how bromocriptine "improves Aß phenotype especially in PSEN1-AD".

(Reply to Reviewer #3)

> We appreciate Reviewer #3's comment. We added an explanation for why we selected bromocriptine and PSEN1-AD patients as the therapeutic target of bromocriptine in the revised manuscript as shown below.

(In the Introduction section in the original manuscript)

As a result, we found that bromocriptine reduces the production of Aβ and the Aβ42/40 ratio. We also compared the effectiveness of bromocriptine among different AD types, and we were able to clarify that bromocriptine improves Aβ phenotypes especially in PSEN1-AD [6,7].

(In the Introduction section in the highlighted revised manuscript p.5, lines 29–33, p.6, lines 1–3)

After the screening, we found bromocriptine to be the most potent modifier of Aβ production for PSEN1-AD neurons among existing drugs. Dose-dependency assay showed that bromocriptine reduced the Aβ42 dose and Aβ42/40 ratio by up to ~50% and ~40%, respectively. Furthermore, we prepared cortical neurons of several patients with PSEN1-AD and sporadic AD to evaluate

the specificity of bromocriptine for PSEN1-AD. Bromocriptine reduced the Aβ42 dose and Aβ42/40 ratio of PSEN1-AD neurons more strongly than those of sporadic AD neurons. From these results, we selected patients with PSEN1-AD as a bromocriptine-responsive subgroup in AD [11,12: same references as original 6,7].

Objectives:

The information stated in point form should be incorporated in the Methods and Analysis section under the various appropriate subheading. Specific hypotheses in terms of the outcomes should also be specified.

(Reply to Reviewer #3)

> We appreciate Reviewer #3's constructive comment. As Reviewer #3 suggested, we incorporated the bullet points in the Methods section under appropriate subheadings. We added a description of hypothesis in the revised manuscript as shown below.

(In the Sample size section in the highlighted revised manuscript p.8, lines 5–6)

There is no formal hypothesis or power sample size calculation due to the exploratory nature of this study.

Study design:

This should be stated explicitly under method section

(Reply to Reviewer #3)

> We appreciate Reviewer #3's constructive comment. We emphasized the rationale for this trial design by explaining the meaning of each of the trial phases in the revised manuscript as shown below.

(In the Intervention and control (previously, the Study design) section in the revised manuscript p.8, lines 10–14)

This clinical trial consists of the screening period (8 weeks), double-blind phase (37 weeks), and extension phase (13 weeks). The double-blind phase for evaluating the efficacy and safety is composed of the low-dose maintenance period (20 weeks), high-dose maintenance period (16

weeks), and tapering period of the trial drug (1 week). Additionally, there is an open-labelled active drug extension period for evaluating long-term safety (Figure 1).

Trial population and rationale for selecting participants:

The rationale for excluding patient who have changed AD drug treatment within 2 months was not explicitly provided. It would be good to provide the reference for use of MMSE-J cut-off equal or less than 25. Also, details of how and where patients would be approached and screened (esp for PSEN 1 mutation) should be included for clarity.

(Reply to Reviewer #3)

> We appreciate Reviewer #3's constructive comment. We added the rationale for excluding patients who have changed AD drug treatment within 2 months. Also, we provided more explanation regarding recruiting in the revised manuscript as shown below.

The rationale is mainly because of the effect on the bromocriptine blood concentration with the use of other AD drug treatments. We stated the rationale for the use of MMSE-J cut-off equal or less than 25. Recruiting will be done by neurologists who are treating PSEN1-AD patients and are blinded to the allocation.

(In the Trial population and rationale for selecting participants section in the revised manuscript p.7, lines 8–11, 12–16, 17–19)

The MMSE-J score will be specified at 25 points or lower to enrol patients with dementia and mild cognitive impairment and exclude those with normal cognitive function. We will exclude MCI participants with MMSE-J scores of 26 or 27, who are reported to have lower risk and longer duration of MCI conversion to AD [Tokuchi R, Hishikawa N, Kurata T, et al. Clinical and demographic predictors of mild cognitive impairment for converting to Alzheimer's disease and reverting to normal cognition. J Neurol Sci 2014;346:288–92.].

The concomitant use of existing drugs for the treatment of AD (donepezil, galantamine, rivastigmine, and memantine) is allowed to evaluate the safety and efficacy of future practical bromocriptine use upon approval, but patients who have changed their regimen within 2 months

before providing informed consent are excluded because the efficacy of bromocriptine alone will be difficult to evaluate.

Recruiting will be done by neurologists who are treating PSEN1-AD patients and are blinded to the allocation. All participants in the study need to be confirmed for the presence of PSEN-1 mutation by genetic analyses.

Sample size:
What is the duration for recruitment and would it be feasible to recruit the sample size proposed given the incidence rate? Also, why is the ratio of 2:1 selected over 1:1 allocation to intervention and control? Also, details on blinding and sequence generation should be provided.
(Reply to Reviewer #3)
> We appreciate Reviewer #3's constructive comment. In response to this comment by Reviewer #3, we added information about the planned feasible recruitment duration of our trial and the rationale for using the 2:1 randomisation in the Randomisation, allocation, and blinding section in the revised manuscript as shown below.

(In the Trial population and rationale for selecting participants section in the revised manuscript p.7, lines 20–21)
Given that PSEN1-AD patients are very few, the planned feasible recruitment duration is 10 months.

(In the Randomisation, allocation, and blinding section in the revised manuscript p.9, lines 22–28)
Owing to the ethical issue for patients assigned to the placebo group, unequal randomisation will be employed. Randomisation will be performed using permuted blocks and stratified based on the important confounding variable of baseline MMSE-J score (< 13 or ≥ 13). The allocation sequence will be prepared by an external contract research organization, and trial participants, care givers, outcome assessors and investigators will be blinded. Registration will be done by investigators etc. using the web enrolment system. When a patient is determined to be eligible

by the web enrolment system, the allocated drug number will be displayed as the enrolment result.

Study design (page 9):

The details contained under this section should be more appropriately titled as “intervention and control”. Details of the screening done during the 8-week screening period is not provided. In the extension phase, what determines increasing the dose to beyond 4 tablets? Page 10: It is not clear what is meant by “not increasing the dose or decreasing the dose is allowed at the discretion of the investigator from the viewpoint of safety of the participants”. Does this not contradict what is going to be done in the extension phase? Also, description and comparison between the intervention and control tablets are not provided. Steps for monitoring of adherence should also be provided.

(Reply to Reviewer #3)

> We appreciate Reviewer #3’s constructive comment. As the reviewer suggested, we changed the title to “Intervention and control”. We added a new table so that the readers can clearly understand the evaluation schedule. We also clarified the dose-monitoring method in the revised manuscript as shown below. In the extension phase, considering the safety of the participants, the investigator can decide the daily dose, for example, 4 tablets/day or more up to 9 tablets/day. If increasing the dose does not cause any adverse reactions and the participant wants to increase the dose, the investigator can increase the dose up to 9 tablets/day. On the other hand, “not increasing the dose” or “decreasing the dose” is allowed at the discretion of the investigator from the viewpoint of safety of the participants. We added quotation marks in the revised manuscript as shown below.

(New table in the revised manuscript pp.19–21)

Table 1. Trial schedule

		Screening period		Double-blind phase																
				Escalation to 4 tablets/day									Escalation to 9 tablets/day							Taper
Timing		Before enrollment	Before start of trial treatment	At start of administration	Week 1	Week 2	Week 3	Week 4	Week 8	Week 12	Week 16	Week 20	Week 21	Week 22	Week 23	Week 24	Week 28	Week 32	Week 36	Week 37
Visit/medical examination		○	○	○	○	Phone	Phone	○	○	○	○	○	○	Phone	Phone	○	○	○	○	○
Informed consent		○																		
Neuropsychological/Motor assessment	ADAS-J cog	○																		
	SIB-J		○	○								○							○	
	NPI		○	○				○	○	○	○	○				○	○	○	○	
	MENFIS		○	○				○	○	○	○	○				○	○	○	○	
	MMSE-J	○	○	○								○							○	
	DAD		○	○								○							○	
	UPDRS part III		○	○								○							○	
	Apathy Scale		○	○								○							○	
	UMNB		○	○								○							○	
Laboratory biomarkers	Plasma biomarkers		○	○						○		○					○		○	
	CSF biomarkers			○															○	
Digital biomarkers	Wearable physical activity		→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	
	Finger tapping		○	○				○	○	○	○	○				○	○	○	○	
PET	Brain amyloid PET		○															○		
	Brain tau PET		○															○		
	Cardiac ultrasound	○										○							○	

Safety assessment	Chest X-ray	○						○				○				○			○	
	ECG	○			▲			○	▲	▲	▲	○	▲			○	▲	▲	○	▲
	Head MRI	○																	○	
	Blood bromocriptine concentration			○ *						○	○	○					○	○	○	
	Laboratory tests	○						○				○				○			○	
	AEs		→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→

		Extension phase										
		Escalation to 4–9 tablets/day †										End of trial
Timing		Week 37	Week 38	Week 39	Week 40	Week 41	Week 42	Week 43	Week 44	Week 45	Week 48	Week 50
Visit/medical examination		○	Phone	Phone	○	Phone	Phone	Phone	○	Phone	○	○
Informed consent												
Neuropsychological/motor assessment	ADAS-J cog											
	SIB-J											
	NPI											
	MENFIS											
	MMSE-J											
	DAD											
	UPDRS part III											
	Apathy Scale											
	UMNB											
Laboratory biomarkers	Plasma biomarkers											
	CSF biomarkers											
Digital biomarkers	Wearable physical activity											
	Finger tapping											
PET	Brain amyloid PET											
	Brain tau PET											
Safety assessment	Cardiac ultrasound											○
	Chest X-ray											○
	ECG	▲			▲				▲		▲	○
	Head MRI											
	Blood bromocriptine concentration											

	Laboratory tests				○							○
	AEs	→	→	→	→	→	→	→	→	→	→	→

○: To be performed

▲: To be performed if donepezil hydrochloride is co-administered.

• After the final visit of the last participant at Week 37, the blind will be broken following data lock to start analyses.

*Schedule for measuring blood bromocriptine concentration on Day 1

Time after first administration of trial drug (h)	Before administration	1	2	3	4	6
Time window (in principle)	−2 hours	± 5 minutes	± 5 minutes	± 5 minutes	± 5 minutes	± 5 minutes
Plasma bromocriptine	○	○	○	○	○	○

†Standard dose is 4 tablets/day, up to 9 tablets/day at maximum.

ADAS-J cog, Alzheimer's Disease Assessment Scale-cognitive subscale Japanese version; AEs, adverse events; DAD, Disability Assessment for Dementia; MENFIS, Mental Function Impairment Scale; MMSE-J, Mini Mental State Examination-Japanese; NPI, Neuropsychiatric Inventory; SIB-J, Severe Impairment Battery-Japanese; UMNB, Upper Motor Neuron Burden Score; UPDRS part III, Movement Disorder Society-sponsored revision of the Unified Parkinson’s Disease Rating Scale Part III.

(In the Intervention and control section in the revised manuscript p.8, line 19; p.9, lines 7–8, line 6)
►Control – placebo (tablets identical in appearance, smell and taste)

Adherence will be monitored by counting empty PTP (press through pack) sheets of study drugs.

In all phases, “not increasing the dose” or “decreasing the dose“ is allowed at the discretion of the investigator from the viewpoint of safety of the participants.

Primary, secondary and reference endpoints:
What are the key pharmacokinetic parameters referred to and what does it mean “for blood bromocriptine concentration on Day 1”? Also, it would be good to provide the purpose and rationale for including the measurement of (1) to (11). Lastly, all items listed are outcome measures, not “endpoints”, further details on when and how they these measures would be made and how reported and analysed would also be needed for clarity.
(Reply to Reviewer #3)
> We appreciate Reviewer #3’s constructive comment. In response to this comment, we added a new table (shown previously) so that readers can clearly understand the evaluation schedule together with the rationale for each evaluation. Our new table also includes the detailed schedule of blood bromocriptine concentration on Day 1, namely -2, 1, 2, 3, 4, and 6 hours after taking the first bromocriptine tablet. We added Cmax and Tmax of bromocriptine blood concentration as the key pharmacokinetic parameters in the revised manuscript as shown below. Obtaining blood concentration is important considering the effect of donepezil on the bromocriptine blood concentration.

(In the Outcomes section in the original manuscript)
For the blood bromocriptine concentration on Day 1, key pharmacokinetic parameters will be calculated.

(In the Outcomes section in the revised manuscript p.10, lines 20–21)
For the blood bromocriptine concentration on Day 1, key pharmacokinetic parameters (Cmax, Tmax) will be calculated (Table 1).

Statistical analysis:
What other stats tests would be used if assumptions of t-test is violated? Any subgroup analysis - since allocation is stratified according to MMSE-J score?
(Reply to Reviewer #3)
> We appreciate Reviewer #3’s constructive comment. We added our plan if the assumption of t-test is violated in the revised manuscript as shown below. As shown in the Statistical Analysis section, we will perform a subgroup analysis in accordance with the baseline MMSE-J score (< 13 or ≥ 13).

(In the Statistical analysis section in the revised manuscript p.12, lines 4–8)
For efficacy endpoints, changes from baseline will be summarised and compared at weeks 20 and 36 using descriptive statistics including point mean differences with 95% confidence intervals and p values derived from Student’s t test with the assumption of normality. If the assumption is violated, nonparametric tests will be considered.

VERSION 2 – REVIEW

REVIEWER	Mehan, Sidharth

	ISF College of Pharmacy, Pharmacology
REVIEW RETURNED	06-May-2021

GENERAL COMMENTS	Dear, I formally acknowledge receipt of your revised manuscript.I note that you have more than appopriately attended to all issues. I have recommended Acceptance

REVIEWER	Besser, Lilah Florida Atlantic University
REVIEW RETURNED	07-May-2021

GENERAL COMMENTS	Thank you for addressing my questions and concerns.

VERSION 2 – AUTHOR RESPONSE

Reviewer: 1

Dr. Sidharth Mehan, ISF College of Pharmacy

Comments to the Author:

Dear,

I formally acknowledge receipt of your revised manuscript.I note that you have more than appopriately attended to all issues.

I have recommended Acceptance

(Reply to Reviewer #1)

> We really appreciate the comment from Reviewer #1.

Reviewer: 2

Dr. Lilah Besser, Florida Atlantic University

Comments to the Author:

Thank you for addressing my questions and concerns.

(Reply to Reviewer #2)

> We really appreciate the comment from Reviewer #2.

1