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# Pivotal trial of low-intensity pulsed ultrasound therapy for early Alzheimer's disease: Rationale and design

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## Abstract

**Background:** There are lines of evidence suggesting that cerebral microcirculatory dysfunction is involved in the pathogenesis of Alzheimer's disease (AD). We have developed a low-intensity pulsed ultrasound (LIPUS) therapy that upregulates endothelial NO synthase with therapeutic angiogenesis. We demonstrated that the LIPUS therapy ameliorates cognitive declines in mouse models of AD and tended to do so in patients with early AD (mild AD and mild cognitive impairment due to AD) in the pilot trial. Thus, the Japanese government has designated our LIPUS device as the first breakthrough medical device in Japan.

**Objective:** We are performing a pivotal clinical trial (LIPUS-AD) to finally address the efficacy and safety of our LIPUS therapy in patients with early AD in Japan.

**Methods:** LIPUS-AD is a randomized, double-blind, placebo-controlled trial, in which a total of 220 patients with early AD, who are positive for amyloid-β (Aβ) PET, will be randomized in a 1:1 fashion. The LIPUS therapy is performed for the whole brain for one hour 3 times a week as one session under the special conditions (32 cycles, 0.5 MHz, 0.25 W/cm$^2$). It is performed for 6 sessions with 3-month intervals in the LIPUS group for 72 weeks, while the placebo group receives placebo therapy. Before and at 72 weeks of the trial, all subjects undergo brain Aβ PET and MRI and 9 cognitive functions tests. The primary efficacy endpoint is the changes in ADAS-J-cog-14 scores from baseline to 72 weeks.

**Conclusions:** LIPUS-AD addresses efficacy and safety of the LIPUS therapy in patients with early AD.

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## Keywords

Alzheimer's disease, endothelial nitric oxide synthase, low-intensity pulsed ultrasound, medical device

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## Introduction

Along with society aging, the prevalence of Alzheimer's disease (AD) has been rapidly increasing worldwide, and effective and safe treatment of AD remains to be developed.[1,2] Based on the amyloid-β (Aβ) cascade hypothesis,[3] a number of pharmacological agents that inhibit Aβ synthesis or promote its degradation have been developed.[4] Indeed, the monoclonal antibodies against Aβ, such as aducanumab,[5] lecanemab,[6] and donanemab,[7] have shown partial efficacy associated with safety concerns (e.g., Aβ-related imaging abnormalities) for the treatment of AD.

On the other hand, AD is widely known to share with vascular dementia common risk factors as well as prevention methods, namely atherosclerotic risk factors, such as hypertension, diabetes mellitus, and lack of exercise.[8] Among the vascular functions, endothelial function plays a central role to suppress progression of atherosclerosis.[9,10]

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Indeed, endothelial dysfunction with reduced nitric oxide (NO) availability has been reported to play an important role in the pathogenesis of AD.[11] Furthermore, the combination of amyloid pathology (e.g., Aβ deposition and neurofibrillary change) and cerebral ischemic pathology has been found as major triggering mechanisms of dementia.[12] Thus, vascular dysfunction, especially cerebral microcirculatory dysfunction, should be regarded as an important pathology of AD.[13]

We have developed a low-intensity pulsed ultrasound (LIPUS) therapy that upregulates endothelial NO synthase (eNOS) with resultant therapeutic angiogenesis and suppression of chronic inflammation.[14,15] We demonstrated that the LIPUS therapy is effective and safe in animal models of chronic myocardial ischemia,[16] myocardial infarction,[17] and left ventricular diastolic dysfunction.[18] We also demonstrated that the LIPUS therapy ameliorates cognitive dysfunctions in mouse models of AD and vascular dementia[19] and also functional recovery in a mouse model of cerebral infarction.[20] The effects of the LIPUS therapy is mainly mediated by its upregulation of eNOS as its beneficial effects are absent in eNOS-deficient mice.[17–20]

Following these encouraging results, we then performed the pilot trial of the LIPUS therapy for patients with early AD (LIPUS-AD pilot trial).[21] We performed two pilot trials of LIPUS therapy for early AD (mild cognitive impairment (MCI) due to AD and mild AD); a roll-in open trial for safety ($N = 5$) and a randomized, double-blind, placebo-controlled (RCT) trial for efficacy and safety ($N = 22$).[21] The LIPUS therapy was applied for the whole brain through the bilateral temporal bones for one hour 3 times a week as one session under the special conditions that we identified (32 cycles, 0.5 MHz, 1.3 MPa, 0.25 W/cm$^2$, 5% duty cycle). The LIPUS therapy was performed for one session in the roll-in trial, and 6 sessions in the RCT trial with 3-month intervals for 1.5 years. The primary endpoint was the changes in the Japanese version of the Alzheimer's disease assessment scale - cognitive subscale (ADAS-J-cog)-11 scores from baseline to week 72. The roll-in trial confirmed the safety of the LIPUS therapy and the RCT trial suggested the efficacy of the LIPUS therapy and again confirmed its safety (Figure 1).[21] Especially, when responders were defined as those with no worsening of ADAS-J-cog scores at week 72, the prevalence was 50% in the LIPUS group and 0% in the placebo groups ($p = 0.053$). Based on these promising results, the Japanese Ministry of Health, Labour, and Welfare designated our LIPUS-Brain machine as "a breakthrough medical device" on September 30, 2022. Thus, we are going to perform the pivotal trial to further address our LIPUS therapy for early AD by increasing the numbers of subjects and institutes (LIPUS-AD trial). In this article, we will report the rationale and design of our pivotal trial.

## Methods

### Study objective and design

LIPUS-AD is a randomized, double-blind, placebo-controlled pivotal trial, in which we aim to confirm our preliminary findings in our pilot study suggesting that the whole-brain LIPUS therapy is effective and safe for the treatment of patients with early AD (MCI and mild AD) (Figure 2).[21]

### Participants

Patients with dementia who meet the inclusion/exclusion criteria will be recruited at the participating institutes in Japan. All study procedures are approved and monitored by an external institutional review board (IRB) with additional oversight by site-local IRBs. The diagnosis of early AD (MCI due to AD and mild AD) will be made by board-certified dementia experts at each participating hospital with imaging biomarkers, including brain Aβ PET and brain MRI (Figure 2). Inclusion and exclusion criteria are as follows:

#### Inclusion criteria

- Written informed consent obtained from the patient or representative consenter
- Age 50–90 years of both sex
- Cognitive function level: MCI due to AD or mild AD by the 2018 NIA/AA criteria when obtaining informed consent[22]
- Guaranteed cooperation from the same partner throughout the trial who will meet the following conditions; (a) living together or getting in touch closely, (b) able to observe patient's daily activity, (c) able to attend the cognitive tests, and (d) able to manage medication confirmed by an attending physician
- Clinical Dementia Rating (CDR) global score of 0.5 $\sim$1.0 at screening[23]
- Japanese version of Mini Mental State Examination (MMSE-J) score greater than 20[23]
- The diagnostic radiology committee confirms the exclusions by brain MRI of organic cerebral diseases, including symptomatic cerebral bleeding, symptomatic cerebral infarction, acute cerebral infarction, and brain tumor and those by brain MRA of severe stenosis or obstruction of the middle cerebral artery.
- When the patient is treated with drugs for mild AD or MCI due to AD, such drugs should not be changed 4 weeks before and after informed consent throughout the trial, including donepezil hydrochloride,

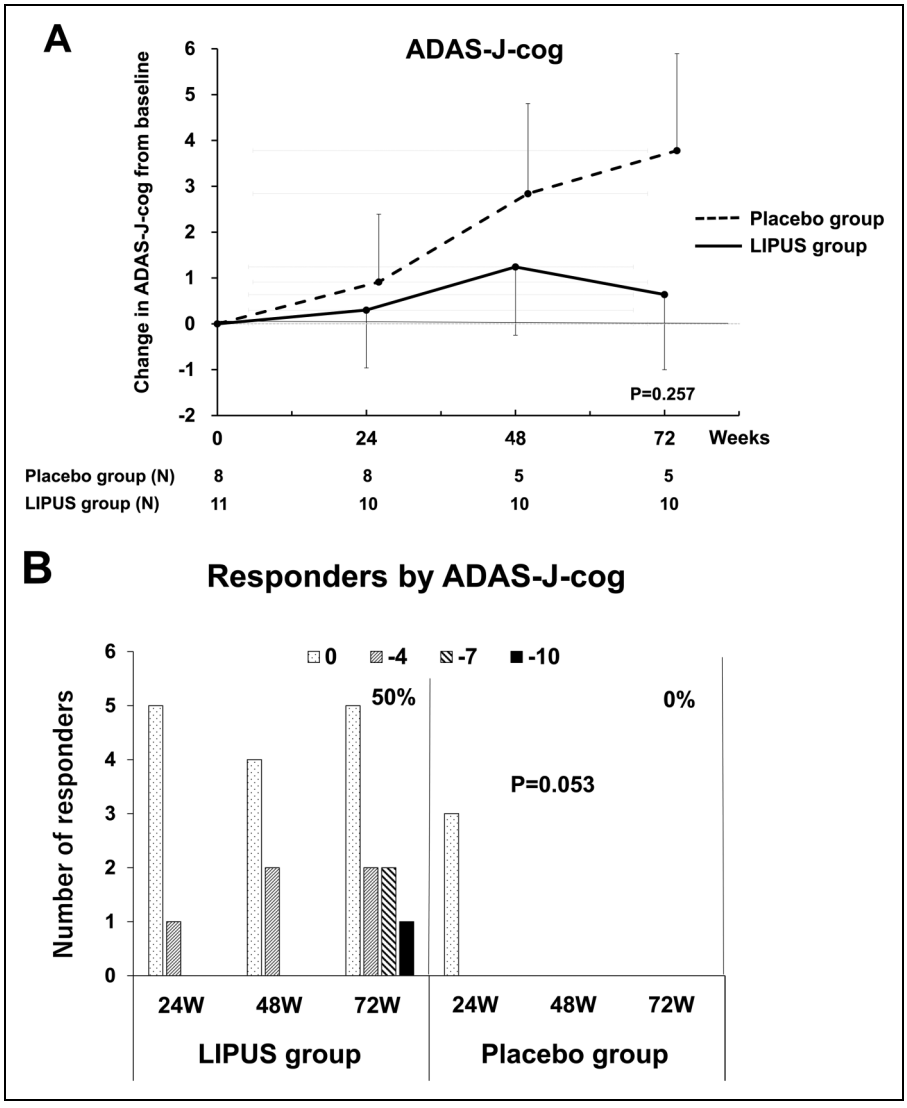


**Figure 1.** Main findings of the exploratory study. (A) Time-course of primary efficacy endpoint (ADAS-J-cog-11). (B) Responder analysis. When responders were defined as subjects who showed no worsening (0), or even improvement (−4〜−10) at week 72 from baseline, the number of responders progressively increased as the number of therapies increased only in the LIPUS group but not in the placebo group. Reproduced with permission from.[21]

donepezil, galantamine hydrobromide, rivastigmine, and memantine hydrochloride.[21]

*Exclusion criteria.*

- Unable to receive the LIPUS therapy for 20 min as determined by a principal or sub investigator
- Unable to undergo brain MRI examination as determined by a principal or sub investigator
- Consciousness disorder with the Glasgow Coma Scale (GCS) below 12 at screening
- Symptomatic cerebral infarction or bleeding within 12 weeks before registration
- Modified Hachinski Ischemic Scale greater than 5 at screening
- Lewy body dementia or frontotemporal lobe dementia
- Severe mental illness as evaluated by a principal or sub investigator
- Unable to participate in the trial due to uncontrolled severe systemic illness (e.g., heart failure, liver failure, kidney failure, vitamin 12 deficiency, hypothyroidism) as determined by a principal or sub investigator
- Uncontrolled diabetic retinopathy (including active fundus hemorrhage)
- Malignant tumor or treatment history of malignant tumor within 5 years of registration (except resected and healed carcinoma in situ)

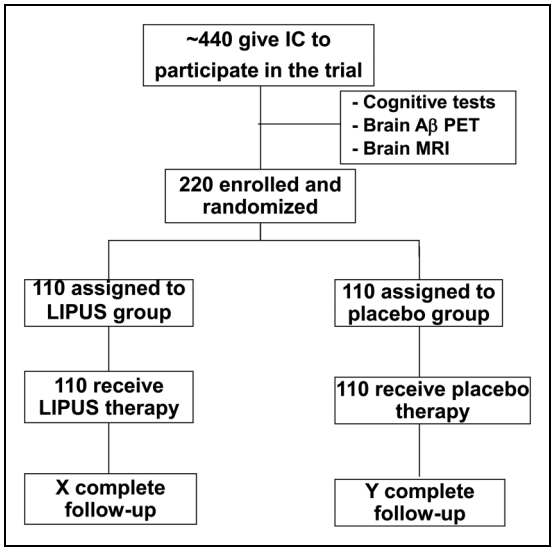


**Figure 2.** Flow chart for enrollment of subjects. Informed consent will be obtained from approximately 440 subjects so that the final number of patients registered in the trial will be 220. They will then be divided into the LIPUS or the placebo group in a blind manner in a 1:1 fashion. They will receive a total of 6 series of the LIPUS therapy or the placebo therapy with 3-month intervals.

- Drug or alcohol addiction and history of such addictions
- Epilepsy or history of epilepsy
- Intracranial implantation of artifact, such as coil, electrode, or stent
- History of neurosurgery operation including intravascular therapy within 5 years
- Pregnancy or intention of pregnancy
- Current participation in other trial except observational study
- Other conditions that a principal or sub investigator evaluate as inappropriate for the trial

## Evaluations

*Primary efficacy endpoint.* Changes in ADAS-J-cog-14 scores from baseline to week 72 after initial therapy. Main symptom of early AD is memory impairment, for which ADAS-J-cog-14 is the most sensitive test. In contrast, since decline of activities of daily living (ADL) is mild in early AD, CDR-SB for ADL is defined as a secondary efficacy endpoint.[24–26]

*Secondary efficacy endpoints.* 1) Changes in ADAS-J-cog-14 scores from baseline to week 24 and 48; 2) Changes in CDR-SB scores from baseline to week 48 and 72; 3) Changes in the Japanese version of the Neuropsychiatric Inventory Questionnaire (NPIQ-J) scores from baseline to week 24, 48, and 72; 4) Changes in Japanese version of the Zarit Burden Interview (J-ZBI) scores from baseline to week 24, 48, and 72; 5) Changes in Wechsler Memory Scale-Revised (WMS-R) scores from baseline to week 24, 48, and 72; 6) Changes in MMSE-J scores from baseline to week 24, 48, and 72; 7) Changes in Functional Activities Questionnaire (FAQ) scores from baseline to week 24, 48, and 72; 8) Changes in EuroQol 5-Dimension 5-level (EQ-5D-5L) scores evaluated by the test of temporal orientation from baseline to week 24, 48, and 72; 9) Changes in ABC dementia scales from baseline to week 24, 48, and 72; 10) Changes in each ADAS-J-cog-14 score from baseline to week 24, 48, and 72; 11) Changes in the prevalence of ADAS-J-cog-14 responders from baseline to week 24, 48, and 72 after initial therapy (especially with regard to the background of hypertension[21]); 12) Conversion rate from MCI to AD at week 72; 13) Prevalence of discontinuation of trial therapy due to worsening of dementia.[24–26]

*Exploratory efficacy endpoints.* 1) Changes in Aβ deposition in the brain evaluated by Aβ PET from baseline to week 72 after initial therapy; 2) Changes in hippocampus volume evaluated by brain MRI from baseline to week 72 after initial therapy.

*Primary safety endpoints.* 1) Prevalence of all events that cannot be ruled out in relation to the trial therapy; 2) Findings by brain MRI at week 72 after initial therapy, such as microbleeding, intracranial bleeding, brain edema, brain infarction, and other organic disorders.

## Clinical trial plan

*Trial design.* The protocol of LIPUS-AD is basically the same as in the pilot trial except Aβ PET before and after trial therapy and 6-month follow-up period (Figure 3).[21] The protocol has been approved by the IRB of each participating hospital and registered as Clinical Trial Gov. No.: NCT05983575 and jRCT No.: jRCT2032230125. LIPUS-AD is a randomized, double-blind, placebo-controlled study, in which patients with early AD (mild AD or MCI due to AD) receive a total of 6 series of the LIPUS therapy with an interval of 3 months; one series consists of 3 days of the LIPUS therapy for a total of 60 min (Figures 2 and 3).[21] There will be 3 periods in this trial, including (1) screening period, (2) trial therapy period, and (3) follow-up period (Figure 3).

1. *Screening period (week −20 to week 0).* Screening will be performed within 20 weeks before the start of trial therapy. After obtaining an informed consent, all subjects will undergo eligibility assessment, including cognitive function tests, brain Aβ-PET, and brain MRI. The results of brain Aβ-PET and brain MRI could be used when the

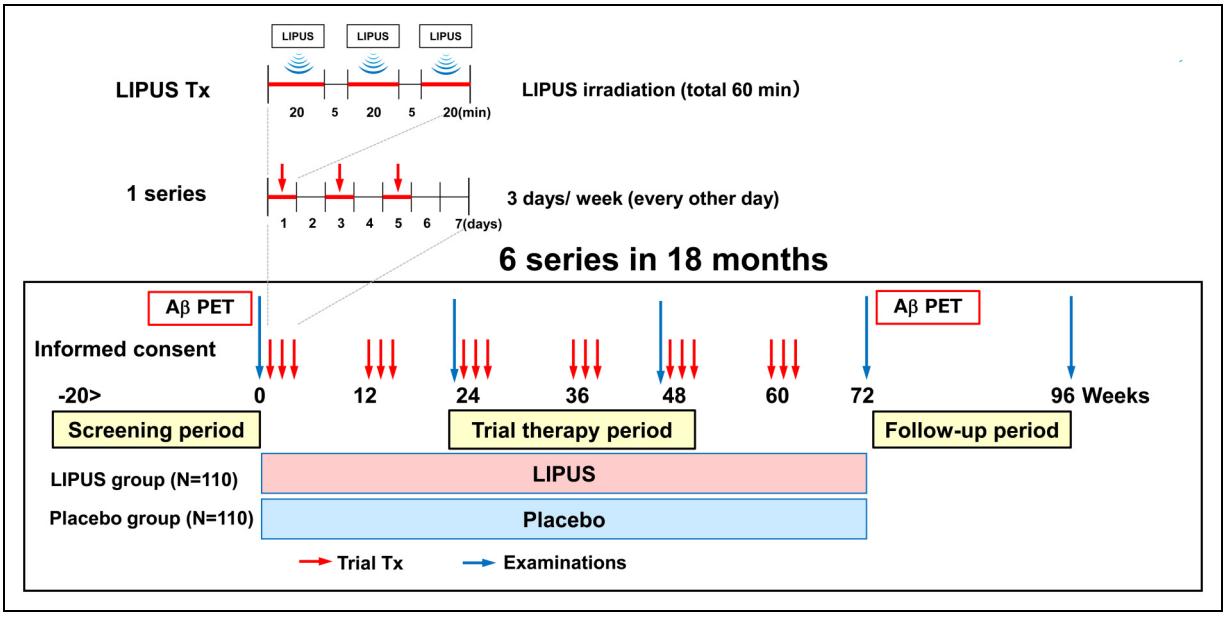


**Figure 3.** Protocol of the LIPUS-AD trial. The LIPUS group will receive a total of 6 series of the LIPUS therapy with 3-month interval, where one series consists of 3 times of the therapy a week every other day. The placebo group will receive the same procedure but without LIPUS irradiation. Before and after the trial therapy, all subjects will undergo Aβ PET and brain MRI examinations, followed by 6-month observation period.

test is performed within 48 weeks before informed consent. When the patients with mild AD or MCI due to AD are treated with drug therapy (donepezil hydrochloride, donepezil, galantamine hydrobromide, rivastigmine, and memantine hydrochloride), those drugs will be allowed to be continued from 4 weeks before informed consent to screening period.

2. *Trial therapy period (week 0 to week 72).* The subjects will undergo 6 series of trial therapy with 3-month intervals for 1.5 years (week 0, 12, 24, 36, 48, and 60). Each series consist of 3 days therapy every other day for 60 min (20 min × 3 with 5-min intervals). The trial therapy will be performed at out-patient clinic in principle. However, hospital treatment will be allowed depending on the situation. All examinations will be repeated at week 72 when 6 series of therapy are completed (Figure 3).
3. *Follow-up period (week 72 to week 96).* The subjects will be followed up for additional 6 months without trial therapy in order to address the sustained efficacy and safety (Figure 3).

*Target sample size.* In the pilot trial, the difference in the primary efficacy endpoint, changes in ADAS-J-cog-11 at week 72, between the LIPUS and the placebo groups was 3.14 (SD: 5.5).[21] Since the sample size was relatively small (N = 22), we employed the conservative policy to calculate the target sample size. Thus, as compared with the pilot trial (shown in parenthesis), we assumed that the group difference would be 2.5 (versus 3.14) and standard deviation 6.5 (versus 5.5), calculating the target sample size as follows: Detection power 70% 170 cases in total; Detection power 80% 216 cases in total; Detection power 90% 288 cases in total. We finally determined the target sample size as 220 cases (110 cases in each LIPUS and placebo group) at the detection power of 80%. Thus, informed consent will be obtained from approximately 440 subjects so that the final sample size registered in the trial will be 220 (Figure 2).

*Randomization.* The allocation ratio of the LIPUS and the placebo groups will be 1:1.[21] Allocation factors will be age (younger than 70 years old versus 70 years old or older), sex (male versus female), and primary disease (mild AD versus MCI due to AD). Allocation will be randomly performed by the stratified sorting block method. When the subject is determined as eligible for the trial, trial therapy will be started within 2 weeks after the randomization.

*Maintenance of blinding.* LIPUS-AD is a double-blind trial for both a patient and an evaluator. At each participating hospital, principal investigator nominates an open-label staff in charge, who can only know the allocation. The open-label staff only makes the setting of the LIPUS machine, either LIPUS or placebo therapy, and is not allowed to contribute to any other works. When the blind staff operates the LIPUS machine, the allocation information will not be shown on the machine for the maintenance of blinding.

## Investigational treatment

*Trial equipment.* The LIPUS machine consists of main body, convex transducers, and head-set (Figure 4). The main body consists of a power switch, connecting terminal, USB memory (data input/output), DC input connection terminal, and touch display. The convex transducers and head-set consist of transducers (ultrasonic transmission), cables, connectors, holding belt, and attachment part.

*Treatment with the LIPUS machine.* After having a check for vital signs and physical findings, the subject assumes supine or sitting position. Then, the operator has the subject wear a fixed headset. Those things that could interfere LIPUS irradiation will not be allowed to wear, such as glasses, wig, earrings, hairpins, and hearing aids, while those that do not interfere will be allowed to wear, such as dentures, dental implants, contact lens, intraocular lens, prosthetic eye, and makeup. After applying acoustic medium (jelly), the operator will fix the probes to the bilateral temporal bones of the subject and starts trial treatment by pressing start button; in the LIPUS group, irradiation conditions are as follows; wave number 32, frequency 0.5 MHz, surface sound pressure 1.3 MPa, pulse pressure, 0.25 W/cm$^2$, duty cycle 5%, and pulse repetition time 1.28 ms (pulse repetition frequency 781 Hz) (Figure 4).[21] The placebo group will be treated in the same manner as in the LIPUS group but without LIPUS irradiation. The LIPUS therapy is non-invasive and appropriate for double-blind manner as it causes no pain or noise. The trial treatment consists 3 times of 20-min therapy with a 5-min interval and this treatment will be repeated 3 times a week as one series (Figure 3).

*Combined equipment and concomitant drugs.* Pacemaker cannot be used together. Drugs prohibited for concomitant use and restricted concomitant use drugs are shown in Supplemental Table 1. In principle, drugs used at the time of registration should be continued throughout the trial without changes. Similarly, cognitive function training and day care services used at the time of registration should be continued throughout the trial without changes.

## Schedule of visit, evaluation, and observation during the trial

The schedules of visit, evaluation, and examination are shown in Table 1 and Supplemental Table 2.

*Items of evaluation.* We will perform 9 cognition tests, including ADAS-J-cog-14, CDR-SB, MMSE-J, WMS-R, NPIQ-J, J-ZBI, FAQ, EQ-5D-5L, and ABC dementia

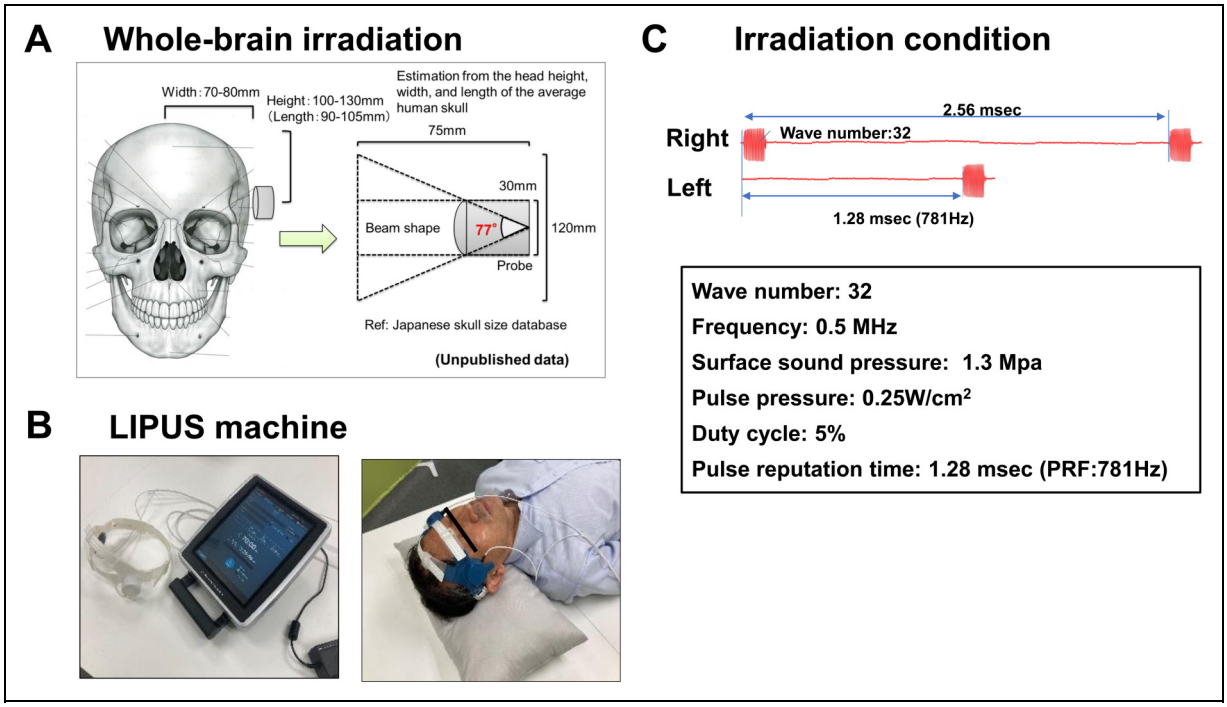


**Figure 4.** LIPUS therapy conditions and equipment. (A) Whole-brain irradiation. Based on the preliminary experiments, we will employ the whole-brain irradiation strategy with convex transducer of 77-degree angle. (B) LIPUS machine. The LIPUS machine consists of main body, convex transducers, and head-set. (C) Irradiation conditions. LIPUS is irradiated alternatively from bilateral temporal bones in the specific conditions, including frequency, 0.5 MHz, intensity, 1.3 MPa, wave number, 32, and pulse reputation time, 1.28 ms (PRF:781 Hz).

**Table 1.** Schedule of visit and evaluation.

| Date | Informed consent −20 weeks ~ 0 days | Screening[d] −20 weeks ~ 0 days | Registration −2 weeks ~ 0 days | Series 1 0 week | Test 1 4 week | Series 2 12 week | Series 3 24 week | Series 4 36 week | Series 5 48 week | Series 6 60 week | Test 2 72 week | Discontinuation - | Follow up 96 week |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Tolerance (weeks) | — | — | — | 0 | +1 | ±1[f] | ±1[f] | ±1[f] | ±1[f] | ±1[f] | ±1 | +2 | +1 |
| Obtaining consent | X | | | | | | | | | | | | |
| Registration | | | X | | | | | | | | | | |
| Investigational treatment[a] | | | | X | | X | X | X | X | X | | | |
| Subject background | | X | | | | | | | | | | | |
| Confirmation of inclusion and exclusion criteria | | X | | | | | | | | | | | |
| Vital signs[a] | | X | | X | X | X | X | X | X | X | X | X | X |
| Pregnancy test | | X | | | | | | | | | | | |
| Physical examination[a] | | X | | X | X | X | X | X | X | X | X | X | X |
| GCS score | | X | | | | | | | | | | | |
| MHIS | | X | | | | | | | | | | | |
| ADAS-J-cog-14[a] | | X | | | | | X | | X | | X | △[g] | X |
| CDR global score / sum of boxes[a,b] | | X | | | | | | | X | | X | △[g] | X |
| MMSE-J score[a] | | X | | | | | X | | X | | X | △[g] | X |
| WMS-R[a] | | X | | | | | X | | X | | X | △[g] | X |
| NPIQ-J[a] | | X | | | | | X | | X | | X | △[g] | X |
| J-ZBI[a] | | X | | | | | X | | X | | X | △[g] | X |
| FAQ[a] | | X | | | | | X | | X | | X | △[g] | X |
| EQ-5D-5L | | X | | | | | X | | X | | X | △[g] | X |
| ABC dementia scale | | X | | | | | X | | X | | X | △[g] | X |
| CAM[c] | | | | △ | △ | △ | △ | △ | △ | △ | △ | △ | △ |
| Malfunction information | | | | | | ◄ | ——— | ——— | ——— | ——— | ——— | ► | |
| Adverse event assessment | | | | | ◄ | ——— | ——— | ——— | ——— | ——— | ——— | ► | |
| Concomitant medication and combination therapy survey | | | | ◄ | ——— | ——— | ——— | ——— | ——— | ——— | ——— | ► | |
| Lab tests Blood test[a] | | X | | | | | X | | | | X | △[g] | |
| *APOE* poly-morphisms | | X | | | | | | | | | | | |
| Resting 12-lead ECG[a] | | X | | | | | | | | | | | |
| Head MRI examination[a,e] | | X[f] | | | | | | | | | X | △[g] | |
| Amyloid PET[h] | | X | | | | | | | | | X | △[g] | |

(continued)

**Table 1.** Continued.

| | Informed consent | Screening[d] | Registration | Series 1 | Test 1 | Series 2 | Series 3 | Series 4 | Series 5 | Series 6 | Test 2 | Discontinuation | Follow up |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | −20 weeks | −20 weeks | −2 weeks | | | | | | | | | | |
| | ~ | ~ | ~ | 0 | 4 | 12 | 24 | 36 | 48 | 60 | 72 | | 96 |
| Date | 0 days | 0 days | 0 days | week | week | week | week | week | week | week | week | - | week |
| Tolerance (weeks) | — | — | — | 0 | +1 | ±1[f] | ±1[f] | ±1[f] | ±1[f] | ±1[f] | ±1 | +2 | +1 |
| Blood biomarkers | | X | | | | | | | | | X | △[g] | |

X: Required, △: When possible
[a]For a detailed schedule of the clinical trial treatment period, please refer to the examination and observation schedule for the clinical trial treatment period.
[b]At screening, global score and box sum will be evaluated. At the start of study treatment 5, examination 2 and discontinuation, only box sum will be evaluated.
[c]Only perform this if an adverse event suspected to be delirium occurs, and do not perform it after recovery.
[d]If the patient is taking prohibited concomitant medication, the screening test will be performed after the 4-week washout period. However, if the patient is taking anti-Parkinson's disease medication, the washout period will be 12 weeks. The screening test will be performed between −8 weeks and 0 days after the start of the first study treatment.
[e]Results from the date of consent up to 48 weeks prior to the date of consent can be used. 3DMRA should only be performed at screening, and it is preferable to also take images at 72 weeks if possible.
[f]For investigational treatments 2 to 6, this is the acceptable range for the date on which the relevant investigational treatment is to be started.
[g]In the event of cancellation, carry out the event if possible.
hResults can be used up to 48 weeks prior to the date of consent.

scale, where changes in ADIS-J-cog-14 at week 72 is the primary efficacy endpoint, while those in other tests at week 24, 48, and 72 are the secondary efficacy endpoints.[24–26]

*APOE ϵ4 (central examination).* We will examine the *APOE* ϵ4 polymorphism to investigate the relationship between the SNP and the response to LIPUS therapy.[27]

*Brain MRI.* We will perform brain MRI before and after the trial therapy for 1.5 years in order to address the presence or absence of brain microbleeding, intracranial hemorrhage, brain edema, and brain infarction and the temporal change in hippocampus volume.[28]

*Brain amyloid PET.* We will perform brain amyloid PET before and after the trial therapy in the same conditions, including PET machine, imaging conditions, and radioactive drug.[29] The images of brain MRI and PET will be evaluated by the independent imaging committee with 3 experienced radiologists.

## Safety evaluation

*Laboratory test results.* The principal and sub investigator evaluate the changes in the laboratory tests compared with the baseline values, based on the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE v4.0) and investigate, if any, the relationship to the trial therapy.

*Adverse events.* Adverse events are defined as all undesirable or unexpected events related and/or unrelated to the trial therapy. Those events are classified as serious and non-serious ones. The relationship of adverse events will be classified into the following 3 judgement standard: related, non-related, and unclear.

*Malfunctions of the trial machine.* All malfunctions of the trial machine, including technical issues, will be recorded on electronic report form (eCRF) and will be dealt with appropriately within 24 h. When needed, the principal investigator should report the events to the PMDA (Pharmaceuticals and Medical Devices Agency).

## Biomarkers (ancillary studies)

In this pivotal trial, 20 ml of peripheral blood will be collected before and after the trial and the serum will be kept frozen for future analysis of possible biomarkers, including Aβ, phosphorylated tau, and molecules related to neurodegeneration and inflammation, etc.

## Data review and data management

*Data monitoring at participating institutes.* During the trial, monitoring staff will regularly visit the participating institutes, examining the medical record, recording to eCRF, and compliance to the clinical trial implementation plan. The principal investigator at each institute should keep all the data related to the trial subjects.

*Data collection.* The principal investigator, sub investigators, and collaborators with approved access to the eCRF, fill in the EDC system (Viedoc system). Data inconsistency will be automatically checked by the validation program and will be informed to the investigators before sending them to CRO.

*Database management and quality control.* The CRO will confirm the accuracy of the data recorded in the eCRF by the principal investigator, sub investigators, and collaborators with approved access to the eCRF. The database will be coded according to the WHO Drug Reference List (Anatomical Therapeutic Chemical classification system). Concomitant treatment technique, non-pharmacological therapy, and adverse events will be coded using the MedDRA (Medical Dictionary for Regulatory Activities).

## Statistical analysis

*Analysis set.* Full analysis set (FAS) includes all subjects who underwent investigational therapy (LIPUS or placebo) at least once and is analyzed for effective analysis. Per protocol set (PPS) is a subgroup of FAS subjects who meet all the clinical implementation plan. Safety assessment set (SAS) is a group of subjects who receive the investigational therapy at least once for safety assessment.

*Analysis of main variables.* Main variable (primary efficacy endpoint) is the temporal change in ADAS-J-cog-14 from baseline to week 72 in the FAS subjects. Reference analysis is performed for the PPS subjects. The primary analysis will involve plotting the change over time from baseline in ADAS-J-cog-14 at 72 weeks after the first treatment, as well as least-squares means, between-group differences in the means, and interval estimates (95% confidence intervals). If the lower limit of the two-sided 95% confidence interval of the between-group difference in the mean is greater than 0, the active treatment will be deemed more effective than placebo treatment. The significance level of the test will be two-sided 5%.

To estimate the mean, between-group differences in the mean, and confidence intervals, a mixed-effects model with repeated measures reference analysis (MMRM) will be used. The change from baseline in the ADAS-J-cog-14 total score will be the outcome variable, and the explanatory variables of the model will include the treatment group, time point, interaction between the treatment group and time point, age, sex, and underlying disease. An unstructured covariance matrix will be used to model within-subject error.

*Sensitivity analysis.* As a supplementary analysis, the primary endpoint will be analyzed for the FAS as follows: 1) The change from baseline to week 72 in the ADAS-J-cog-14 total score imputed by the last observation carried forward (LOCF) method will be analyzed using t-tests. The estimated mean change difference will be shown with 95% confidence intervals and p-values.; 2) Means, mean differences, and confidence intervals will be estimated using a mixed-effects repeated measures model (MMRM) with the change from baseline to week 72 in the ADAS-J-cog-14 total score imputed by the last observation carried forward (LOCF) method as the outcome variable. The model will consider treatment group, time point, interaction between treatment group and time point, age, sex, and underlying disease as explanatory variables. An unstructured covariance matrix will be used to model within-subject error. The p-values obtained in the supplementary analysis will be descriptive in nature. Subgroup analyses will be performed as necessary.

*Analysis of secondary variables.* Secondary variable (secondary efficacy endpoint) are analyzed in the FAS subjects as in the main variables. Reference analysis will also be performed for the PPS subjects.

*Responder analysis.* For ADAS-J-cog-14, responders will be analyzed at 24, 48, and 72 weeks after the first treatment. Those who showed a decrease in change from baseline that exceeded a threshold will be defined as responders, and the rest as non-responders. The time course of the responder rate will be plotted and the rate estimated. The thresholds will be set to "0", "−4", "−7", and "−10", and the number of cases and rate will be calculated for each threshold and compared between groups. The treatment effect will be estimated by longitudinal data analysis using a GEE logistic regression model. The binary outcome variable will be "responder" or "non-responder", and the explanatory variables will include treatment group, time point, interaction between treatment group and time point, age, sex, and underlying disease. An unstructured covariance matrix will be used to model within-subject error.

*Conversion rate from MCI due to AD to mild AD.* Conversion rate from MCI to mild AD (CDR global score 1.0) at week 72 after the first treatment will be analyzed.

*Discontinuation rate due to cognitive worsening.* The discontinuation rate and number of subjects due to cognitive worsening will be analyzed between groups.

*Analysis of exploratory efficacy endpoints.* The exploratory endpoints, including the changes at week 72 in Aβ deposition by PET and hippocampal volume by brain MRI, will be examined as in the major variables.

*Analysis of safety endpoints.* The safety endpoints that cannot be ruled out in relation to the trial therapy during the trial and follow-up period will be reviewed with SAS with regard to severity, outcome, and organs involved. Brain MRI findings at week 72 (e.g., microbleeding, cerebral hemorrhage, cerebral edema, and other organ damage) will also be reviewed with SAS.

## Discussion

In this pivotal LIPUS-AD trial, based on our pilot trial,[21] we will address the efficacy and safety of our LIPUS therapy for the treatment of early AD (mild AD and MCI due to AD). To the best of our knowledge, LIPUS-AD is the first non-pharmacological trial to address whether the LIPUS therapy is effective and safe for early AD

The protocol of this pivotal trial is basically the same as in the pilot trial.[21] The differences from the pilot trial are that only subjects with positive Aβ PET will be included in the pivotal trial with additional Aβ PET also performed at the end of the trial therapy and that 6-month follow-up period will be added after the 18-momth LIPUS therapy. The former point further secures the trial protocol so that only Aβ-positive patients with early AD are included as they are likely to have AD progression[30] and that the relationship between the effects of the LIPUS therapy and the extent of reduction in Aβ deposition can be evaluated. The latter point addresses the natural course of AD after cessation of the LIPUS therapy, an important point in the clinical settings.

For the efficacy of the LIPUS therapy, in addition to the cognitive function tests, the responder analysis will provide further insights into the therapy. Indeed, in the pilot trial, as the LIPUS therapy number increased, the number of responders also increased with their final prevalence of approximately 50%, suggesting the dose-response relationship in the LIPUS therapy for early AD.[21] Furthermore, our experimental findings suggest that our LIPUS therapy is effective for both AD and vascular dementia.[19] Considering the fact that elderly patients with AD have more or less vascular pathology,[31] our LIPUS therapy may exerts neuroprotective effects for those patients through up-regulation of eNOS and related molecules.[17–20] Additionally, it is possible that our LIPUS therapy also exerts beneficial effects on neurovascular unit in the brain, which has been reported to play an important role in cognitive functions.[32]

For the safety, there were no LIPUS-related adverse events in the pilot trial.[21] Indeed, the sound pressure used in this trial is within the range of sound pressure of abdominal or cardiac echography.[21,33,34] We will confirm the safety of the LIPUS therapy in this pivotal trial as well.

The LIPUS therapy appears to have multiple future indications as it ameliorates microcirculatory disorders caused by chronic inflammation, including dementia in the brain, heart failure in the heart, and chronic kidney disease in the kidney.[15] The therapeutic strategy to up-regulate eNOS in the target organ is important as it not only enhances endothelial production of NO but also ameliorates the balance between NO and endothelium-derived hyperpolarizing factor (EDHF) in microvasculatures.[10]

If the efficacy and safety of the LIPUS therapy are confirmed in this LIPUS-AD trial, it is expected that a new era of non-pharmacological therapy will be opened for the treatment of early AD. Also, the importance of vascular pathology in the pathogenesis of early AD will be re-confirmed.

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### Statements and declarations

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## Supplemental material

Supplemental material for this article is available online.

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