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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

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Title

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

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Key words

Alzheimer disease, clinical trial, presenilin-1, induced pluripotent stem cells, bromocriptine

3844 words

ABSTRACT

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 a 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 a 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.

Ethics and dissemination

The proposed trial is conducted according to the Declaration of Helsinki, and was approved by the Institutional Review Board (K070). The study results are expected to be disseminated at international or national conferences and published in international journals following the peer-review process.

Trial registration number

jRCT2041200008 (Japan Registry of Clinical Trials), NCT 04413344 (ClinicalTrials.gov)

ARTICLE SUMMARY

Strengths and limitations of this study

- This trial will provide novel data regarding the safety and efficacy of bromocriptine for PSEN1-AD.
- The trial design includes enrichment of the patient population by iPSC technology.
- Various promising biomarkers will be investigated.
- A possible limitation may be a difficulty in recruiting a large-enough number of patients due to the rarity of PSEN1-AD.

INTRODUCTION

Alzheimer's disease (AD) was first reported by Dr. Alios Alzheimer in 1906, and it is now regarded as the most prevalent neurodegenerative disease, accounting for 60–80% of dementia cases. Over 50 million people are estimated to live with dementia globally, a figure set to increase to 152 million by 2050 [1]. The number of AD patients is also expected to increase explosively in Japan in line with our super-aging society. Although this disease should be treated promptly, available treatments are limited to several symptomatic therapies [2].

AD is a neurodegenerative disease that presents clinical symptoms, primarily including progressive decline in memory. Elucidation of the central pathology of AD began in the 1980s with the identification of amyloid β-protein (Aβ) by biochemical methods. Aβ is a major component of senile plaques, which represent a pathological characteristic of AD. Subsequently, in the 1990s, genetic approaches identified the Aβ precursor protein (APP), presenilin 1 (PSEN1), and presenilin 2 (PSEN2) as causative genes for familial AD, thereby rapidly advancing the understanding of such condition [3]. 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 [5,6,7]. 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 [8,9].

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 [10]. We utilised an existing drug library the safety and pharmacokinetic profile of which had already been confirmed clinically and approved for use in humans [10]. 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].

Bromocriptine is an already approved drug with few safety concerns and a long history of usage in clinical settings. Bromocriptine was approved for the treatment of Parkinson's disease (approved dosage 22.5mg/day) [13], pituitary tumour, and hyperprolactinaemia, and was proven to penetrate the blood-brain barrier. Based on iPSC studies, as we hypothesised that bromocriptine might attenuate the clinical symptoms of PSEN1-AD patients, we decided to conduct a clinical trial to evaluate the safety and efficacy of the first-time administration to PSEN1-AD patients as an investigator-initiated clinical trial (exploratory study), which will confirm its safety and provide evidence of its efficacy.

OBJECTIVES

The aim of this study is to investigate the safety and efficacy of an orally administered dose of bromocriptine in patients with PSEN1-AD using a placebo group as control. In addition, long-term safety will be examined in an open-label extension trial.

METHODS AND ANALYSIS

Trial population and rationale for selecting participants

The following patients will be included in this study. Detailed eligibility criteria are presented in "Box 1". This ongoing clinical trial started to enrol participants in June 2020.

►Alzheimer's disease patients with PSEN1 mutations (PSEN1-AD)

►Patients diagnosed with "probable AD" according to the diagnostic guideline of NIA-AA [14] or "probable Alzheimer-type dementia" according to the diagnostic criteria for Alzheimer's disease specified in DSM-5 [15]

► Mini Mental State Examination-Japanese (MMSE-J) score of ≤ 25

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 [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 their regimen within 2 months before providing informed consent will be excluded because the efficacy of bromocriptine alone will be difficult to evaluate. 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. Recruiting will be done by neurologists who treat PSEN1-AD patients and are blinded to their allocation. All participants in the study need to be confirmed for the presence of PSEN-1 mutation by genetic analyses. Given that PSEN1-AD patients are very few, the planned feasible recruitment duration will be 10 months.

Sample size

The number of PSEN1-AD patients confirmed by genetic diagnosis in Japan is approximately 100 according to the Japanese Familial Alzheimer's Disease (JFAD) database [17] and a report of a research project on the support system for individuals with familial AD and their families in Japan [18]. This number corresponds to the global data of reported cases from Asia, the UK and the US [19,20,21]. In addition, compared with patients with sporadic AD without PSEN1 mutations, PSEN1-AD patients have a more rapid progression of cognitive decline and a more substantial decrease in the ability to perform daily living activities (ADLs) owing to the overlap of various neurologic symptoms, including spastic paralysis and extrapyramidal symptoms. Hence, the number of patients is more limited to those who meet the inclusion criteria (Box 1) in the limited trial duration. 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 sufficient participants complete the trial, clinical research coordinators will communicate well and frequently both with the central coordinating centre and the participants/care givers.

Intervention and control

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.

►Intervention – bromocriptine (TW-012R, each tablet contains 2.5 mg of bromocriptine, Towa Pharmaceutical) [13]

►Control – placebo (tablets identical in appearance, smell and taste)

Participants will receive the trial drug in the double-blind phase, during which the low-dose maintenance period (from the start of trial treatment until Week 20) will be started with 1 tablet of TW-012R or placebo administered orally once daily. The daily dose will be increased by 1 tablet per week up to 4 tablets (10 mg) daily until Week 20 as the maintenance dose. The safety and efficacy of bromocriptine orally administered at 10 mg/day will be evaluated in comparison with the placebo. Then, the high-dose maintenance period (weeks 20–36) will be started with 5 tablets of TW-012R or placebo daily (further increased by 1 tablet from the maintenance dose of the low-dose maintenance period). The daily dose will be increased by 1 tablet per week, and a maximum of 9 tablets (22.5 mg) daily will be continued as the maintenance dose until Week 36. The safety and efficacy of bromocriptine orally administered at a dose of 22.5 mg/day will be evaluated in comparison with the placebo. During the tapering period of the trial drug (weeks 36–37), the dose of bromocriptine or placebo will be tapered to 1 tablet from the previous dose during 1 week.

In the extension phase (weeks 37–50), the participants in both groups will start oral administration of 1 tablet of TW-012R (2.5 mg) once daily. The daily dose will be increased by 1 tablet per week, and the administration will be continued until Week 48 at a standard dose of 4 tablets daily, with a maximum of 9 tablets, as maintenance dose to evaluate the safety of the long-term administration of bromocriptine. Then, the dose will be tapered during about 1–2 weeks to complete the treatment.

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

As for drug interactions, CYP3A4 inhibitors/inducers and dopamine antagonists are prohibited because bromocriptine is primarily metabolised by CYP3A4 and drug interaction is anticipated. Benzodiazepines are also prohibited because they might result in reduced efficacy in the clinical phenotype, especially cognitive function.

This study will be conducted at 6 academic hospitals and 1 community hospital in Japan.

Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT checklist) was followed in designing the study protocol.

Randomisation, allocation, and blinding

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 a patient is determined to be eligible by the web enrolment system, the allocated drug number will be displayed as the enrolment result. Under emergency circumstances such as severe adverse events, unblinding is permissible by the discretion of the coordinating investigators.

Outcomes

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 [22].

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 [23,24,25]. Higher scores indicate better cognitive function (range 0–100). Participants will be questioned and assessed by the investigator, sub-investigator, clinical psychologist or speech therapist. 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 (Table 1).

Primary endpoint 3) Neuropsychiatric Inventory (NPI)

NPI (Japanese version) is a validated clinical instrument for evaluating psychopathology in dementia [26,27,28]. Higher scores indicate more severe neuropsychiatric derangements (range 10–120). Caregivers will be interviewed and assessed by the investigator, sub-investigator, clinical psychologist or speech therapist. Individual time courses of observed values and changes from baseline in NPI score up to Weeks 20 and 36 after trial drug administration will be shown (Table 1).

Secondary endpoints

Changes in the following 1) to 11) will be shown individually. For the blood bromocriptine concentration on Day 1, key pharmacokinetic parameters (Cmax, Tmax) will be calculated (Table 1).

1) Mental Function Impairment Scale (MENFIS) [29]
2) MMSE-J [30,31,32]
3) Disability Assessment for Dementia (DAD) [33]
4) Movement Disorder Society-sponsored revision of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS) Part III [34]
5) Apathy Evaluation Scale Informant Version [35,36]
6) Plasma Aβ protein [37]
7) Plasma neurofilament light chain protein (NfL) [38]
8) Plasma total tau and plasma phosphorylated tau protein [37,39,40,41]
9) Cerebrospinal fluid Aβ (CSF Aβ) [42,43]
10) Cerebrospinal fluid total tau and phosphorylated tau protein (CSF total/p-tau) [42,43]
11) Blood bromocriptine concentration

Reference endpoints

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 [44], brain tau PET [45], Upper Motor Neuron Burden Score (UMNB) [46], and plasma Aβ-related peptides [47] will be assessed as reference endpoints (Table 1). The wearable physical activity meter continuously monitors activity, sleep habits, amount of speech, time of physical movement, and heart rate variability. Finger tapping is correlated with

cognitive function and parkinsonian signs, and it allows obtaining parameters directly related to ADLs not limited to cognitive function evaluated by questionnaires.

All neuropsychological testing will be conducted by certified physicians or psychologists who completed the assessment training of this trial. 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 [48,49].

Data management, monitoring, and auditing

The Data Centre will manage the data using the participant identification code or enrolment number. In direct access to source data related to the conduct of this trial, the participants' informed consent forms, and publication of the trial results, full consideration should be given to the protection of privacy and personal information, such as the participants' names and diseases. The full dataset without personal identifiable information will be accessed by data managers, statisticians and investigators. An independent data monitoring committee has been established to assess the safety data.

Trial auditing will be conducted multiple times by an outside contract research organization. Biological specimens will be preserved for future use in ancillary studies with the participants' consent.

Statistical analysis

Safety analyses will be performed for patients who undergo at least part of the trial treatment. Efficacy analyses will be performed among patients who undergo at least part of the trial treatment, provide efficacy information, and satisfy study inclusion criteria. Descriptive statistics will be used to analyse safety data; results will be summarised as counts and percentages. 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. A subgroup analysis in accordance with the baseline MMSE-J score (< 13 or ≥ 13) will be performed to investigate the patient response to bromocriptine. All statistical analyses will be performed using SAS software,

version 9.4 or higher (SAS Institute, Cary, NC). The statistical analysis plan will be developed prior to database lock.

Patient and public involvement

No patient or public were involved in this study design. 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.

ETHICS AND DISSEMINATION

This trial will be conducted in compliance with the ethical principles that have their origin in the Declaration of Helsinki (1964) and its revisions, namely, "Act on Securing Quality, Efficacy and Safety of Products including Pharmaceuticals and Medical Devices," "Ministerial Ordinance on Good Clinical Practice for Drugs," and its related notifications, written procedures, and this protocol. This trial was approved by the Institutional Review Board (K070, Kyoto University Hospital; F-2020-016, Mie University Hospital; 209001-A, Osaka University Hospital; 2105 (02-5), Tokushima University Hospital; T19-04 Tokyo Metropolitan Geriatric Hospital; 20200421, Kawasaki Medical School Hospital; 20200624, Asakayama Hospital).

The investigator will prepare the written information for participants and the informed consent form and will obtain approval from the Institutional Review Board in advance. Items to be described in the written information shall be prepared based on Article 51 of the "Ministerial Ordinance on Good Clinical Practice for Drugs". If the investigator, etc. obtains any information that may affect the intention of the participant or the participant's legally acceptable representative to continue to participate in the trial, he/she will promptly provide the information to the participant or the participant's legally acceptable representative to confirm the intention of whether to continue to participate in the trial. The investigator will also promptly revise the written information, submit it to the head of the trial site, and obtain approval from the Institutional Review Board. The investigator will provide another explanation to the participant who has already been participating in the trial or his/her legally acceptable representative using the revised written information, confirm the participant's will as to whether or not to continue participation in the trial, and obtain written consent from the participant. If an adverse event occurs due to the conduct of the trial, leading to health injury in participants, the investigator will immediately provide the participant with appropriate diagnosis, treatment, and necessary measures including compensation insurance.

Persons involved in this trial shall comply with applicable laws and ordinances, shall exert their utmost efforts to protect personal information and privacy of the participants, and shall not leak personal information obtained in the conduct of the trial without justifiable reasons. The same shall apply after their retirement. When submitting the patient enrolment forms and case report forms, the investigator, sub-investigator, and clinical trial collaborator will use the participant identification code and will not enter information that allows persons outside the trial site to identify the participant (e.g., name, address, and telephone number). The central trial office, data centre and statisticians will use only the participant identification codes and cannot access the participants' personal information.

The proposed study will evaluate both the safety and efficacy of bromocriptine in PSEN1-AD, and the study results are expected to be disseminated at international and/or national conferences and published in international journals following the peer-review process.

DISCUSSION

This study evaluates the safety and explores the efficacy of bromocriptine for patients with PSEN1-AD. The potential efficacy of bromocriptine for PSEN1-AD patients has already been identified by an approach applying iPSC-based drug repurposing [10]. We previously developed a phenotypic screening system to evaluate compounds with a readout of Aβ42 reduction using familial and sporadic AD patient-iPSCs. Our high-throughput screening system revealed that bromocriptine, among 1,258 pharmaceutical compounds, reduced the Aβ42 level most effectively. This drug reduced Aβ42 by 50% in PSEN1-AD iPSCs, and by 20-30% in sporadic patient-iPSC models [10]. A recent report implies that inhibition of less than 50 percent of Aβ might preserve neuronal signalling because the APP family plays an important role in synaptic plasticity underlying learning and memory [50]. Thus, we designed the clinical trial for PSEN1-AD with the expectation that bromocriptine will become a candidate as a molecularly targeted drug for AD, and especially for PSEN1-AD.

Bromocriptine is a therapeutic agent used for the treatment of patients with Parkinson's disease, and the accumulated data of long usage verifies its safety for patients with Parkinson's disease. However, evaluation of the safety of bromocriptine in AD patients is required in order to support its development for the treatment of AD. This clinical trial consists of a 20-week low-dose maintenance period followed by a 16-week high-dose maintenance period to assess its safety. For recruitment and ethical considerations, we also added a 13-week open extension period to continue to follow the safety of the trial drug.

Safety information has been accumulated from long usage of bromocriptine since the 1970s. While nausea and vomiting are the most commonly reported adverse events, it is generally manageable by slow titration of the medication and use of antiemetics. While the occurrence and management of adverse events in AD patients seem to be comparable to those with Parkinson's disease, this study was designed to evaluate any unacceptable adverse events that specifically occur in PSEN1-AD patients. During the recruiting period of this study, the COVID-19 pandemic is influencing study recruitment, onsite monitoring, and participants' visit schedules all over the world. Our study protocol enabled the delivery of the study drugs. 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 [51].

In this study we will limit the included patients to those with PSEN1-AD, because bromocriptine is most effective in PSEN1-AD patient-derived iPSCs compared to other types of familial AD and sporadic AD patients' iPSCs [11,12]. This enrichment will make it difficult to recruit many patients for this study, considering the rarity of such patients in Japan. Although the variety of clinical severities among patients is one of the reasons that cause difficulty for clinical trial evaluation, our inclusion criteria admit a broad disease-stage as we consider that PSEN1-AD patients would be the best candidates for bromocriptine administration. To explore the efficacy of the trial drug, various promising biomarkers in AD, plasma Aβ, plasma Aβ-related peptide, plasma NfL, plasma total/p-tau, CSF Aβ, CSF total/p-tau, amyloid/tau PET, and digital biomarkers will be evaluated in this study. These biomarkers might indicate the target engagement, and its alteration might reflect the effect of bromocriptine treatment. Of note, digital biomarkers will potentially reduce participants' and caregivers' burden in future trials. 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.

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 detections. This is a phase I/IIa study for the safety and early efficacy evaluation with a small number of participants. Therefore, a large-scale study will be needed in the following phases. This study may pave the way for a new and practical disease-modifying AD therapy.

Author contributions

TK, HB, TO, YA, KE, AN, RU, AK, H Tada, SM, SS, TW, and H Inoue conceived and designed the study. AN and RU will conduct the statistical analysis. H Ishikawa, AS, TM, KY, YT, KM, TS, MI, KF, YI, KK, KI, KS, YK, YS, SK, OU, RT and H Tomimoto provided critical advice on the study design. All authors critically revised the draft and approved the final version of this manuscript.

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Competing interests

TK has a patent, Agent for preventing and/or treating Alzheimer's disease, licensed to H Inoue and TK.; HB reports funding for this clinical trial from Time Therapeutics, Inc., trial drugs from Towa Pharmaceutical Co., Ltd., during the conduct of the study; personal fees from Sumitomo Dainippon Pharma Co., Ltd., outside the submitted work; RU reports personal fees from Eisai, Sawai Pharmaceutical Co., Ltd., and CAC Croit, outside the submitted work; SM reports personal fees from AstraZeneca K.K., Bristol-Myers Squibb Company, Chugai Pharmaceutical Co. Ltd., Eli Lilly Japan K.K., MSD K.K., Nippon Boehringer Ingelheim Co. Ltd., Ono Pharmaceutical Co. Ltd., Pfizer Japan Inc., and Taiho Pharmaceutical Co. Ltd.; YT reports personal fees from Sumitomo Dainippon Pharma Co., Ltd., Otsuka Pharmaceutical Co., Ltd., AbbVie GK, Kyowa Kirin Co., Ltd., Takeda Pharmaceutical Company Limited, Tsumura & Co., Eisai Co., Ltd., Sanofi K.K., Mylan EPD G.K., and Ono Pharmaceutical Co., Ltd., outside the submitted work; TM reports personal fees from Bayer

Yakuhin, Ltd., and Otsuka Pharmaceutical Co., Ltd., outside the submitted work; MI reports grants and personal fees from Eisai Co., Ltd., Sumitomo Dainippon Pharma Co., Ltd., Otsuka Pharmaceutical Co., Ltd., MSD K.K., Daiichi Sankyo Co., Ltd., and Takeda Pharmaceutical Company Limited, grants from Mitsubishi Tanabe Pharma Corporation, personal fees from Janssen Pharmaceutical K.K., Nihon Medi-Physics Co., ltd., Fujifilm, Novartis Japan, Meiji Seika Pharma Co., Ltd., Nippon Chemiphar Co., Ltd., Eli Lily Japan K.K., and Chugai Pharmaceutical Co., Ltd., outside the submitted work; KF reports grants from Novartis, outside the submitted work; YI reports grants from Sumitomo Dainippon Pharma Co., Ltd., Eisai Co., Ltd., Japan Blood Products Organization, Otsuka Pharmaceutical Co., Ltd., Kyowa Kirin Co., Ltd., Teijin Pharma Limited, Nihon Pharmaceutical Co., Ltd., and FP Pharmaceutical Corporation, outside the submitted work; KI reports grants, personal fees and other from GE Healthcare, during the conduct of the study; grants and personal fees from Nihon Medi-Physics Co. Ltd., and Eli Lilly Japan K.K., personal fees and other from Eisai Co., Ltd., and Chugai Pharmaceutical Co., other from Biogen, personal fees from Novartis, outside the submitted work; YK reports personal fees from Tsumura & Co., Novartis Japan, UCB Japan Co. Ltd., and Janssen Pharmaceutical K.K., outside the submitted work; YS reports grants from Nippon Shinyaku Co., Ltd., and The Nakatomi Foundation, personal fees from FP Pharmaceutical Corporation, Sumitomo Dainippon Pharma Co., Ltd., and Novartis Japan, outside the submitted work; SK reports grants and personal fees from Eisai Co., Ltd., personal fees from Janssen Pharmaceutical K.K., Novartis Japan, Daiichi-Sankyo, Sumitomo Dainippon Pharma Co., Ltd., Fujifilm Toyama Chemical Co., ltd., Nippon Chemiphar, Nihon Medi-Physics Co., ltd., Tsumura & Co., and Eli Lily Japan K.K., outside the submitted work; SS is an employee of Time Therapeutics, Inc.; TW is an employee of Time Therapeutics, Inc., during the conduct of the study; TW reports personal fees from KanonCure Inc., Tsubota Laboratory, Inc., Dompé Farmaceutici S.p.A., and Novaliq GmbH, outside the submitted work; OU is an employee of Towa Pharmaceutical Co., Ltd.; RT reports grants and personal fees from Takeda Pharmaceutical Co., Ltd., Nippon Boehringer Ingelheim Co., Ltd., Sumitomo Dainippon Pharma Co., Ltd., Eisai Co., Ltd., Kyowa Kirin Co., Ltd., Otsuka Pharmaceutical Co., Ltd., and Sanofi K.K, grants from Astellas Pharma Inc., Novartis Japan, and Nihon Medi-Physics Co. Ltd., personal fees from AbbVie GK, Mitsubishi Tanabe Pharma Corporation, Mylan N.V., Japan Blood Products Organization, Sanwa Kagaku Kenkyusho Co., Ltd., FP Pharmaceutical Corporation, Tsumura & Co., KAN Research Institute, Inc., Kissei Pharmaceutical Co., Ltd., Chugai Pharmaceutical Co., Ltd., and Biogen Inc., outside the submitted work; H Tomimoto reports personal fees from Daiichi Sankyo Co., Ltd., outside the submitted work; H Inoue possesses reports grants and personal fees from Takeda

Pharmaceutical Co., Ltd., Eisai Co., Ltd., Suntory Wellness Limited, Institute for Health Care Science, and Mitsubishi Tanabe Pharma Corporation, grants from Taisho Pharmaceutical Co., Ltd., Toray Industries, Inc., KAN Research Institute, Inc., Shimadzu Corporation, MicroBiopharm Japan Co., Ltd., Kaneka Corporation, Panasonic Corporation, Biogen Inc., and Stem Cell & Device Laboratory, Inc. (SCAD), personal fees from Nomura Securities Co., Ltd., FP Pharmaceutical Corporation, Nippon Chemiphar Co., Ltd., Kansai Pharmaceutical Industries Association, Otsuka Pharmaceutical Co., Ltd., Kyowa Kirin Co., Ltd., outside the submitted work. H Inoue possesses unlisted stocks of Time Therapeutics, Inc. In addition, Kyoto University grants an exclusive license to Time Therapeutics, Inc. through iPS Academia Japan, Inc. regarding the invention of the trial drug (intellectual property) which was discovered through drug screening by the principal investigator (H Inoue). Thereby, Kyoto University and the principal investigator obtain a patent income from Time Therapeutics, Inc. H Inoue does not engage in data management, monitoring and statistical analyses. The coordinating investigators (H Tomimoto and HB) and Time Therapeutics, Inc. will conduct the trial under the investigator-initiated clinical trial agreement. Prior to the trial, the principal investigator and the coordinating investigators underwent a review and received approval by the Conflict of Interest Review Committee based on the conflict of interest management policy at each site. All remaining authors have declared no conflicts of interest.

Data statement

The study protocol, a model informed consent form, and the checklist based on the SPIRIT guidelines were attached as supplements.

Figure legends

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.

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.

Box 1

ELIGIBILITY CRITERIA

Patients who meet all the following inclusion criteria and do not meet any of the exclusion criteria will be enrolled as eligible participants.

Inclusion criteria

1) Alzheimer's disease patients with PSEN1 mutations
2) Patients diagnosed with "probable AD" according to the diagnostic guideline of NIA-AA or "probable Alzheimer-type dementia" according to the diagnostic criteria for Alzheimer's disease specified in DSM-5
3) An MMSE-J score of ≤ 25
4) Patients whose cognitive function and every-day function are obviously impaired based on their medical record or information provided by a person well-acquainted with the patient
5) Patients for whom intellectual disability and mental disorders other than dementia can be ruled out based on their academic background, work history, and life history
6) Patients with a reliable and close relationship with a partner/caregiver
7) Age ≥ 20 years at the time of providing informed consent
8) Written informed consent has been obtained from the patient or his/her legally acceptable representative to participate in this trial

Exclusion criteria

1) Difficulty with the oral intake of tablets
2) Patients receiving anti-dementia drugs who have changed the dosing regimen during the 2 months prior to providing informed consent
3) Patients with dementia due to a pathology other than AD (e.g., vascular dementia, FTD, Lewy body dementia, progressive supranuclear palsy, corticobasal degeneration, Huntington's disease, prion disease)
4) Presence of clinically relevant or unstable mental disorders. Patients with major depression in remission can be enrolled
5) Imminent risk of self-harm or harm to others
6) Body mass index of ≤ 17 or ≥ 35
7) Patients with a history of alcohol dependence, drug dependence, or drug abuse within 5 years before providing informed consent
8) HBs antigen positive
9) Anti-HIV antibody positive
10) Anti-HTLV-1 antibody positive
11) Patients with an active infection such as hepatitis C and syphilis (STS/TPHA)
12) Patients with the following liver function values by testing before enrolment

- AST (GOT) > 4.0 × Upper limit of institutional reference range or
- ALT (GPT) > 4.0 × Upper limit of institutional reference range

13) Patients with uncontrolled, clinically significant medical conditions (e.g., diabetes mellitus, hypertension, thyroid/endocrine disease, congestive cardiac failure, angina pectoris, cardiac/gastrointestinal disease, dialysis, and abnormal renal function with an estimated CLcr < 30 mL/min) within 3 months prior to providing informed consent in addition to the underlying disease to be investigated in the trial and for whom the investigator or sub-investigator considers that there is a significant medical risk in the patient's participation in the trial
14) Patients with long QT syndrome or tendency toward prolonged QTc interval (male: ≥ 470 msec, female: ≥ 480 msec), or patients with a history/complication of torsades de pointes
15) Patients with a history of malignancies within 5 years prior to providing informed consent However, patients with the following diseases can be enrolled if they are treated appropriately:
    a. Skin cancer (basal cell, squamous cell)
    b. Cervical carcinoma in situ
    c. Localised prostate cancer
    d. Malignancies that have not recurred for at least 3 years since surgery and the patient's physician has determined that the risk of recurrence is low
16) Patients with clinically significant vitamin B1/B12 deficiency or folic acid deficiency within 6 months prior to providing informed consent
17) Patients who participated in other clinical research/trials involving interventions within 3 months prior to providing informed consent
18) Patients who previously received bromocriptine or TW-012R
19) Patients with a history of hypersensitivity to bromocriptine or ergot alkaloids
20) Patients with current or a history of thickened heart valve cusps, restricted heart valve motion, and associated heart valve lesions, such as stenosis, confirmed by echocardiography
21) Pregnant females, lactating females, and females who may be pregnant (pregnancy test will be performed to confirm this status), and females who wish to become pregnant
22) Other patients who are considered inappropriate for participating in this trial at the discretion of the investigator or sub-investigator

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

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Figure 1

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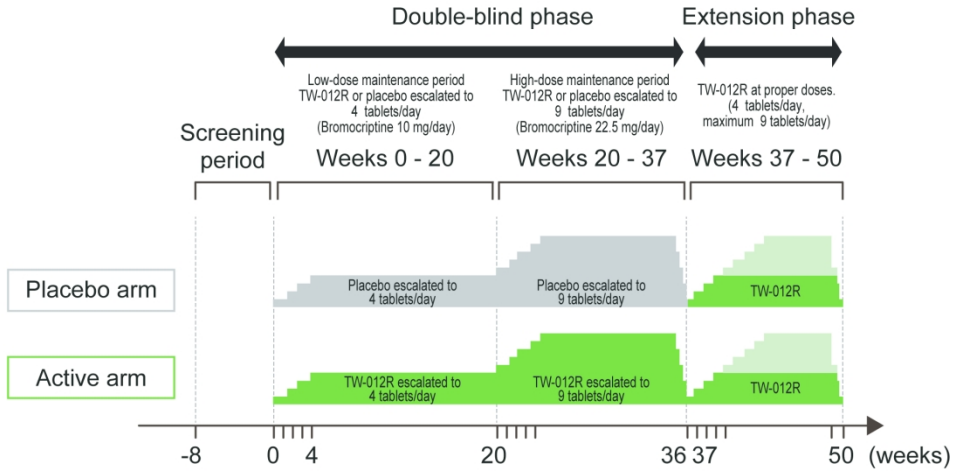

Figure 1. Design of REBRAnD study
The study consists of the screening period (8 weeks), double-blind phase (37 weeks), and extension phase (13 weeks). The double-blind phase for evaluating the efficacy and safety is composed of the low-dose maintenance period (up to 10 mg/day), high-dose maintenance 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.

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	3
	2b	All items from the World Health Organization Trial Registration Data Set	6-10, Table 1
Protocol version	3	Date and version identifier	Suppl (protocol)
Funding	4	Sources and types of financial, material, and other support	15
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1,14
	5b	Name and contact information for the trial sponsor	1,15
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	16,17
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	11

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	5, 6
	6b	Explanation for choice of comparators	6
Objectives	7	Specific objectives or hypotheses	6
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	6
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	1, 3, 8
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	6, 22, 23
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	7, 8, Table 1
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	8
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	7, 8
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	6, 8
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	9, 10, Table 1
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Figure 1, Table 1

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	7
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	6, 7
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	9
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	9
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	9
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	9
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	9
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	9, 10, Table 1
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	7, 8

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	11
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	11
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	11
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	11
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	11
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	N/A
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	9, Table 1
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	11
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	12
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	12

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	12
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	11
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	12, 13
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	15, 16, 17
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	11, 16
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	12
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	13
	31b	Authorship eligibility guidelines and any intended use of professional writers	14
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	Suppl.(protocol)
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Suppl. (model consent form)
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	11

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

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

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Primary Subject Heading:	Neurology
Secondary Subject Heading:	Mental health, Pharmacology and therapeutics
Keywords:	Dementia < NEUROLOGY, Clinical trials < THERAPEUTICS, Neurogenetics < NEUROLOGY, Neurology < INTERNAL MEDICINE, Neurobiology < NATURAL SCIENCE DISCIPLINES

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Title

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

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Key words

Alzheimer disease, clinical trial, presenilin-1, induced pluripotent stem cells, bromocriptine

3853 words

ABSTRACT

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 a 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 a 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.

Ethics and dissemination

The proposed trial is conducted according to the Declaration of Helsinki, and was approved by the Institutional Review Board (K070). The study results are expected to be disseminated at international or national conferences and published in international journals following the peer-review process.

Trial registration number

jRCT2041200008 (Japan Registry of Clinical Trials), NCT 04413344 (ClinicalTrials.gov)

ARTICLE SUMMARY

Strengths and limitations of this study

- This trial will provide the safety and efficacy data of bromocriptine for Alzheimer's disease patients with mutations in the PSEN1 gene (PSEN1-AD).
- Bromocriptine is an approved drug with safety information accumulated over decades.
- The trial design includes enrichment of the patient population by induced pluripotent stem cell (iPSC) technology.
- Minimally invasive biomarkers, using plasma samples, PET and digital tools, will be investigated in addition to Aβ and tau in cerebrospinal fluid samples.
- A possible limitation may be a difficulty in recruiting a large-enough number of patients due to the rarity of PSEN1-AD.

INTRODUCTION

Alzheimer's disease (AD) was first reported by Dr. Alios Alzheimer in 1906, and it is now regarded as the most prevalent neurodegenerative disease, accounting for 60–80% of dementia cases. Over 50 million people are estimated to live with dementia globally, a figure set to increase to 152 million by 2050 [1]. The number of AD patients is also expected to increase explosively in Japan in line with our super-aging society. Although this disease should be treated promptly, available treatments are limited to several symptomatic therapies [2].

AD is a neurodegenerative disease that presents clinical symptoms, primarily including progressive decline in memory. Elucidation of the central pathology of AD began in the 1980s with the identification of amyloid β-protein (Aβ) by biochemical methods. Aβ is a major component of senile plaques, which represent a pathological characteristic of AD. Subsequently, in the 1990s, genetic approaches identified the Aβ precursor protein (APP), presenilin 1 (PSEN1), and presenilin 2 (PSEN2) as causative genes for familial AD, thereby rapidly advancing the understanding of such condition [3]. 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 [5,6,7]. 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 [8,9].

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 [10]. We utilised an existing drug library the safety and pharmacokinetic profile of which had already been confirmed clinically and approved for use in humans [10]. 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].

Bromocriptine is an already approved drug with few safety concerns and a long history of usage in clinical settings. Bromocriptine was approved for the treatment of Parkinson's disease (approved dosage 22.5mg/day) [13], pituitary tumour, and hyperprolactinaemia, and was proven to penetrate the blood-brain barrier. Based on iPSC studies, as we hypothesised that bromocriptine might attenuate the clinical symptoms of PSEN1-AD patients, we decided to conduct a clinical trial to evaluate the safety and efficacy of the first-time administration to PSEN1-AD patients as an investigator-initiated clinical trial (exploratory study), which will confirm its safety and provide evidence of its efficacy.

OBJECTIVES

The aim of this study is to investigate the safety and efficacy of an orally administered dose of bromocriptine in patients with PSEN1-AD using a placebo group as control. In addition, long-term safety will be examined in an open-label extension trial.

METHODS AND ANALYSIS

Trial population and rationale for selecting participants

The following patients will be included in this study. Detailed eligibility criteria are presented in "Box 1". This ongoing clinical trial started to enrol participants in June 2020. The study is planned to end in March 2022.

►Alzheimer's disease patients with PSEN1 mutations (PSEN1-AD)

►Patients diagnosed with "probable AD" according to the diagnostic guideline of NIA-AA [14] or "probable Alzheimer-type dementia" according to the diagnostic criteria for Alzheimer's disease specified in DSM-5 [15]

► Mini Mental State Examination-Japanese (MMSE-J) score of ≤ 25

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 [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 their regimen within 2 months before providing informed consent will be excluded because the efficacy of bromocriptine alone will be difficult to evaluate. 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. Recruiting will be done by neurologists who treat PSEN1-AD patients and are blinded to their allocation. All participants in the study need to be confirmed for the presence of PSEN-1 mutation by genetic analyses. Given that PSEN1-AD patients are very few, the planned feasible recruitment duration will be 10 months.

Sample size

The number of PSEN1-AD patients confirmed by genetic diagnosis in Japan is approximately 100 according to the Japanese Familial Alzheimer's Disease (JFAD) database [17] and a report of a research project on the support system for individuals with familial AD and their families in Japan [18]. This number corresponds to the global data of reported cases from Asia, the UK and the US [19,20,21]. In addition, compared with patients with sporadic AD without PSEN1 mutations, PSEN1-AD patients have a more rapid progression of cognitive decline and a more substantial decrease in the ability to perform daily living activities (ADLs) owing to the overlap of various neurologic symptoms, including spastic paralysis and extrapyramidal symptoms. Hence, the number of patients is more limited to those who meet the inclusion criteria (Box 1) in the limited trial duration. 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 sufficient participants complete the trial, clinical research coordinators will communicate well and frequently both with the central coordinating centre and the participants/care givers.

Intervention and control

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.

►Intervention – bromocriptine (TW-012R, each tablet contains 2.5 mg of bromocriptine, Towa Pharmaceutical) [13]

►Control – placebo (tablets identical in appearance, smell and taste)

Participants will receive the trial drug in the double-blind phase, during which the low-dose maintenance period (from the start of trial treatment until Week 20) will be started with 1 tablet of TW-012R or placebo administered orally once daily. The daily dose will be increased by 1 tablet per week up to 4 tablets (10 mg) daily until Week 20 as the maintenance dose. The safety and efficacy of bromocriptine orally administered at 10 mg/day will be evaluated in comparison with the placebo. Then, the high-dose maintenance period (weeks 20–36) will be started with 5 tablets of TW-012R or placebo daily (further increased by 1 tablet from the maintenance dose of the low-dose maintenance period). The daily dose will be increased by 1 tablet per week, and a maximum of 9 tablets (22.5 mg) daily will be continued as the maintenance dose until Week 36. The safety and efficacy of bromocriptine orally administered at a dose of 22.5 mg/day will be evaluated in comparison with the placebo. During the tapering period of the trial drug (weeks 36–37), the dose of bromocriptine or placebo will be tapered to 1 tablet from the previous dose during 1 week.

In the extension phase (weeks 37–50), the participants in both groups will start oral administration of 1 tablet of TW-012R (2.5 mg) once daily. The daily dose will be increased by 1 tablet per week, and the administration will be continued until Week 48 at a standard dose of 4 tablets daily, with a maximum of 9 tablets, as maintenance dose to evaluate the safety of the long-term administration of bromocriptine. Then, the dose will be tapered during about 1–2 weeks to complete the treatment.

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

As for drug interactions, CYP3A4 inhibitors/inducers and dopamine antagonists are prohibited because bromocriptine is primarily metabolised by CYP3A4 and drug interaction is anticipated.

Benzodiazepines are also prohibited because they might result in reduced efficacy in the clinical phenotype, especially cognitive function.

This study will be conducted at 6 academic hospitals and 1 community hospital in Japan.

Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT checklist) was followed in designing the study protocol.

Randomisation, allocation, and blinding

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 a patient is determined to be eligible by the web enrolment system, the allocated drug number will be displayed as the enrolment result. Under emergency circumstances such as severe adverse events, unblinding is permissible by the discretion of the coordinating investigators.

Outcomes

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 [22].

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 [23,24,25]. Higher scores indicate better cognitive function (range 0–100). Participants will be questioned and assessed by the investigator, sub-investigator, clinical psychologist or speech therapist. 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 (Table 1).

Primary endpoint 3) Neuropsychiatric Inventory (NPI)

NPI (Japanese version) is a validated clinical instrument for evaluating psychopathology in dementia [26,27,28]. Higher scores indicate more severe neuropsychiatric derangements (range 10–120). Caregivers will be interviewed and assessed by the investigator, sub-investigator, clinical psychologist or speech therapist. Individual time courses of observed values and changes from baseline in NPI score up to Weeks 20 and 36 after trial drug administration will be shown (Table 1).

Secondary endpoints

Changes in the following 1) to 11) will be shown individually. For the blood bromocriptine concentration on Day 1, key pharmacokinetic parameters (Cmax, Tmax) will be calculated (Table 1).

1) Mental Function Impairment Scale (MENFIS) [29]
2) MMSE-J [30,31,32]
3) Disability Assessment for Dementia (DAD) [33]
4) Movement Disorder Society-sponsored revision of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS) Part III [34]
5) Apathy Evaluation Scale Informant Version [35,36]
6) Plasma Aβ protein [37]
7) Plasma neurofilament light chain protein (NfL) [38]
8) Plasma total tau and plasma phosphorylated tau protein [37,39,40,41]
9) Cerebrospinal fluid Aβ (CSF Aβ) [42,43]
10) Cerebrospinal fluid total tau and phosphorylated tau protein (CSF total/p-tau) [42,43]
11) Blood bromocriptine concentration

Reference endpoints

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 [44], brain tau PET [45], Upper Motor Neuron Burden Score (UMNB) [46], and plasma Aβ-related peptides [47] will be assessed as reference endpoints

(Table 1). The wearable physical activity meter continuously monitors activity, sleep habits, amount of speech, time of physical movement, and heart rate variability. Finger tapping is correlated with cognitive function and parkinsonian signs, and it allows obtaining parameters directly related to ADLs not limited to cognitive function evaluated by questionnaires.

All neuropsychological testing will be conducted by certified physicians or psychologists who completed the assessment training of this trial. 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 [48,49].

Data management, monitoring, and auditing

The Data Centre will manage the data using the participant identification code or enrolment number. In direct access to source data related to the conduct of this trial, the participants' informed consent forms, and publication of the trial results, full consideration should be given to the protection of privacy and personal information, such as the participants' names and diseases. The full dataset without personal identifiable information will be accessed by data managers, statisticians and investigators. An independent data monitoring committee has been established to assess the safety data.

Trial auditing will be conducted multiple times by an outside contract research organization. Biological specimens will be preserved for future use in ancillary studies with the participants' consent.

Statistical analysis

Safety analyses will be performed for patients who undergo at least part of the trial treatment. Efficacy analyses will be performed among patients who undergo at least part of the trial treatment, provide efficacy information, and satisfy study inclusion criteria. Descriptive statistics will be used to analyse safety data; results will be summarised as counts and percentages. 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. A subgroup analysis in accordance with the baseline MMSE-J score (< 13 or ≥ 13) will be performed to investigate the

patient response to bromocriptine. All statistical analyses will be performed using SAS software, version 9.4 or higher (SAS Institute, Cary, NC). The statistical analysis plan will be developed prior to database lock.

Patient and public involvement

No patient or public were involved in this study design. 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.

ETHICS AND DISSEMINATION

This trial will be conducted in compliance with the ethical principles that have their origin in the Declaration of Helsinki (1964) and its revisions, namely, "Act on Securing Quality, Efficacy and Safety of Products including Pharmaceuticals and Medical Devices," "Ministerial Ordinance on Good Clinical Practice for Drugs," and its related notifications, written procedures, and this protocol. This trial was approved by the Institutional Review Board (K070, Kyoto University Hospital; F-2020-016, Mie University Hospital; 209001-A, Osaka University Hospital; 2105 (02-5), Tokushima University Hospital; T19-04 Tokyo Metropolitan Geriatric Hospital; 20200421, Kawasaki Medical School Hospital; 20200624, Asakayama Hospital).

The investigator will prepare the written information for participants and the informed consent form and will obtain approval from the Institutional Review Board in advance. Items to be described in the written information shall be prepared based on Article 51 of the "Ministerial Ordinance on Good Clinical Practice for Drugs". If the investigator, etc. obtains any information that may affect the intention of the participant or the participant's legally acceptable representative to continue to participate in the trial, he/she will promptly provide the information to the participant or the participant's legally acceptable representative to confirm the intention of whether to continue to participate in the trial. The investigator will also promptly revise the written information, submit it to the head of the trial site, and obtain approval from the Institutional Review Board. The investigator will provide another explanation to the participant who has already been participating in the trial or his/her legally acceptable representative using the revised written information, confirm the participant's will as to whether or not to continue participation in the trial, and obtain written consent from the participant. If an adverse event occurs due to the conduct of the trial, leading to health injury

in participants, the investigator will immediately provide the participant with appropriate diagnosis, treatment, and necessary measures including compensation insurance.

Persons involved in this trial shall comply with applicable laws and ordinances, shall exert their utmost efforts to protect personal information and privacy of the participants, and shall not leak personal information obtained in the conduct of the trial without justifiable reasons. The same shall apply after their retirement. When submitting the patient enrolment forms and case report forms, the investigator, sub-investigator, and clinical trial collaborator will use the participant identification code and will not enter information that allows persons outside the trial site to identify the participant (e.g., name, address, and telephone number). The central trial office, data centre and statisticians will use only the participant identification codes and cannot access the participants' personal information.

The proposed study will evaluate both the safety and efficacy of bromocriptine in PSEN1-AD, and the study results are expected to be disseminated at international and/or national conferences and published in international journals following the peer-review process.

DISCUSSION

This study evaluates the safety and explores the efficacy of bromocriptine for patients with PSEN1-AD. The potential efficacy of bromocriptine for PSEN1-AD patients has already been identified by an approach applying iPSC-based drug repurposing [10]. We previously developed a phenotypic screening system to evaluate compounds with a readout of Aβ42 reduction using familial and sporadic AD patient-iPSCs. Our high-throughput screening system revealed that bromocriptine, among 1,258 pharmaceutical compounds, reduced the Aβ42 level most effectively. This drug reduced Aβ42 by 50% in PSEN1-AD iPSCs, and by 20-30% in sporadic patient-iPSC models [10]. A recent report implies that inhibition of less than 50 percent of Aβ might preserve neuronal signalling because the APP family plays an important role in synaptic plasticity underlying learning and memory [50]. Thus, we designed the clinical trial for PSEN1-AD with the expectation that bromocriptine will become a candidate as a molecularly targeted drug for AD, and especially for PSEN1-AD.

Bromocriptine is a therapeutic agent used for the treatment of patients with Parkinson's disease, and the accumulated data of long usage verifies its safety for patients with Parkinson's disease. However, evaluation of the safety of bromocriptine in AD patients is required in order to support its development for the treatment of AD. This clinical trial consists of a 20-week low-dose maintenance period followed by a 16-week high-dose maintenance period to assess its safety. For recruitment and

ethical considerations, we also added a 13-week open extension period to continue to follow the safety of the trial drug.

Safety information has been accumulated from long usage of bromocriptine since the 1970s. While nausea and vomiting are the most commonly reported adverse events, it is generally manageable by slow titration of the medication and use of antiemetics. While the occurrence and management of adverse events in AD patients seem to be comparable to those with Parkinson's disease, this study was designed to evaluate any unacceptable adverse events that specifically occur in PSEN1-AD patients. During the recruiting period of this study, the COVID-19 pandemic is influencing study recruitment, onsite monitoring, and participants' visit schedules all over the world. Our study protocol enabled the delivery of the study drugs. 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 [51].

In this study we will limit the included patients to those with PSEN1-AD, because bromocriptine is most effective in PSEN1-AD patient-derived iPSCs compared to other types of familial AD and sporadic AD patients' iPSCs [11,12]. This enrichment will make it difficult to recruit many patients for this study, considering the rarity of such patients in Japan. Although the variety of clinical severities among patients is one of the reasons that cause difficulty for clinical trial evaluation, our inclusion criteria admit a broad disease-stage as we consider that PSEN1-AD patients would be the best candidates for bromocriptine administration. To explore the efficacy of the trial drug, various promising biomarkers in AD, plasma Aβ, plasma Aβ-related peptide, plasma NfL, plasma total/p-tau, CSF Aβ, CSF total/p-tau, amyloid/tau PET, and digital biomarkers will be evaluated in this study. These biomarkers might indicate the target engagement, and its alteration might reflect the effect of bromocriptine treatment. Of note, digital biomarkers will potentially reduce participants' and caregivers' burden in future trials. 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.

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 detections. This is a phase I/IIa study for the safety and early efficacy evaluation with a small number of participants. Therefore, a large-scale study will be needed in the following phases. This study may pave the way for a new and practical disease-modifying AD therapy.

Author contributions

TK, HB, TO, YA, KE, AN, RU, AK, H Tada, SM, SS, TW, and H Inoue conceived and designed the study. AN and RU will conduct the statistical analysis. H Ishikawa, AS, TM, KY, YT, KM, TS, MI, KF, YI, KK, KI, KS, YK, YS, SK, OU, RT and H Tomimoto provided critical advice on the study design. All authors critically revised the draft and approved the final version of this manuscript.

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Competing interests

TK has a patent, Agent for preventing and/or treating Alzheimer's disease, licensed to H Inoue and TK.; HB reports funding for this clinical trial from Time Therapeutics, Inc., trial drugs from Towa Pharmaceutical Co., Ltd., during the conduct of the study; personal fees from Sumitomo Dainippon Pharma Co., Ltd., outside the submitted work; RU reports personal fees from Eisai, Sawai Pharmaceutical Co., Ltd., and CAC Croit, outside the submitted work; SM reports personal fees from AstraZeneca K.K., Bristol-Myers Squibb Company, Chugai Pharmaceutical Co. Ltd., Eli Lilly Japan K.K., MSD K.K., Nippon Boehringer Ingelheim Co. Ltd., Ono Pharmaceutical Co. Ltd., Pfizer Japan Inc., and Taiho Pharmaceutical Co. Ltd.; YT reports personal fees from Sumitomo Dainippon Pharma Co., Ltd., Otsuka Pharmaceutical Co., Ltd., AbbVie GK, Kyowa Kirin Co., Ltd., Takeda

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Data statement

The study protocol, a model informed consent form, and the checklist based on the SPIRIT guidelines were attached as supplements.

Figure legends

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.

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.

Box 1

ELIGIBILITY CRITERIA

Patients who meet all the following inclusion criteria and do not meet any of the exclusion criteria will be enrolled as eligible participants.

Inclusion criteria

1) Alzheimer's disease patients with PSEN1 mutations
2) Patients diagnosed with "probable AD" according to the diagnostic guideline of NIA-AA or "probable Alzheimer-type dementia" according to the diagnostic criteria for Alzheimer's disease specified in DSM-5
3) An MMSE-J score of ≤ 25
4) Patients whose cognitive function and every-day function are obviously impaired based on their medical record or information provided by a person well-acquainted with the patient
5) Patients for whom intellectual disability and mental disorders other than dementia can be ruled out based on their academic background, work history, and life history
6) Patients with a reliable and close relationship with a partner/caregiver
7) Age ≥ 20 years at the time of providing informed consent
8) Written informed consent has been obtained from the patient or his/her legally acceptable representative to participate in this trial

Exclusion criteria

1) Difficulty with the oral intake of tablets
2) Patients receiving anti-dementia drugs who have changed the dosing regimen during the 2 months prior to providing informed consent
3) Patients with dementia due to a pathology other than AD (e.g., vascular dementia, FTD, Lewy body dementia, progressive supranuclear palsy, corticobasal degeneration, Huntington's disease, prion disease)
4) Presence of clinically relevant or unstable mental disorders. Patients with major depression in remission can be enrolled
5) Imminent risk of self-harm or harm to others
6) Body mass index of ≤ 17 or ≥ 35
7) Patients with a history of alcohol dependence, drug dependence, or drug abuse within 5 years before providing informed consent
8) HBs antigen positive
9) Anti-HIV antibody positive
10) Anti-HTLV-1 antibody positive
11) Patients with an active infection such as hepatitis C and syphilis (STS/TPHA)
12) Patients with the following liver function values by testing before enrolment

- AST (GOT) > 4.0 × Upper limit of institutional reference range or
- ALT (GPT) > 4.0 × Upper limit of institutional reference range

13) Patients with uncontrolled, clinically significant medical conditions (e.g., diabetes mellitus, hypertension, thyroid/endocrine disease, congestive cardiac failure, angina pectoris, cardiac/gastrointestinal disease, dialysis, and abnormal renal function with an estimated CLcr < 30 mL/min) within 3 months prior to providing informed consent in addition to the underlying disease to be investigated in the trial and for whom the investigator or sub-investigator considers that there is a significant medical risk in the patient's participation in the trial
14) Patients with long QT syndrome or tendency toward prolonged QTc interval (male: ≥ 470 msec, female: ≥ 480 msec), or patients with a history/complication of torsades de pointes
15) Patients with a history of malignancies within 5 years prior to providing informed consent However, patients with the following diseases can be enrolled if they are treated appropriately:
    a. Skin cancer (basal cell, squamous cell)
    b. Cervical carcinoma in situ
    c. Localised prostate cancer
    d. Malignancies that have not recurred for at least 3 years since surgery and the patient's physician has determined that the risk of recurrence is low
16) Patients with clinically significant vitamin B1/B12 deficiency or folic acid deficiency within 6 months prior to providing informed consent
17) Patients who participated in other clinical research/trials involving interventions within 3 months prior to providing informed consent
18) Patients who previously received bromocriptine or TW-012R
19) Patients with a history of hypersensitivity to bromocriptine or ergot alkaloids
20) Patients with current or a history of thickened heart valve cusps, restricted heart valve motion, and associated heart valve lesions, such as stenosis, confirmed by echocardiography
21) Pregnant females, lactating females, and females who may be pregnant (pregnancy test will be performed to confirm this status), and females who wish to become pregnant
22) Other patients who are considered inappropriate for participating in this trial at the discretion of the investigator or sub-investigator

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

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Figure 1

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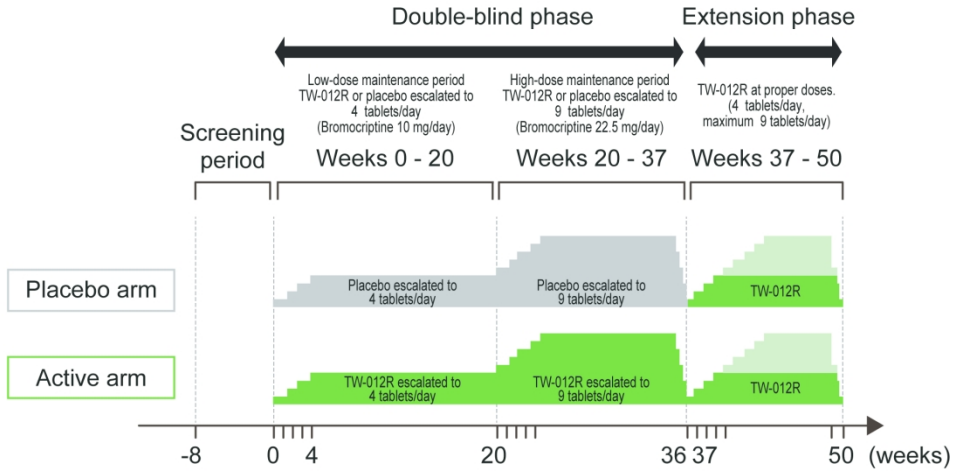

Figure 1. Design of REBRAnD study
The study consists of the screening period (8 weeks), double-blind phase (37 weeks), and extension phase (13 weeks). The double-blind phase for evaluating the efficacy and safety is composed of the low-dose maintenance period (up to 10 mg/day), high-dose maintenance 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.

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	3
	2b	All items from the World Health Organization Trial Registration Data Set	6-10, Table 1
Protocol version	3	Date and version identifier	Suppl (protocol)
Funding	4	Sources and types of financial, material, and other support	15
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1,14
	5b	Name and contact information for the trial sponsor	1,15
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	16,17
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	11

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	5, 6
	6b	Explanation for choice of comparators	6
Objectives	7	Specific objectives or hypotheses	6
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	6
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	1, 3, 8
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	6, 22, 23
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	7, 8, Table 1
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	8
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	7, 8
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	6, 8
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	9, 10, Table 1
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Figure 1, Table 1

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	7
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	6, 7
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	9
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	9
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	9
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	9
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	9
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	9, 10, Table 1
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	7, 8

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	11
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	11
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	11
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	11
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	11
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	N/A
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	9, Table 1
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	11
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	12
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	12

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	12
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	11
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	12, 13
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	15, 16, 17
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	11, 16
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	12
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	13
	31b	Authorship eligibility guidelines and any intended use of professional writers	14
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	Suppl.(protocol)
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Suppl. (model consent form)
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	11

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.