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Safety and efficacy of plasma transfusion from exercise-trained donors in patients with early Alzheimer's disease: protocol for the ExPlas Study

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ABSTRACT

Introduction

Given that exercise training reduces the risk of developing Alzheimer's disease (AD), induces changes in the blood composition and has widespread systemic benefits, it is reasonable to hypothesize that exercised plasma may have rejuvenative properties. The main objective is to test safety and tolerability of transfusing exercised plasma (ExPlas) from young, healthy, fit adults to patients with mild cognitive impairment or early AD. The study is a pilot for a future efficacy study. The key secondary objectives are examining the effect of plasma transfusions on cognitive function, fitness level, vascular risk profile, assessment of cerebral blood flow and hippocampal volume, quality of life, functional connectivity assessed by resting state functional MRI and biomarkers in blood and cerebrospinal fluid.

Methods and analysis

ExPlas is a double-blinded, randomized controlled clinical single center trial. Patients aged 50-75 years with diagnosis mild cognitive impairment or early AD will be recruited from two Norwegian hospitals. ExPlas is plasma drawn by plasmapheresis once a month for 4 months, from a total of 30 donors (aged 18-40, BMI ≤27 kg/m2 and VO2max >50 mL/kg/min). All units will be virus inactivated by the Intercept method in accordance with procedures at St. Olavs Hospital. Comparison with isotonic saline allows differentiation from a non-blood product. The main study consists of 6 rounds of examinations in addition to 12 plasma transfusions divided over three 4-weeks periods during study year-1. Follow-up examinations after 2 and 5 years after baseline is also planned.

Ethics and dissemination

Written informed consent will be obtained from all participants and participation is voluntary. All participants have a next of kin who will follow them throughout the study and represent the patient's interest. The study is approved by the Regional Committee for Medical and Health Research Ethics (REK 2018/702) and the Norwegian Medicines Agency (EudraCT No. 2018-000148-24). The study will be published in a leading clinical journal and results will be presented at numerous national and international meetings as well as on social media platforms.

STRENGHT AND LIMITATIONS OF THIS SUDY

- First double blinded, randomized controlled clinical phase II trial to examine safety and explore therapeutic effects of "exercised blood" in 60 AD patients
- Relatively long follow-up (up to 5 years) in patients diagnosed with AD according to the IWG-2 criteria, making the study group homogenous
- Active participation in study design, recruitment and dissamination by a user board consisting of next kin of present and past AD patients
- ExPlas is innovative and potentially groundbreaking if it is found to be safe with few side effects and with similar promising results as seen in preclinical AD models
- Uncertainties in the assumptions for power calculation and in addition to being a safety study, ExPlas must be regarded as a pilot for a future efficacy study

BACKGROUND

The forecast of about 2 billion people being above the age of 60 by the year 2050 (1) implies an expected increased prevalence of Alzheimer's disease (AD) from today's 36 million to 108 (2-4). New estimates from Norway show a higher prevalence of dementia and mild cognitive impairment (MCI) in both younger adults and elderly than previous calculations (5, 6). Recent American data show that deaths from AD increased by 145% during the last two decades (7); for comparison, deaths from heart disease decreased by 7.3% (7). During the Covid-19 pandemic, deaths from AD or other dementias have additionally increased by 16% from that expected based on previous years (7). As of 2021 there is no proven cure for AD (8-10) and the World Health Organization has stated that AD is a global crisis that requires a global solution. Without intervention, the expected rise in AD adds a major burden to public health and health care costs globally.

It is hypothesized that around 40-50% of dementia cases worldwide are caused by modifiable risk factors (11, 12), and that many factors associated with higher risk of cardiovascular disease, such as obesity, hyperlipidaemia, hypertension, smoking, diabetes and physical inactivity are also associated with increased risk of AD (12-20). These factors have in common that they can be substantially modified through physical activity that secures above average age- and sex-specific levels of cardiorespiratory fitness (CRF) (12, 19, 21-23) measured as peak oxygen uptake ($PeakVO_2$). In line with this we demonstrated in a prospective cohort study of 30 695 adults that participants who increased or sustained high $PeakVO_2$ over time (10 years apart) had 40-50% reduced risk of incident dementia, 30-40% reduced risk of dementia-related mortality, approximately 2.0 years delayed dementia onset, and 2-3 years of life gained when compared to persistently unfit individuals (24). Thus, at present exercise training leading to a high age-relative $PeakVO_2$ may be the most promising preventive "AD-medicine" (25, 26).

Although it is well established that exercise positively influences brain neurogenesis, plasticity (27, 28), and cognition (21, 27, 29) it is not well understood how these effects are mediated. The beneficial effects of exercise on the brain have traditionally been thought not to be mediated through systemic changes (30). However, a number of studies in both rodents and humans (31-33) demonstrates direct effects on the brain of exercised induced blood-born molecules crossing the the blood-brain barrier (34). For instance, systemic administration of blood from young mice into old mice counteracts age-related changes in the brain (35, 36). Furthermore, direct evidence of beneficial effects of young blood treatment for preserving brain health has been provided in two different mouse models of AD (37, 38), suggesting beneficial effects also in the AD brain. Of particular interest, a small clinical trial (39) reported that plasma from young donors transfused to patients with mild cognitive impairment or early AD is safe and possibly beneficial. An exploratory endpoint analysis showed improvements on functional abilities, although no changes were found in global cognition, mood, or functional connectivity.

Although evidence suggests beneficial effects of *young blood* treatment in *aged* animals, less is known about the effects of *exercised blood* treatment in the aging or *diseased* brain. A recent study demonstrated that administration of blood from exercised, *aged* donor mice into sedentary, *aged* mice conferred beneficial effects of exercise on hippocampal neurogenesis and cognition (40). Given that exercise training reduces the risk of AD development, induces changes in the blood composition and has widespread systemic benefits, it is reasonable to hypothesize that exercised plasma may have rejuvenative properties similar to, or stronger than young blood. In this study, we aim to test the safety profile of transfusing exercised plasma from young, healthy adults in AD patients, and perform a pilot test for potential therapeutic effects.

ENDPOINTS

The purpose of this study is to explore the safety of transfusion of plasma from exercise trained donors (ExPlas) compared to Octaplasma®, a commercially available virus inactivated plasma product pooled from approximately 1000 donors, and Sodium Chloride 0.9% (saline) in patients in the early symptomatic phase of AD and provide pilot data regarding efficacy. An additional aim is to provide advancements to the field by exploring therapeutical effects on AD of blood-borne factors.

Primary endpoint of ExPlas

Proportion of patients with adverse events after 1 year as a measure of safety and tolerability, and number of subjects who comply with the research protocol as a measure of feasibility.

Secondary endpoints of ExPlas after 1, 2 and 5 years

- Change in performance in the CERAD (The Consortium to Establish a Registry for Alzheimer's Disease) Ten word Test
- Change in the Mini-Mental State Examination Score
- Change in performance in Trail-Making test A and B
- Change in scores in other cognitive tests: the Clock Drawing Test, Controlled Oral Word Association Test (COWAT)-FAS, Visual Object and Space Perception (VOSP) Silhouettes
- Change in Clinical Dementia Rating Scale Global score and Sum of Boxes, and The Lawton Instrumental Activities of Daily Living Scale (IADL)
- Change in performance in the 6-minutes walk-test
- Change in/Reduced hippocampal atrophy and preservation of functional connectivity assessed by resting state functional MRI
- Change in score of quality-of-Life SF-36 Questionnaire
- Change in biomarkers in blood and cerebrospinal fluid
- Change in cardiac dimensions, volumes and functional indices

Hypothesis, primary outcome:

I) ExPlas transfusions to patients in early symptomatic phase of AD is safe

Hypotheses secondary outcome:

II) ExPlas transfusions to patients in early symptomatic phase of AD ameliorates the biomarker-profile in blood and cerebrospinal fluid, structural- and functional MRI, cognitive function, functional capacity, fitness, and quality of life

METHODS

Design

The study is a double blinded, randomized controlled clinical phase II trial. The estimated date of first patient enrolled and randomized is September 15th, 2021. The anticipated recruitment period is 36 months and the estimated date of last patient enrolled is September 1th, 2024. The treatment duration is 1 year, follow-up period: 61 months, including screening. There are 3 study arms with patient ratio 1:1:1, stratified by *APOE* genotype. The flow chart of the ExPlas Study is given in Figure 1.

Settings and participants – Plasmapheresis and Cardiopulmonary testing (donors)

Exercised plasma collected by plasmapheresis from male donors who have not themselves received plasma will be used in the study. The rationale for these selection criteria is that women may have developed antibodies during pregnancies and men may have developed antibodies during plasma transfusions. Thus, selection reduces the risk of antibody-induced transfusion complications. All donors have been recruited from the existing donor corps at St. Olavs Hospital Blood Bank. Donors must fulfill all requirements in the Norwegian laws and guidelines for blood donors.

Potential donors will perform a cardiopulmonary exercise test to voluntary exhaustion on a treadmill (PPS55 Med, Woodway GmbH, Germany) with continuous gas and minute ventilation analysis using the Cortex MetaMax II (Cortex Biophysik Gmbh, Leipzig, Germany). The individualized steady-state test protocol starts at a speed and inclination that will be defined during a 15-minute warm-up. The first stage of the test will be held for three minutes, or longer until steady state is reached. Thereafter, speed is increased by 1 km/h every minute until maximal oxygen uptake is reached, defined as flattening of oxygen uptake despite increased workload. Test procedure has been described in detail previously (41). Plasma donation will be performed within one month after the cardiopulmonary test.

Donor inclusion criteria:

- Healthy male donors
- Age 18-40 years
- BMI ≤27 kg/m^2
- Maximal oxygen uptake ≥50 mL/kg/min
- Already an approved donor at the St. Olav's Hospital Blood Bank

Donor exclusion criteria:

- Injury or other incident preventing regular exercise during the last month
- Previous recipient of blood transfusion
- PeakVO2 ≤50 mL/kg/min

Settings and participants - Patients

Patients will be recruited from the Department of Neurology or Geriatrics out-patient clinics at St. Olavs Hospital, and from the Department of Geriatric psychiatry, Levanger Hospital, both Norway. The diagnostic work up of the patient will decide if the patient is eligible for inclusion. Eligible patients who sign informed consent to join the study go through a further screening and are evaluated regarding the defined inclusion and exclusion criteria.

Patient inclusion criteria:

Patients will be included in the study if they meet **all the following criteria**:

- Signed informed consent
- Age 50-75 years
- Diagnosis AD in early phase according to the IWG-2 criteria (42)
- In-vivo evidence of Alzheimer´s pathology (one of the following):
  - Decreased Aβ42 together with increased t-tau or p-tau in CSF
  - Increased tracer retention on amyloid PET
- Mini-Mental State Examination (MMSE) Score ≥20
- Availability of a next of kin who knows the patient well and is willing to accompany the subject to all trial visits and give information about the patient's functional level
- The patient is judged fit for the study and capable to cooperate in treatment and follow-up.
- Ability to communicate in Norwegian or another Scandinavian language

Patient exclusion criteria:

Patients will be excluded from the study if they meet **any of the following criteria**:

- Pregnancy or unwilling to use adequate birth control for the duration of and 6 months beyond study participation. Defined according to Clinical Trial Facilitation Group document "Recommendations related to contraception and pregnancy testing in clinical trials"
- Positive for Hepatitis B, Hepatitis C or HIV at screening
- Not qualified to give consent at inclusion
- Any other condition judged to interfere with the safety of the patient or the intent and conduct of the study

Related to medical history:

- Stroke
- Anaphylaxis
- Prior adverse reaction to any human blood product
- Any history of a blood coagulation disorder or hypercoagulability
- Congestive heart failure, defined as any previous heart failure hospitalization, or current symptomatic heart failure in New York heart Association class ≥II with reduced, mid-range or preserved ejection fraction
- Coagulation defect or hypercoagulopathy
- Uncontrolled hypertension
- Renal failure
- Prior intolerance to intravenous fluids
- Recent history of uncontrolled atrial fibrillation
- Bone marrow transplant
- IgA deficiency
- Severe protein S deficiency
- Thrombocytopenia (platelets < 40 x 10^9/L)
- Contraindication for Octaplasma

Related to medications or other treatments:

- Any concurrent use of anticoagulant therapy, clopidogrel or acetylsalicylic acid/dipyridamole in combination
- Initiation or change in the dosage of a acetylcholine esterase inhibitor (AChEI) or memantine during the trial (week 0-52). Participants will be urged to start on AChEI when diagnosis is communicated, and must be on a stable dose for at least one month prior to screening
- Concurrent participation in another treatment trial for AD. If there was prior participation, the last dose of the investigational agent must have been given at least 6 months prior to screening, except if the patient received placebo medication
- Treatment with any human blood product, including intravenous immunoglobulin, during the 6 months prior to screening or during the trial
- Concurrent daily treatment with benzodiazepines, typical or atypical antipsychotics, long-acting opioids, or other medications that are judged to interfere with cognition. Intermittent treatment with short-acting benzodiazepines or atypical antipsychotics may be permitted, provided that no dose is administered within 72 hours prior to cognitive assessment

Related to magnetic resonance imaging:

- Claustrophobia
- Any metallic surgical implant, like a pacemaker or clip incompatible with MRI

Certain metallic implants like joint prostheses may be permitted, provided that specific manufacturer specifications are available, and that the device is known to be safe for 7T MRI. In case a patient is not eligible for the 7T scanner, the 3T scanner will be used

Treatment and examinations

The main study consists of 6 rounds of examinations in addition to plasma transfusions, mainly during the time span of one year, and once 2 years after baseline. A follow-up visit is also planned 5 years after baseline.

Treatment

For this study, ExPlas (plasma from fit donors) and Octaplasma are defined as Investigational Medicinal Products. ExPlas is plasma drawn by plasmapheresis once a month for 4 months, from a total of 30 donors (aged 18-40, BMI ≤27 kg/m^2 and VO_{2max} >50 mL/kg/min). All units will be virus inactivated by the Intercept method (CERUS corporation, US), in accordance with the instructions from the manufacturer and the procedures at the Blood Bank at St. Olavs Hospital.

Octaplasma is human pooled plasma produced by Octapharma (Lachen, Switzerland). The rationale for including Octaplasma is to separate the effect of ExPlas from the "general untrained" plasma pooled from thousands of donors. Placebo for this study is isotonic saline (0.9% sodium chloride) (Fresenius Kabi, Halden, Norway). Comparison with isotonic saline allows differentiation from a non-blood product. ExPlas and Octaplasma are stored at ≤-18°C until the time of transfusion. The transfusion volume will be 200 mL at every time point for all three treatments.

Cognitive test battery

All tests will be undertaken at baseline, weeks 24, 52 and 104 after inclusion, and performance at all time points will be evaluated against baseline values.

CERAD Ten-word test will be used as a measure of objective evidence of an amnestic syndrome of the hippocampal type (43).

Mini-Mental State Examination Score – (MMSE-NR-3) will be used as a screening tool for cognitive function (44). The test consists of standardized questions within five areas: orientation for time and place, short-term memory, attention, short-term recall and language. The test may help to evaluate degree of cognitive impairment. The maximum score is 30 (45).

Trail-Making test A and B – (TMT-NR3) will be used to measure visual attention, processing speed and executive function (46, 47).

Clock Drawing Test is a cognitive screening tool and will be used as a supplement for examining visuospatial function and executive function (48).

COWAT-FAS. The Controlled Oral Word Association Test (COWAT)-FAS will be used to measure verbal fluency and executive function (49, 50).

VOSP - Visuospatial abilities will be evaluated with the silhouettes test from the Visual Object and Space Perception Battery. The test also assesses semantic memory and name retrieval (51, 52).

Clinical Dementia Rating Scale (CDR) is a clinical scale for the staging of dementia. The participant is rated from 0-3 on six cognitive and behavioral categories: memory, orientation, judgement and problem solving, community affairs, home and hobbies, and personal care. The Global score is calculated according to an established algorithm, where memory is considered the primary category and all others are secondary categories. A global score of 0 equals no dementia, 0,5 questionable dementia, 1 mild dementia, 2 moderate dementia and 3 severe dementia (53, 54). The Sum of Boxes score is a continuous measure of dementia severity and ranges from 0-18. The CDR Sum of Boxes are found to be adequate for use in prodromal AD and continued use is warranted and recommended in

clinical trials because it is continuous and provides a greater variation in values (45). Both the CDR-Global score and Sum of Boxes will be calculated.

The Lawton Instrumental Activities of Daily Living Scale (IADL). This IADL scale evaluate eight items, related to complex everyday activities, and each can be scored 0 that equals "dependent" and 1 that reflect "completely independent". Change from 0 to 1 in any of the eight items is considered a "clinically relevant change" (55).

Unified Parkinson's Disease Rating Scale (UPDRS). The motor examination part of UPDRS will be used to detect development of parkinsonian motor signs, such as tremor, rigidity, bradykinesia, and postural-gait abnormalities (56).

The 6-minute walk test

Fitness level will be measured using the 6 minute walk-test which is a good alternative to direct measurement of PeakVO_2 (57). The 6-minute walk test is considered safer for the current patient group than a treadmill test.

Structural- and functional MRI

For increased sensitivity we will use multiparameric MRI at 7T MRI to assess brain structure and function to uncover both neurodegenerative and cerbrovasular changes from baseline to 1, 2 and 5 years. The 7T protocol consists of the following scans: whole brain 3D MP2RAGE, gradient echo SWI/Quantitative susceptibility mapping (QSM), and FLAIR, high resolution T2 weighted spin echo sequence of the medial temporal lobe, multishell DTI, asl FLAIR, and rs-fMRI.The primary outcomes are hippocampal and entorhinal cortex structure ((subfield)-volume/thickness, diffusion characteristics, T2*-mapping) and function (blood flow, connectivity and BOLD signal). Other quantitative measures include brain morphometry (e.g., parenchymal fraction, at-risk AD pattern volume loss), and diffusion metrics in white and grey matter. Cerebrovascular pathology (perfusion, white matter hyperintensities, perivascular spaces (quantitative from mulitshell DTI), microhaemorrhages, micro- and macro- infarction) will be evaluated. For participants where 7T is contraindicated, but not 3T, a similar examination will take place using 3T.

A secondary aim is to identify any effect of treatment group on MRI markers of both neurodegenerative and cerebrovascular disease. MRI at 7T allows for a stronger signal and better anatomical localization in less time, but with the stronger magnetic field there are also more contraindications.

Quality of Life

Quality of life will be evaluated by the questionnaire SF-36, which contains questions about physical and mental health, pain, vitality and general health perceptions (58, 59).

Echocardiography

All patients will undergo echocardiography examination at screening and four times during the first year, and potentially at 2 and 5-years follow up. Screening echo is performed to ensure safety of transfusions for patients included in the study. Patients with reduced cardiac function will be excluded due to risk of developing heart failure triggered by fluid overload. Screening echocardiography serve as baseline for included patients. Following echocardiography will be performed at week 5 (one week after 4 weeks of transfusion treatment), week 20 (before treatment period two), week 48 (before treatment period three), week 52 (one week after 4 weeks of transfusion treatment), and assumedly at 2 and 5-years follow up. The echocardiography examination will be a complete examitation of cardiac structure and function, including ultrahigh framerate recordings at each time point.

Biomarkers in blood and spinal fluid

Although no single ideal biomarker yet exists for AD, there are substances currently considered to be 'core' biomarkers for AD. According to a landmark paper published in 2017 from the joint proceedings of the International Working Group (IWG) and the American Alzheimer's Association, the most important early indicators of incipient AD are changes in two key proteins: reduced amyloid-beta 1-42 (Aβ42) and increased total tau protein and hyperphosphorylated tau measured biochemically in cerebrospinal fluid (CSF), or increased deposition of amyloid plaque and neurofibrillary tangles of tau protein in brain as shown by PET. Since PET is exceptionally expensive, we plan to analyse these substances in CSF. Collected cerebrospinal fluid and blood will be analysed for established AD risk markers (including APOE genotyping in Blood and Amyloid Beta 1-42, Amyloid Beta 1-40, phosphor tau and total tau in spinal fluid). Individuals apparently without clinical symptoms of cognitive decline but with pathological levels of both these biomarkers are considered to have "preclinical AD". If only one of the biomarkers is found to have a pathological level, the individual is considered to be "at risk of AD" (the difference being that the individual is only at risk of AD, whereas preclinical AD is considered to be an early manifestation of the disease itself). In order to better understand the potential link between the cardiovascular system and the brain, the collected blood will also be analysed with respect to cardiovascular profile (Albumin, Ferritin, Natrium, Kalium, Kreatinin, Glukose, ALAT, GT, Kolesterol, Triglyserider, HDL, Hs-CRP, NT-proBNP Troponin, Leukocytter, Trombocytter, HB, HbA1c). Some of the biological material will be stored for future analysis in the search for new biomarkers. For instance, the study group has previously identified potential "fitness-microRNAs" that could distinguish high- and low-fitness individuals (60). In the ExPlas Study we aim (as a start) to detect microRNAs that show a significant change in expression concomitant with ExPlas treatment and examine changes in level in relation to speed of AD progression. Unbiased screenings of microRNAs will be performed at the NTNU Genomics Core Facility and analysed together with the NTNU Bioinformatics Core Facility. Identification of other circulating factors will be performed by gel-free shotgun proteomics analysis. Proteomic RNA analyses will be performed at the NTNU Proteomics and Modomics Experimental Core Facility and analysed together with the NTNU Bioinformatics Core Facility.

Blood sampling procedures

All blood samples will be taken by trained biomedical engineer or nurse. Serum and plasma samples are collected with venous puncture using sterile disposable equipment in serum-gel, EDTA plasma and litsium heparine tubes. The tubes are centrifuged and stored on ice while shipped to further handling and analysis. If not analysed right away, samples are to be stored at -80ºC (in the established Trønderbrain biobank, Director of the biobank is Geir Bråthen; geir.brathen@ntnu.no, tel. 72575077). Blood tests will be taken on 7 occasions in addition to screening and after 2 years as well as offered 5 years after baseline (Figure 1).

Spinal puncture procedures

Lumbar puncture will be performed by neuorolgist. A thin needle is inserted into the spinal canal in the lowerback, while the patient is lying down on the side. The procedure is done using sterile technique (the area is cleaned with chlorhexidine and air dried before inserting the needle). The sample is collected directly into polypropylene tubes (used for dementia markers), and stored on ice until shipped to further handling. The samples are to be stored at -80ºC (Trønderbrain biobank, Geir Bråthen) until analysis. The sample will be analysed for risk genes and AD related biomarkers. Some portion of the sample will be stored for future analysis. Spinal puncture will be performed at baseline, week 24, week 52, after 2 years, and offered 5 years after baseline (Figure 1). All sample collection, handling and analysis will be performed in accordance with hospital/laboratory standard procedures.

Sample size and statistics

We do not expect that ExPlas will cause more adverse effects than Octaplasma, but have made the following considerations about power calculations related to safety. The most common reaction to transfusion of Octaplasma is allergy and occurs in up to 1% of the elderly. If we consider that ExPlas would cause a dramatic increase in allergic reactions, of e.g. 35% vs. 1% after Octaplasma treatment, 19 participants will be needed in each group to show significant differences (with alpha 0.05 and power of 0.8). There are substantial uncertainties in the assumptions for this power calculation. But considering that another study found that transfusing plasma from young donors to patients (n=18) with mild cognitive impairment or early AD was safe with no adverse events (39) we find it likely that 20 patients in each group will be enough to test safety in ExPlas.

The magnitude of a possible treatment effect of ExPlas is currently not established. The following information has been established: i) a difference of 2 points on the Mini-Mental State Examination Score (MMSE) primarily between those receiving ExPlas vs. Octaplasma will be clinically relevant after 1 and 2 years; ii) Based on several clinical studies in this population, we expect an average MMSE-NR-3 of about 24 in our population, iii) Based on previous studies, we expect a standard deviation of MMSE-NR-3 score of approximately 3. These assumptions suggest that 17 participants are needed in each group (with alpha 0.05, and power 0.8) to demonstrate a clinically relevant effect of ExPlas. Given potential dropouts, it is reasonable to include 20 in each group. The plan is therefore to include 60 patients in the study.

For the primary endpoints, counts will be reported and compared using recommended methods for analysis of contingency tables (56). Secondary and other endpoints will be analyzed using mixed models with the outcome variable as dependent variable, treatment group, time and their interaction as categorical covariates, and patient as random effect. In these analyses, we will adjust for the baseline value of the outcome variable, as recommended (61, 62).

Ethics

The study will be performed according to the Declaration of Helsinki. Written informed consent will be obtained from all participants and participation is voluntary. Patients will be insured according to Norwegian regulations for patients involved in medical research (npe.no). The patients' abilities to keep track of the objectives of the project and assess its relevance will progressively deteriorate during the project period. In view of this, all participants are required to include a next of kin who will follow them throughout the study and represent the patient's interest. The burden from participation, number of tests and time points of conducting tests during the study have been planned in dialogue with the user group consisting of three next of kin of current and previous AD-patients. The study is approved by the Regional Committee for Medical and Health Research Ethics (REK 2018/702) and the Norwegian Medicines Agency (Statens Legemiddelverk, EudraCT No. 2018-000148-24).

Organization

The steering committee of ExPlas has developed the study protocol and is responsible for overall study management, data collection, analyses, publications, and the final data set.

A safety committee consisting of two clinicians (one neurologist and one specialist in Transfusion Medicine) has been appointed to ensure the safety of study participants. In case of adverse events, the safety committee will evaluate whether treatments can continue or must be stopped. A study nurse will observe the patients during and for one hour after infusion and a physician will evaluate the patients in case of adverse effects. Neither the safety committee nor the attending physician responsible for each infusion are involved in other parts of the study and they will not be blinded for the treatment given.

Study monitors

The primary goal of the study monitors is to ensure that the site follows the standardized operation procedures described for the trial, and to report and manage any deviations that may occur from the investigational plan. The ExPlas Study has been appointed two study monitors by the Unit for Applied Clinical research at NTNU, one who has the overall overview of the study, and is blinded to the treatment randomization, and one who is unblinded. A study monitoring plan has been developed and includes regular visits by the Clinical Study Monitors (headed by Torbjørn Øvreness, Torbjorn.Ovreness@stolav.no), who will check the following:

- Informed consent process
- Reporting of adverse events and all other safety data
- Adherence to protocol
- Maintenance of required regulatory documents
- Study Supply accountability
- Facilities and equipments (treatment storage and manufacturing at the Blood Bank)
- Data completion on the CRFs including source data verification (SDV).

The monitor will review the relevant CRFs for accuracy and completeness and will ask the site staff to adjust any discrepancies as required. Sponsor's representatives (e.g., monitors, auditors) and/or competent authorities will be allowed access to source data for source data verification in which case a review of those parts of the hospital records relevant to the study will be required.

Data management

The Clinical Data Management System (CDMS) used for the electronic case report forms (eCRF) in this study is The Unit for Applied Clinical Research at NTNU. The setup of the study specific eCRF in the CDMS will be performed by The Unit for Applied Clinical Research at NTNU. The eCRF system will be FDA Code of Federal Regulations 21 Part 11 compliant. The designated investigator staff will enter the data required by the protocol into the eCRF. The Investigator is responsible for assuring that data entered into the eCRF is complete, accurate, and that entry is performed in a timely manner. The signature of the investigator will attest the accuracy of the data on each eCRF. If any assessments are omitted, the reason for such omissions will be noted on the eCRFs. Corrections, with the reason for the corrections will also be recorded. After database lock, the investigator will receive a digital copy of the subject data for archiving at the investigational site.

The medical records of each patient will clearly describe at least:

- That the patient is participating in the study, by including the enrollment number and the study code or other study identification;
- Date when Informed Consent was obtained from the patient and statement that patient received a copy of the signed and dated Informed Consent;
- Results of all assessments confirming a patient's eligibility for the study;
- Diseases (past and current; both the disease studied and others, as relevant);
- Surgical history, as relevant;
- Treatments withdrawn/withheld due to participation in the study;
- Results of assessments performed during the study;
- Treatments given, changes in treatments during the study and the time points for the changes;
- Visits to the clinic / telephone contacts during the study, including those for study purposes only;
- Non-Serious Adverse Events and Serious Adverse Events (if any) including causality assessments;
- Date of, and reason for, discontinuation from study treatment;
- Date of, and reason for, withdrawal from study;
- Date of death and cause of death, if available;
- Additional information according to local regulations and practice.

Data management will be performed by the Unit for Applied Clinical Research at NTNU (Berit Bjelkåsen). The Data management procedures will be performed in accordance with the department's SOPs and ICH guidelines. The data management process will be described in the study specific data handling plan and the study specific data handling report after database closure. Data entered into the eCRF will be validated as defined in the data validation plan. Validation includes, but is not limited to, validity checks (e.g. range checks), consistency checks and customised checks (logical checks between variables to ensure that study data are accurately reported) for eCRF data and external data (e.g. laboratory data). A majority of edit checks will be triggered during data entry and will therefore facilitate efficient 'point of entry' data cleaning. Data management personnel will perform both manual eCRF review and review of additional electronic edit checks to ensure that the data are complete, consistent and reasonable. The electronic edit checks will run continually throughout the course of the study and the issues will be reviewed manually online to determine what action needs to be taken. Manual queries may be added to the system by clinical data management or study monitor. Clinical data managers and study monitors are able to remotely and proactively monitor the patient eCRFs to improve data quality. All updates to queried data will be made by authorised study centre personnel only and all modifications to the database will be recorded in an audit trail. Once the queries have been resolved, eCRFs will be signed by electronic signature Any changes to signed eCRFs will be approved and resigned by the Investigator. Once the full set of eCRFs have been completed and locked, the Sponsor will authorise database lock and all electronic data will be sent to the designated statistician for analysis. Subsequent changes to the database will then be made only by written agreement. The data will be stored in a dedicated and secured area at NTNU. Data will be stored in a de-identified manner, where each study participant is recognisable by his/her unique trial subject number. The data will be stored until 15 years after database lock.

Patients and public involvement

To ensure a high study quality and relevance a user board consisting of three next kin of present and past AD patients has been established. As we conduct research on a patient group that is considered vulnerable, this board is particularly important. We have met the user group on several occasions while working on the study protocol (first meeting in Feb 2017) and have received input on several matters, such as how to ensure a tolerable load of participation for the individual patient/relatives. The board will continue to consult the study team twice annually throughout the study period, on implementation, results, and future developments. They are encouraged to give their opinions regarding the project as a whole and particularly on the patients' well-being. The study group has already made changes and adjustments in the planned protocols based on feedback from the user group. The study has a user representative who participates in meetings and presentations of the study to the general public. On initiative from the ExPlas user group, we are currently making three information videos about "AD and participation in research studies", for AD patients and their families, where patients and their next of kin tell their story to help new patients and their next of kin in the coming process. These videos will also be used in the recruitment phase of the study to inform and motivate to take part in ExPlas.

Dissemination

Direct communication with users and patient organisations: ExPlas Study group regularly present at various meetings of patient organizations (such as the National Association for Public Health) and for senior citizens' societies. This type of contact with the public, patients and relatives has proven mutually useful. We plan to intensify participation in such meetings to inform about current knowledge about prevention and treatment of AD, particularly via the established user groups. Communication via Internet: One of the most important media for spreading the news and awareness will be the Internet. The results and information (including videos) about the studies will be presented on CERG's webpage (ntnu.edu/cerg), one of the most visited sites at Scandinavian universities; and ExPlas's own Norweian webpage (ntnu.no/cerg/explas). Scientific and non-scientific communication: General

communication activities include publication in open access peer reviewed journals, non-scientific journals and at national- and international meetings, to reach the general public, patients, scientists, and policy makers. Importantly, our group is closely linked and active partners in the Norwegian Research School in Neuroscience, Physical Activity and Health (master program) and medical education where we actively will present our research to the next generation of health care personnel and scientists. We also have a journalist at CERG (Anders Revdal), available for ExPlas, who will be responsible for communication.

DISCUSSION

To our knowledge, ExPlas will be the first study with the aim of studying safety and efficacy of transfusion of plasma from well endurance trained donors to patients in the early symptomatic phase of AD. Even if prevention probably will be the most effective way to reduce numbers of patients with AD worldwide, we need a cure for this devastating disease which impacts the lives for both patients and their families substantially. There is also a need to understand the mechanisms behind the beneficial effect of physical exercise on the brain, and it seems logically to try to exploit this effect in treatment of the early phase of symptomatic AD.

On June 7th, 2021, the U.S. Food and Drug Administration, approved aducanumab (marketed as Aduhelm) for use in treatment of AD (63), due to its ability to reduce amyloid plaques in the brain, under an accelerated approval pathway (64). Confirmation of the clinical benefit is still required to be confirmed for continued approval (63). Independent of the usefulness of aducanumab in AD therapeutics, other interventions capable of delaying the clinical onset of AD dementia should continue to be studied. The findings from preclinical AD models (36-38, 65), and a small clinical trial (39) clearly indicate that there is communication between the systemic environment and the hippocampus. Systemic factors are capable of inducing changes, and even therapeutic effects, in the brain without hindrance by the blood-brain barrier; potentially an effective strategy to reverse neurodegeneration in the AD-brain (30). There are myriads of factors and processes that are set in motion during and after exercise training, and much of this is reflected in the composition of the blood (66). Thus, it is not likely that it is a single factor is orchestrating the beneficial effects of exercise, but rather an interplay between several molecular factors that need to be discovered and understood to develop the first-generation of exercise-mimicking drugs. This is a promising idea as a large population of patients are simply unable to exercise, such as patients in the intensive care unit, the critically ill, patients recovering from accidents, the morbidly obese and paralyzed patients. For these patients, innovative exercise-mimicking therapies could be of benefit.

However, development of exercise-mimicking therapies is a very complex and time-consuming undertaking, that should not delay the testing of a potential benefit of exercise trained plasma, with most of its natural components, on safety and therapeutic effect in patients with AD. In the context of lack of disease-modifying treatments, ExPlas is innovative and potentially groundbreaking if it is found to be safe with few side effects and with similar promising results as seen in preclinical AD models (35-38).

Another key question is at what stage of AD interventions such as ExPlas treatment can be expected to have an effect. Today we know that AD-related changes in the brain are present 10-30 years before symptoms develop. The optimal time window for treatment is probably as early as possible during this period, to decelerate or stop the progressive loss of neurons before functions are lost. As in all diseases, prevention will always be the optimal path. Depending upon outcomes in the ExPlas Study, a natural next step may be treatment with ExPlas of patients in the preclinical phase of the disease (67).

A small clinical trial found that plasma from young donors (young blood) transfused to patients with mild to moderate AD dementia (MMSE score ranging from 12-24) was safe with no adverse events and possibly beneficial with improvement in functional activity. In this study, 9 patients were randomized to a cross-over cohort, receiving 4 once-weekly infusions of either 250 mL of plasma from male donors (aged 18-30 years) or 250 mL of saline, followed by a 6-week washout and crossover to 4 once-weekly infusions of the alternate treatment. In addition, 9 patients were included in an open-label design in which patients received 4 once-weekly infusions of only young plasma. Considering the low number of patients, short follow-up period and promising findings in the study by Sha *et al.* (39) there is reason to believe that transfusion of exercise-trained plasma also is safe. With increased treatment periods and extended follow-up, we believe the ExPlas Study is well designed also to evaluate the potential therapeutic effect of exercise trained plasma. The relatively large number of patients will also likely enable us to assess whether endpoints become differentially affected by *APOE* 4 status. As the ExPlas Study is the first of its kind it is not straightforward to undertake power calculations, and the results of our study may be useful for planning of an appropriately sample sized study in the future.

In conclusion, we expect the ExPlas study to give new knowledge about whether transfusion of plasma from exercise-trained donors is safe and indications on whether it has therapeutic effects. ExPlas will also contribute to pioneering the discovery of molecular targets to potentially treat AD and lay the foundation for first-generation exercise-mimicking drugs, by capturing the molecular signature of high-fitness and molecular mechanisms provided by exercise.

Contributor statement

Atefe R. Tari Conception and design of the study, obtained funding, drafting the manuscript and applications to etchical committee and Norwegian Medicine Agency for study approval

Helene H. Berg Conception of the study, drafting the manuscript, critical review of manuscript, applications to etchical committee and Norwegian Medicine Agency for study approval

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Håvard Dalen Critical review of the manuscript, established echocardiographic procedures for AD patients in ExPlas, supervision, obtained funding

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Geir Selbæk Conception and design of the study, critical review of the manuscript, supervision, obtained funding, applications to etchical committee and Norwegian Medicine Agency for study approval

Stian Lydersen Conception and design of the study, critical review of the manuscript, supervision, obtained funding, applications to etchical committee and Norwegian Medicine Agency for study approval, making the statistical plan and analyses in ExPlas

Emrah Duzel Critical review of the manuscript, supervision, established methods and operating procedures for the study, supervision, obtained funding

Sverre Bergh Conception and design of the study, critical review of the manuscript, supervision, obtained funding, applications to etchical committee and Norwegian Medicine Agency for study approval

Kjell Rune Halvorsrud Critical review of the manuscript, supervision, established methods and operating procedures for the blood-donor part of ExPlas, supervision, obtained funding

Sigrid B. Sando Conception and design of the study, critical review of the manuscript, supervision, obtained funding, applications to etchical committee and Norwegian Medicine Agency for study approval, Co-PI of ExPlas

Ulrik Wisløff Conception and design of the study, critical review of the manuscript, supervision, obtained funding, applications to etchical committee and Norwegian Medicine Agency for study approval, Co-PI of ExPlas

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Competing interests

None of the authors reports any competing interests or had financial relationships with any organisations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work. All authors will complete the ICMJE uniform disclosure form in due time.

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Authors reports no financial relationships with any organisations that might have an interest in the submitted work in the previous three years, or any other relationships or activities that could appear to have influenced the submitted work.

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Data sharing

We are not permitted to share individual data from the current trial, but we are open to collaborative research with researchers worldwide, who can have access to analysed data from our university. We have also established a biobank of blood and genetic material that we plan to share with researchers worldwide, but individual data must be analysed within our university only.

References

1. World Health Organization. Aging and Life Course 2015 [Available from: http://www.who.int/ageing/en/.
2. Associaton As. Alzheimer's Disease Facts and Figures. Report. 2018 2018.
3. Andrieu S, Coley N, Lovestone S, Aisen PS, Vellas B. Prevention of sporadic Alzheimer's disease: lessons learned from clinical trials and future directions. Lancet Neurol. 2015;14(9):926-44.
4. Helsedirektoratet d.
5. Gjøra L, Strand BH, Bergh S, Borza T, Brækhus A, Engedal K, et al. Current and Future Prevalence Estimates of Mild Cognitive Impairment, Dementia, and Its Subtypes in a Population-Based Sample of People 70 Years and Older in Norway: The HUNT Study. J Alzheimers Dis. 2021;79(3):1213-26.
6. Kvello-Alme M, Bråthen G, White LR, Sando SB. The Prevalence and Subtypes of Young Onset Dementia in Central Norway: A Population-Based Study. J Alzheimers Dis. 2019;69(2):479-87.
7. Association As. 2021 Alzheimer's Disease Facts and Figures. 2021.
8. Potter PE. Investigational medications for treatment of patients with Alzheimer disease. J Am Osteopath Assoc. 2010;110(9 Suppl 8):S27-36.
9. Mullane K, Williams M. Alzheimer's therapeutics: continued clinical failures question the validity of the amyloid hypothesis-but what lies beyond? Biochem Pharmacol. 2013;85(3):289-305.
10. Cummings JL, Morstorf T, Zhong K. Alzheimer's disease drug-development pipeline: few candidates, frequent failures. Alzheimers Res Ther. 2014;6(4):37.
11. Livingston G, Huntley J, Sommerlad A, Ames D, Ballard C, Banerjee S, et al. Dementia prevention, intervention, and care: 2020 report of the Lancet Commission. Lancet. 2020;396(10248):413-46.
12. Barnes DE, Yaffe K. The projected effect of risk factor reduction on Alzheimer's disease prevalence. Lancet Neurol. 2011;10(9):819-28.
13. Colcombe SJ, Erickson KI, Raz N, Webb AG, Cohen NJ, McAuley E, et al. Aerobic fitness reduces brain tissue loss in aging humans. The journals of gerontology. 2003;58(2):176-80.
14. de Bruijn RF, Ikram MA. Cardiovascular risk factors and future risk of Alzheimer's disease. BMC Med. 2014;12:130.
15. Levine DA, Langa KM. Vascular cognitive impairment: disease mechanisms and therapeutic implications. Neurotherapeutics. 2011;8(3):361-73.
16. Rovio S, Kareholt I, Helkala EL, Viitanen M, Winblad B, Tuomilehto J, et al. Leisure-time physical activity at midlife and the risk of dementia and Alzheimer's disease. The Lancet Neurology. 2005;4(11):705-11.
17. Qiu C, Xu W, Fratiglioni L. Vascular and psychosocial factors in Alzheimer's disease: epidemiological evidence toward intervention. J Alzheimers Dis. 2010;20(3):689-97.
18. Qiu C. Preventing Alzheimer's disease by targeting vascular risk factors: hope and gap. J Alzheimers Dis. 2012;32(3):721-31.
19. Exalto LG, Quesenberry CP, Barnes D, Kivipelto M, Biessels GJ, Whitmer RA. Midlife risk score for the prediction of dementia four decades later. Alzheimer's & dementia : the journal of the Alzheimer's Association. 2014;10(5):562-70.

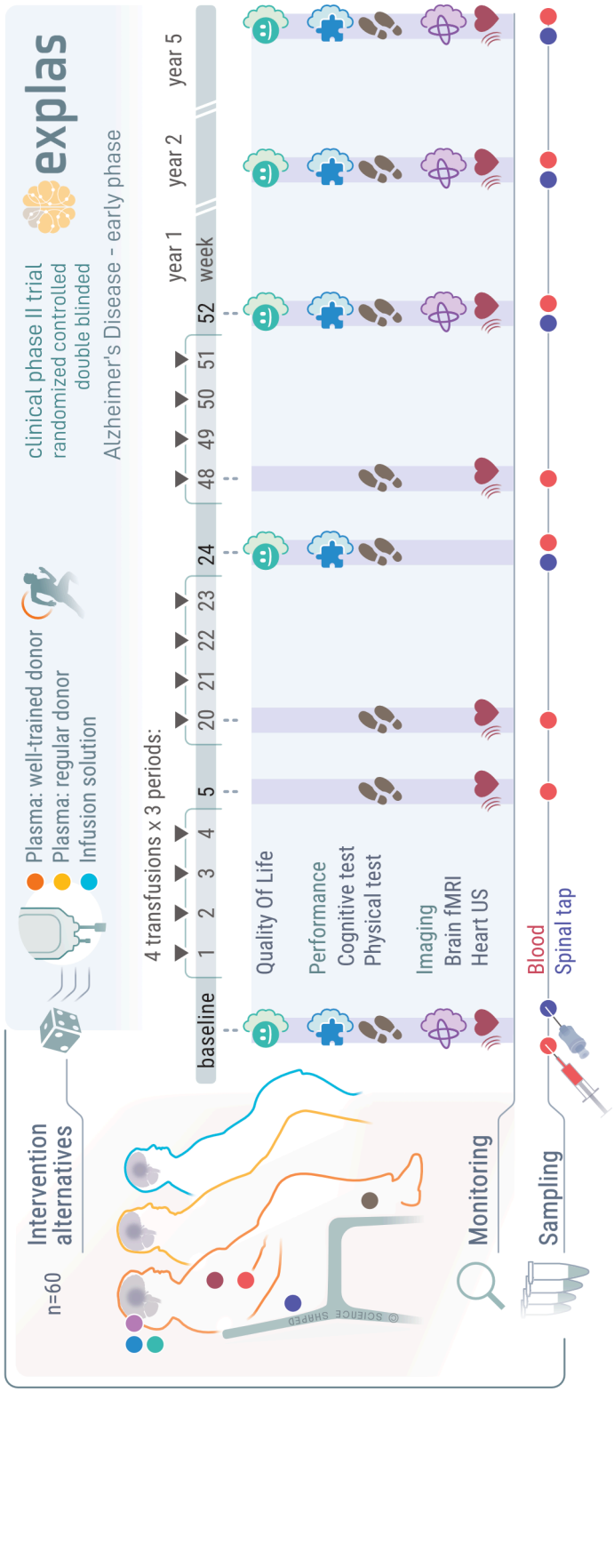
20. Tolppanen AM, Solomon A, Soininen H, Kivipelto M. Midlife vascular risk factors and Alzheimer's disease: evidence from epidemiological studies. J Alzheimers Dis. 2012;32(3):531-40.
21. Norton S, Matthews FE, Barnes DE, Yaffe K, Brayne C. Potential for primary prevention of Alzheimer's disease: an analysis of population-based data. Lancet Neurol. 2014;13(8):788-94.
22. Kaminsky LA, Arena R, Beckie TM, Brubaker PH, Church TS, Forman DE, et al. The importance of cardiorespiratory fitness in the United States: the need for a national registry: a policy statement from the American Heart Association. Circulation. 2013;127(5):652-62.
23. Larson EB, Wang L, Bowen JD, McCormick WC, Teri L, Crane P, et al. Exercise is associated with reduced risk for incident dementia among persons 65 years of age and older. Ann Intern Med. 2006;144(2):73-81.
24. Tari AR, Nauman J, Zisko N, Skjellegrind HK, Bosnes I, Bergh S, et al. Temporal changes in cardiorespiratory fitness and risk of dementia incidence and mortality: a population-based prospective cohort study. The Lancet Public Health. 2019;4(11):e565-e74.
25. Hoffmann K, Frederiksen KS, Sobol NA, Beyer N, Vogel A, Simonsen AH, et al. Preserving cognition, quality of life, physical health and functional ability in Alzheimer's disease: the effect of physical exercise (ADEX trial): rationale and design. Neuroepidemiology. 2013;41(3-4):198-207.
26. Lautenschlager NT, Cox KL, Flicker L, Foster JK, van Bockxmeer FM, Xiao J, et al. Effect of physical activity on cognitive function in older adults at risk for Alzheimer disease: a randomized trial. Jama. 2008;300(9):1027-37.
27. van Praag H, Shubert T, Zhao C, Gage FH. Exercise enhances learning and hippocampal neurogenesis in aged mice. J Neurosci. 2005;25(38):8680-5.
28. van Praag H, Christie BR, Sejnowski TJ, Gage FH. Running enhances neurogenesis, learning, and long-term potentiation in mice. Proc Natl Acad Sci U S A. 1999;96(23):13427-31.
29. Maass A, Duzel S, Goerke M, Becke A, Sobieray U, Neumann K, et al. Vascular hippocampal plasticity after aerobic exercise in older adults. Molecular psychiatry. 2015;20(5):585-93.
30. Bouchard J, Villeda SA. Aging and brain rejuvenation as systemic events. J Neurochem. 2015;132(1):5-19.
31. Pereira AC, Huddleston DE, Brickman AM, Sosunov AA, Hen R, McKhann GM, et al. An in vivo correlate of exercise-induced neurogenesis in the adult dentate gyrus. Proc Natl Acad Sci U S A. 2007;104(13):5638-43.
32. Fabel K, Fabel K, Tam B, Kaufer D, Baiker A, Simmons N, et al. VEGF is necessary for exercise-induced adult hippocampal neurogenesis. The European journal of neuroscience. 2003;18(10):2803-12.
33. Castellano JM, Mosher KI, Abbey RJ, McBride AA, James ML, Berdnik D, et al. Human umbilical cord plasma proteins revitalize hippocampal function in aged mice. Nature. 2017;544(7651):488-92.
34. Tari AR, Norevik CS, Scrimgeour NR, Kobro-Flatmoen A, Storm-Mathisen J, Bergersen LH, et al. Are the neuroprotective effects of exercise training systemically mediated? Progress in cardiovascular diseases. 2019.
35. Villeda SA, Plambeck KE, Middeldorp J, Castellano JM, Mosher KI, Luo J, et al. Young blood reverses age-related impairments in cognitive function and synaptic plasticity in mice. Nat Med. 2014;20(6):659-63.

36. Katsimpardi L, Litterman NK, Schein PA, Miller CM, Loffredo FS, Wojtkiewicz GR, et al. Vascular and neurogenic rejuvenation of the aging mouse brain by young systemic factors. Science. 2014;344(6184):630-4.
37. Xia E, Xu F, Hu C, Kumal JPP, Tang X, Mao D, et al. Young Blood Rescues the Cognition of Alzheimer's Model Mice by Restoring the Hippocampal Cholinergic Circuit. Neuroscience. 2019;417:57-69.
38. Zhao Y, Qian R, Zhang J, Liu F, Iqbal K, Dai CL, et al. Young blood plasma reduces Alzheimer's disease-like brain pathologies and ameliorates cognitive impairment in 3×Tg-AD mice. Alzheimers Res Ther. 2020;12(1):70.
39. Sha SJ, Deutsch GK, Tian L, Richardson K, Coburn M, Gaudioso JL, et al. Safety, Tolerability, and Feasibility of Young Plasma Infusion in the Plasma for Alzheimer Symptom Amelioration Study: A Randomized Clinical Trial. JAMA Neurol. 2018.
40. Horowitz AM, Fan X, Bieri G, Smith LK, Sanchez-Diaz CI, Schroer AB, et al. Blood factors transfer beneficial effects of exercise on neurogenesis and cognition to the aged brain. Science. 2020;369(6500):167-73.
41. Aspenes ST, Nilsen TI, Skaug EA, Bertheussen GF, Ellingsen Ø, Vatten L, et al. Peak oxygen uptake and cardiovascular risk factors in 4631 healthy women and men. Med Sci Sports Exerc. 2011;43(8):1465-73.
42. Dubois B, Feldman HH, Jacova C, Hampel H, Molinuevo JL, Blennow K, et al. Advancing research diagnostic criteria for Alzheimer's disease: the IWG-2 criteria. Lancet Neurol. 2014;13(6):614-29.
43. Morris JC, Heyman A, Mohs RC, Hughes JP, van Belle G, Fillenbaum G, et al. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part I. Clinical and neuropsychological assessment of Alzheimer's disease. Neurology. 1989;39(9):1159-65.
44. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res. 1975;12(3):189-98.
45. McDougall F, Edgar C, Mertes M, Delmar P, Fontoura P, Abi-Saab D, et al. Psychometric Properties of the Clinical Dementia Rating - Sum of Boxes and Other Cognitive and Functional Outcomes in a Prodromal Alzheimer's Disease Population. J Prev Alzheimers Dis. 2021;8(2):151-60.
46. Reitan RM. Validity of the Trail Making Test as an indication of organic brain damage. Perceptual and motor skills. 1958;8(3):271-6.
47. Espenes J, Hessen E, Eliassen IV, Waterloo K, Eckerström M, Sando SB, et al. Demographically adjusted trail making test norms in a Scandinavian sample from 41 to 84 years. Clin Neuropsychol. 2020;34(sup1):110-26.
48. Brodaty H, Moore CM. The Clock Drawing Test for dementia of the Alzheimer's type: A comparison of three scoring methods in a memory disorders clinic. Int J Geriatr Psychiatry. 1997;12(6):619-27.
49. Nutter-Upham KE, Saykin AJ, Rabin LA, Roth RM, Wishart HA, Pare N, et al. Verbal fluency performance in amnestic MCI and older adults with cognitive complaints. Arch Clin Neuropsychol. 2008;23(3):229-41.
50. Benton ALH, K. deS. Multilingual Aphasia Examination: AJA Associates; 1989.
51. Elizabeth K Warrington MJ. Visual Object and Space Perception Battery (VOSP)1991.
52. Binetti T. Disorders of visual and spatial perception in the early stage of Alzheimers disease1996.
53. Hughes CP, Berg L, Danziger WL, Coben LA, Martin RL. A new clinical scale for the staging of dementia. Br J Psychiatry. 1982;140:566-72.

54. O'Bryant SE, Humphreys JD, Smith GE, Ivnik RJ, Graff-Radford NR, Petersen RC, et al. Detecting dementia with the mini-mental state examination in highly educated individuals. Arch Neurol. 2008;65(7):963-7.
55. Lawton MP, Brody EM. Assessment of older people: self-maintaining and instrumental activities of daily living. Gerontologist. 1969;9(3):179-86.
56. Fagerland M, Lydersen, S., & Laake, P. Statistical Analysis of Contingency Tables. Chapman and Hall/CRC2017.
57. Ries JD, Echternach JL, Nof L, Gagnon Blodgett M. Test-retest reliability and minimal detectable change scores for the timed "up & go" test, the six-minute walk test, and gait speed in people with Alzheimer disease. Phys Ther. 2009;89(6):569-79.
58. Geschke K, Fellgiebel A, Laux N, Schermuly I, Scheurich A. Quality of life in dementia: impact of cognition and insight on applicability of the SF-36. Am J Geriatr Psychiatry. 2013;21(7):646-54.
59. Ware JE, Jr., Sherbourne CD. The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. Med Care. 1992;30(6):473-83.
60. Bye A, Røsjø H, Aspenes ST, Condorelli G, Omland T, Wisløff U. Circulating microRNAs and aerobic fitness--the HUNT-Study. PLoS One. 2013;8(2):e57496.
61. J T, L B, T H, J R, M W, M H. Different ways to estimate treatment effects in randomised controlled trials. Contemp Clin Trials Commun. 2018;10:80-5.
62. Coffman CJ, Edelman D, Woolson RF. To condition or not condition? Analysing 'change' in longitudinal randomised controlled trials. BMJ Open. 2016;6(12):e013096.
63. Cavazzoni P. FDA's Decision to Approve New Treatment for Alzheimer's Disease: fda.gov; 06/07/2021 [Available from: https://www.fda.gov/drugs/news-events-human-drugs/fdas-decision-approve-new-treatment-alzheimers-disease.
64. Sevigny J, Chiao P, Bussière T, Weinreb PH, Williams L, Maier M, et al. The antibody aducanumab reduces Aβ plaques in Alzheimer's disease. Nature. 2016;537(7618):50-6.
65. Villeda SA, Plambeck KE, Middeldorp J, Castellano JM, Mosher KI, Luo J, et al. Young blood reverses age-related impairments in cognitive function and synaptic plasticity in mice. Nature Medicine. 2014;20:659.
66. Tari AR, Norevik CS, Scrimgeour NR, Kobro-Flatmoen A, Storm-Mathisen J, Bergersen LH, et al. Are the neuroprotective effects of exercise training systemically mediated? Progress in cardiovascular diseases. 2019;62(2):94-101.
67. Dubois B, Hampel H, Feldman HH, Scheltens P, Aisen P, Andrieu S, et al. Preclinical Alzheimer's disease: Definition, natural history, and diagnostic criteria. Alzheimer's & dementia : the journal of the Alzheimer's Association. 2016;12(3):292-323.

Figure text

Figure 1. Flow chart of the ExPlas Study. Infusion solution, Sodium Chloride 0.9% (Saline); US, ultrasound of the heart; fMRI, functional magnetic resonance imaging.


explas
clinical phase II trial
randomized controlled
double blinded
Alzheimer's Disease - early phase
Plasma: well-trained donor
Plasma: regular donor
Infusion solution
4 transfusions x 3 periods:
baseline 1 2 3 4 5 20 21 22 23 24 48 49 50 51 52 week
year 1 year 2 year 5
Quality Of Life
Performance
Cognitive test
Physical test
Imaging
Brain fMRI
Heart US
Blood
Spinal tap
n=60
Intervention alternatives
Monitoring
Sampling

BMJ Open

Safety and efficacy of plasma transfusion from exercise-trained donors in patients with early Alzheimer's disease: protocol for the ExPlas Study

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Safety and efficacy of plasma transfusion from exercise-trained donors in patients with early Alzheimer's disease: protocol for the ExPlas Study

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ABSTRACT

Introduction

Given that exercise training reduces the risk of developing Alzheimer's disease (AD), induces changes in the blood composition and has widespread systemic benefits, it is reasonable to hypothesize that exercised plasma may have rejuvenative properties. The main objective is to test safety and tolerability of transfusing exercised plasma (ExPlas) from young, healthy, fit adults to patients with mild cognitive impairment or early AD. The study is a pilot for a future efficacy study. The key secondary objectives are examining the effect of plasma transfusions on cognitive function, fitness level, vascular risk profile, assessment of cerebral blood flow and hippocampal volume, quality of life, functional connectivity assessed by resting state functional MRI and biomarkers in blood and cerebrospinal fluid.

Methods and analysis

ExPlas is a double-blinded, randomized controlled clinical single center trial. Patients aged 50-75 years with diagnosis mild cognitive impairment or early AD will be recruited from two Norwegian hospitals. ExPlas is plasma drawn by plasmapheresis once a month for 4 months, from a total of 30 donors (aged 18-40, BMI ≤27 kg/m2 and VO2max >55 mL/kg/min). All units will be virus inactivated by the Intercept method in accordance with procedures at St. Olavs Hospital. Comparison with isotonic saline allows differentiation from a non-blood product. The main study consists of 6 rounds of examinations in addition to 12 plasma transfusions divided over three 4-weeks periods during study year-1. Follow-up examinations after 2 and 5 years after baseline is also planned.

Ethics and dissemination

Written informed consent will be obtained from all participants and participation is voluntary. All participants have a next of kin who will follow them throughout the study and represent the patient's interest. The study is approved by the Regional Committee for Medical and Health Research Ethics (REK 2018/702) and the Norwegian Medicines Agency (EudraCT No. 2018-000148-24). The study will be published in a leading clinical journal and results will be presented at numerous national and international meetings as well as on social media platforms.

STRENGTH AND LIMITATIONS OF THIS STUDY

- First double blinded, randomized controlled clinical phase II trial to examine safety and explore therapeutic effects of "exercised blood" in 60 AD patients.
- Relatively long follow-up (up to 5 years) in patients diagnosed with AD according to the IWG-2 criteria, making the study group homogenous.
- We have an active user board, consisting of next kin of present and past AD patients, that has taken part in study design, recruitment and dissemination. We have received input on several matters, such as how to ensure a tolerable load of participation for the individual patient/relatives and made changes and adjustments in the planned protocols based on feedback from the user group. The board will continue to consult the study team throughout the study period, on implementation, results, and future developments.
- ExPlas is innovative and potentially groundbreaking if it is found to be safe with few side effects and with similar promising results as seen in preclinical AD models.
- Uncertainties in the assumptions for power calculation and in addition to being a safety study, ExPlas must be regarded as a pilot for a future efficacy study.

BACKGROUND

The forecast of about 2 billion people being above the age of 60 by the year 2050 (1) implies an expected increased prevalence of Alzheimer's disease (AD) from today's 36 million to 108 (2-4). New estimates from Norway show a higher prevalence of dementia and mild cognitive impairment (MCI) in both younger adults and elderly than previous calculations (5, 6). Recent American data show that deaths from AD increased by 145% during the last two decades (7); for comparison, deaths from heart disease decreased by 7.3% (7). During the Covid-19 pandemic, deaths from AD or other dementias have additionally increased by 16% from that expected based on previous years (7). As of 2021 there is no proven cure for AD (8-10) and the World Health Organization has stated that AD is a global crisis that requires a global solution. Without intervention, the expected rise in AD adds a major burden to public health and health care costs globally.

It is hypothesized that around 40-50% of dementia cases worldwide are caused by modifiable risk factors (11, 12), and that many factors associated with higher risk of cardiovascular disease, such as obesity, hyperlipidaemia, hypertension, smoking, diabetes and physical inactivity are also associated with increased risk of AD (12-20). These factors have in common that they can be substantially modified through physical activity that secures above average age- and sex-specific levels of cardiorespiratory fitness (CRF) (12, 19, 21-23) measured as peak oxygen uptake ($PeakVO_2$). In line with this we demonstrated in a prospective cohort study of 30 695 adults that participants who increased or sustained high $PeakVO_2$ over time (10 years apart) had 40-50% reduced risk of incident dementia, 30-40% reduced risk of dementia-related mortality, approximately 2.0 years delayed dementia onset, and 2-3 years of life gained when compared to persistently unfit individuals (24). Thus, at present exercise training leading to a high age-relative $PeakVO_2$ may be the most promising preventive "AD-medicine" (25, 26).

Although it is well established that exercise positively influences brain neurogenesis, plasticity (27, 28), and cognition (21, 27, 29) it is not well understood how these effects are mediated. The beneficial effects of exercise on the brain have traditionally been thought not to be mediated through systemic changes (30). However, a number of studies in both rodents and humans (31-33) demonstrates direct effects on the brain of exercised induced blood-born molecules crossing the the blood-brain barrier (34). For instance, systemic administration of blood from young mice into old mice counteracts age-related changes in the brain (35, 36). Furthermore, direct evidence of beneficial effects of young blood treatment for preserving brain health has been provided in two different mouse models of AD (37, 38), suggesting beneficial effects also in the AD brain. Of particular interest, a small clinical trial (39) reported that plasma from young donors transfused to patients with mild cognitive impairment or early AD is safe and possibly beneficial. An exploratory endpoint analysis showed improvements on functional abilities, although no changes were found in global cognition, mood, or functional connectivity.

Although evidence suggests beneficial effects of *young blood* treatment in *aged* animals, less is known about the effects of *exercised blood* treatment in the aging or *diseased* brain. A recent study demonstrated that administration of blood from exercised, *aged* donor mice into sedentary, *aged* mice conferred beneficial effects of exercise on hippocampal neurogenesis and cognition (40). Given that exercise training reduces the risk of AD development, induces changes in the blood composition and has widespread systemic benefits, it is reasonable to hypothesize that exercised plasma may have rejuvenative properties similar to, or stronger than young blood. In this study, we aim to test the safety profile of transfusing exercised plasma from young, healthy adults in AD patients, and perform a pilot test for potential therapeutic effects.

ENDPOINTS

The purpose of this study is to explore the safety of transfusion of plasma from exercise trained donors (ExPlas) compared to Octaplasma®, a commercially available virus inactivated plasma product pooled from approximately 1000 donors, and Sodium Chloride 0.9% (saline) in patients in the early symptomatic phase of AD and provide pilot data regarding efficacy. An additional aim is to provide advancements to the field by exploring therapeutical effects on AD of blood-borne factors.

Primary endpoint of ExPlas

Proportion of patients with adverse events after 1 year as a measure of safety and tolerability, and number of subjects who comply with the research protocol as a measure of feasibility.

Secondary endpoints of ExPlas after 1, 2 and 5 years

- Change in performance in the CERAD (The Consortium to Establish a Registry for Alzheimer's Disease) Ten word Test
- Change in the Mini-Mental State Examination Score
- Change in performance in Trail-Making test A and B
- Change in scores in other cognitive tests: the Clock Drawing Test, Controlled Oral Word Association Test (COWAT)-FAS, Visual Object and Space Perception (VOSP) Silhouettes
- Change in Clinical Dementia Rating Scale Global score and Sum of Boxes, and The Lawton Instrumental Activities of Daily Living Scale (IADL)
- Change in performance in the 6-minutes walk-test
- Change in/Reduced hippocampal atrophy and preservation of functional connectivity assessed by resting state functional MRI
- Change in score of quality-of-Life SF-36 Questionnaire
- Change in biomarkers in blood and cerebrospinal fluid
- Change in cardiac dimensions, volumes and functional indices

Hypothesis, primary outcome:

I) ExPlas transfusions to patients in early symptomatic phase of AD is safe

Hypotheses secondary outcome:

II) ExPlas transfusions to patients in early symptomatic phase of AD ameliorates the biomarker-profile in blood and cerebrospinal fluid, structural- and functional MRI, cognitive function, functional capacity, fitness, and quality of life

METHODS

Design

The study is a double blinded, randomized controlled clinical phase II trial recruiting at a single study site at St. Olavs Hospital in Norway . The first patient was enrolled and randomized on September 15th, 2021. The anticipated recruitment period is 36 months and the estimated date of last patient enrolled is September 1th, 2024. The treatment duration is 1 year, follow-up period: 61 months, including screening. There are 3 study arms with patient ratio 1:1:1 (ExPlas, Octaplasma, saline), stratified by *APOE* genotype. Electronic randomization, provided by the Unit for Applied Clinical Research at NTNU, ensures that allocation of patients to a treatment group is random. Electronic randomization is conducted by the blinded ExPlas study nurse. The results of the randomization is not visible to any member of the study group. An automated message on each patient allocation is sent directly to the study nurses who undertake transfusions. The flow chart of the ExPlas Study is given in Figure 1.

Settings and participants – Plasmapheresis, Cardiopulmonary testing and physical (donors)

Exercised plasma collected by plasmapheresis from male donors who have not themselves received plasma will be used in the study. The rationale for these selection criteria is that women may have developed antibodies during pregnancies and men may have developed antibodies during plasma transfusions. Thus, selection reduces the risk of antibody-induced transfusion complications. All donors have been recruited from the existing donor corps at St. Olavs Hospital Blood Bank. Donors must fulfill all requirements in the Norwegian laws and guidelines for blood donors.

Potential donors will perform a cardiopulmonary exercise test to voluntary exhaustion on a treadmill (PPS55 Med, Woodway GmbH, Germany) with continuous gas and minute ventilation analysis using the Cortex MetaMax II (Cortex Biophysik Gmbh, Leipzig, Germany). The individualized steady-state test protocol starts at a speed and inclination that will be defined during a 15-minute warm-up. The first stage of the test will be held for three minutes, or longer until steady state is reached. Thereafter, speed is increased by 1 km/h every minute until maximal oxygen uptake is reached, defined as flattening of oxygen uptake despite increased workload. Test procedure has been described in detail previously (41). The first plasma donation will be performed within one month after the cardiopulmonary test. To ensure that the donors sustained a high physical activity level in between the 4 donations (within 4 months from first donation) they were equipped with a wristworn heart rate monitor (Huami GTS2, Huami North America Inc, Irvine, CA, USA) and required to have a physical activity level above 100 weekly Personalized Activity Intelligence (PAI) points, to sustain a high maximal oxygen uptake, as described in detail elsewhere (42), using the Zepp mobile Application downloaded from Apple Store or Google Play.

Donor inclusion criteria:

- Healthy male donors
- Age 18-40 years
- BMI ≤27 kg/m^2
- Maximal oxygen uptake ≥55 mL/kg/min
- Already an approved donor at the St. Olav's Hospital Blood Bank

Donor exclusion criteria:

- Injury or other incident preventing regular exercise during the last month
- Previous recipient of blood transfusion
- PeakVO2 ≤55 mL/kg/min

Settings and participants - Patients

Patients will be recruited from the Department of Neurology or Geriatrics out-patient clinics at St. Olavs Hospital, and from the Department of Geriatric psychiatry, Levanger Hospital, both Norway. The diagnostic work up of the patient will decide if the patient is eligible for inclusion. Eligible patients who sign informed consent to join the study go through a further screening and are evaluated regarding the defined inclusion and exclusion criteria.

Patient inclusion criteria:

Patients will be included in the study if they meet **all the following criteria**:

- Signed informed consent
- Age 50-75 years
- Diagnosis AD in early phase according to the IWG-2 criteria (43)
- In-vivo evidence of Alzheimer´s pathology (one of the following):
- Decreased Aβ42 together with increased t-tau or p-tau in CSF
- Increased tracer retention on amyloid PET
- Mini-Mental State Examination (MMSE) Score ≥20
- Availability of a next of kin who knows the patient well and is willing to accompany the subject to all trial visits and give information about the patient's functional level
- The patient is judged fit for the study and capable to cooperate in treatment and follow-up.
- Ability to communicate in Norwegian or another Scandinavian language

Patient exclusion criteria:

Patients will be excluded from the study if they meet **any of the following criteria**:

- Pregnancy or unwilling to use adequate birth control for the duration of and 6 months beyond study participation. Defined according to Clinical Trial Facilitation Group document "Recommendations related to contraception and pregnancy testing in clinical trials"
- Positive for Hepatitis B, Hepatitis C or HIV at screening
- Not qualified to give consent at inclusionAny other condition judged to interfere with the safety of the patient or the intent and conduct of the study

Related to medical history:

- Stroke
- Anaphylaxis
- Prior adverse reaction to any human blood product
- Any history of a blood coagulation disorder or hypercoagulability
- Congestive heart failure, defined as any previous heart failure hospitalization, or current symptomatic heart failure in New York heart Association class ≥II with reduced, mid-range or preserved ejection fraction
- Coagulation defect or hypercoagulopathy
- Uncontrolled hypertension
- Renal failure
- Prior intolerance to intravenous fluids
- Recent history of uncontrolled atrial fibrillation
- Bone marrow transplant
- IgA deficiency
- Severe protein S deficiency
- Thrombocytopenia (platelets < 40 x 10^9/L)
- Contraindication for Octaplasma

Related to medications or other treatments:

- Any concurrent use of anticoagulant therapy, clopidogrel or acetylsalicylic acid/dipyridamole in combination
- Initiation or change in the dosage of a acetylcholine esterase inhibitor (AChEI) or memantine during the trial (week 0-52). Participants will be urged to start on AChEI when diagnosis is communicated, and must be on a stable dose for at least one month prior to screening
- Concurrent participation in another treatment trial for AD. If there was prior participation, the last dose of the investigational agent must have been given at least 6 months prior to screening, except if the patient received placebo medication
- Prior or concurrent participation in amyloid antibody trials, except if the patient received placebo medication

- Treatment with any human blood product, including intravenous immunoglobulin, during the 6 months prior to screening or during the trial
- Concurrent daily treatment with benzodiazepines, typical or atypical antipsychotics, long-acting opioids, or other medications that are judged to interfere with cognition. Intermittent treatment with short-acting benzodiazepines or atypical antipsychotics may be permitted, provided that no dose is administered within 72 hours prior to cognitive assessment

Related to magnetic resonance imaging:

- Claustrophobia
- Any metallic surgical implant, like a pacemaker or clip incompatible with MRI
  Certain metallic implants like joint prostheses may be permitted, provided that specific manufacturer specifications are available, and that the device is known to be safe for 7T MRI. In case a patient is not eligible for the 7T scanner, the 3T scanner will be used

189

Treatment and examinations

The main study consists of 6 rounds of examinations in addition to plasma transfusions, mainly during the time span of one year, and once 2 years after baseline. A follow-up visit is also planned 5 years after baseline.

Treatment

For this study, ExPlas (plasma from fit donors) and Octaplasma are defined as Investigational10 Medicinal Products. ExPlas is plasma drawn by plasmapheresis once a month for 4 months, from a total of 30 donors (aged 18-40, BMI ≤27 kg/m^2 and VO_{2max} >55 mL/kg/min). All units will be virus inactivated by the Intercept method (CERUS corporation, US), in accordance with the instructions from the manufacturer and the procedures at the Blood Bank at St. Olavs Hospital.

Octaplasma is human pooled plasma produced by Octapharma (Lachen, Switzerland). The rationale for including Octaplasma is to separate the effect of ExPlas from the "general untrained" plasma pooled from thousands of donors (relatively young men). Placebo for this study is isotonic saline (0.9% sodium chloride) (Fresenius Kabi, Halden, Norway). Comparison with isotonic saline allows differentiation from a non-blood product. ExPlas and Octaplasma are stored at ≤-18°C until the time of transfusion. The transfusion volume will be 200 mL at every time point for all three treatments.

Cognitive test battery

All tests will be undertaken at baseline, weeks 24, 52 and 104 after inclusion, and performance at all time points will be evaluated against baseline values.

CERAD Ten-word test will be used as a measure of objective evidence of an amnestic syndrome of the hippocampal type (44).

Mini-Mental State Examination Score – (MMSE-NR-3) will be used as a screening tool for cognitive function (45). The test consists of standardized questions within five areas: orientation for time and place, short-term memory, attention, short-term recall and language. The test may help to evaluate degree of cognitive impairment. The maximum score is 30 (46).

Trail-Making test A and B – (TMT-NR3) will be used to measure visual attention, processing speed and executive function (47, 48).

Clock Drawing Test is a cognitive screening tool and will be used as a supplement for examining visuospatial function and executive function (49).

COWAT-FAS. The Controlled Oral Word Association Test (COWAT)-FAS will be used to measure verbal fluency and executive function (50, 51).

VOSP - Visuospatial abilities will be evaluated with the silhouettes test from the Visual Object and Space Perception Battery. The test also assesses semantic memory and name retrieval (52, 53).

Clinical Dementia Rating Scale (CDR) is a clinical scale for the staging of dementia. The participant is rated from 0-3 on six cognitive and behavioral categories: memory, orientation, judgement and problem solving, community affairs, home and hobbies, and personal care. The Global score is calculated according to an established algorithm, where memory is considered the primary category and all others are secondary categories. A global score of 0 equals no dementia, 0,5 questionable dementia, 1 mild dementia, 2 moderate dementia and 3 severe dementia (54, 55). The Sum of Boxes score is a continuous measure of dementia severity and ranges from 0-18. The CDR Sum of Boxes are found to be adequate for use in prodromal AD and continued use is warranted and recommended in

clinical trials because it is continuous and provides a greater variation in values (46). Both the CDR-Global score and Sum of Boxes will be calculated.

The Lawton Instrumental Activities of Daily Living Scale (IADL). This IADL scale evaluate eight items, related to complex everyday activities, and each can be scored 0 that equals "dependent" and 1 that reflect "completely independent". Change from 0 to 1 in any of the eight items is considered a "clinically relevant change" (56).

Unified Parkinson's Disease Rating Scale (UPDRS). The motor examination part of UPDRS will be used to detect development of parkinsonian motor signs, such as tremor, rigidity, bradykinesia, and postural-gait abnormalities (57).

The 6-minute walk test

Fitness level will be measured using the 6 minute walk-test which is a good alternative to direct measurement of PeakVO_2 (58). The 6-minute walk test is considered safer for the current patient group than a treadmill test.

Structural- and functional MRI

For increased sensitivity we will use multiparameric MRI at 7T MRI to assess brain structure and function to uncover both neurodegenerative and cerbrovasular changes from baseline to 1, 2 and 5 years. The 7T protocol consists of the following scans: whole brain 3D MP2RAGE, gradient echo SWI/Quantitative susceptibility mapping (QSM), and FLAIR, high resolution T2 weighted spin echo sequence of the medial temporal lobe, multishell DTI, asl FLAIR, and rs-fMRI.The primary outcomes are hippocampal and entorhinal cortex structure ((subfield)-volume/thickness, diffusion characteristics, T2*-mapping) and function (blood flow, connectivity and BOLD signal). Other quantitative measures include brain morphometry (e.g., parenchymal fraction, at-risk AD pattern volume loss), and diffusion metrics in white and grey matter. Cerebrovascular pathology (perfusion, white matter hyperintensities, perivascular spaces (quantitative from mulitshell DTI), microhaemorrhages, micro- and macro- infarction) will be evaluated. For participants where 7T is contraindicated, but not 3T, a similar examination will take place using 3T.

A secondary aim is to identify any effect of treatment group on MRI markers of both neurodegenerative and cerebrovascular disease. MRI at 7T allows for a stronger signal and better anatomical localization in less time, but with the stronger magnetic field there are also more contraindications.

Quality of Life

Quality of life will be evaluated by the questionnaire SF-36, which contains questions about physical and mental health, pain, vitality and general health perceptions (59, 60).

Echocardiography

All patients will undergo echocardiography examination at screening and four times during the first year, and potentially at 2 and 5-years follow up. Screening echo is performed to ensure safety of transfusions for patients included in the study. Patients with reduced cardiac function will be excluded due to risk of developing heart failure triggered by fluid overload. Screening echocardiography serve as baseline for included patients. Following echocardiography will be performed at week 5 (one week after 4 weeks of transfusion treatment), week 20 (before treatment period two), week 48 (before treatment period three), week 52 (one week after 4 weeks of transfusion treatment), and assumedly at 2 and 5-years follow up. The echocardiography examination will be a complete examitation of cardiac structure and function, including ultrahigh framerate recordings at each time point.

Biomarkers in blood and spinal fluid

Although no single ideal biomarker yet exists for AD, there are substances currently considered to be 'core' biomarkers for AD. According to a landmark paper published in 2017 from the joint proceedings of the International Working Group (IWG) and the American Alzheimer's Association, the most important early indicators of incipient AD are changes in two key proteins: reduced amyloid-beta 1-42 (Aβ42) and increased total tau protein and hyperphosphorylated tau measured biochemically in cerebrospinal fluid (CSF), or increased deposition of amyloid plaque and neurofibrillary tangles of tau protein in brain as shown by PET(61-63). Since PET is exceptionally expensive, we plan to analyse these substances in CSF. Collected cerebrospinal fluid and blood will be analysed for established AD risk markers (including APOE genotyping in Blood and Amyloid Beta 1-42, Amyloid Beta 1-40, phosphor tau and total tau in spinal fluid). Individuals apparently without clinical symptoms of cognitive decline but with pathological levels of both these biomarkers are considered to have "preclinical AD". If only one of the biomarkers is found to have a pathological level, the individual is considered to be "at risk of AD" (the difference being that the individual is only at risk of AD, whereas preclinical AD is considered to be an early manifestation of the disease itself). In order to better understand the potential link between the cardiovascular system and the brain, the collected blood will also be analysed with respect to cardiovascular profile (Albumin, Ferritin, Natrium, Kalium, Kreatinin, Glukose, ALAT, GT, Kolesterol, Triglyserider, HDL, Hs-CRP, NT-proBNP, Troponin, Leukocytter, Trombocytter, HB, HbA1c). Some of the biological material will be stored for future analysis in the search for new biomarkers. For instance, the study group has previously identified potential "fitness-microRNAs" that could distinguish high- and low-fitness individuals (64). In the ExPlas Study we aim (as a start) to detect microRNAs that show a significant change in expression concomitant with ExPlas treatment and examine changes in level in relation to speed of AD progression. Unbiased screenings of microRNAs will be performed at the NTNU Genomics Core Facility and analysed together with the NTNU Bioinformatics Core Facility. Identification of other circulating factors will be performed by gel-free shotgun proteomics analysis. Proteomic RNA analyses will be performed at the NTNU Proteomics and Modomics Experimental Core Facility and analysed together with the NTNU Bioinformatics Core Facility.

Blood sampling procedures

All blood samples will be taken by trained biomedical engineer or nurse. Serum and plasma samples are collected with venous puncture using sterile disposable equipment in serum-gel, EDTA plasma and litsium heparine tubes. The tubes are centrifuged and stored on ice while shipped to further handling and analysis. If not analysed right away, samples are to be stored at -80ºC (in the established Trønderbrain biobank, Director of the biobank is Geir Bråthen; geir.brathen@ntnu.no, tel. 72575077). Blood tests will be taken on 7 occasions in addition to screening and after 2 years as well as offered 5 years after baseline (Figure 1).

Spinal puncture procedures

Lumbar puncture will be performed by neuorolgist. A thin needle is inserted into the spinal canal in the lowerback, while the patient is lying down on the side. The procedure is done using sterile technique (the area is cleaned with chlorhexidine and air dried before inserting the needle). The sample is collected directly into polypropylene tubes (used for dementia markers), and stored on ice until shipped to further handling. The samples are to be stored at -80ºC (Trønderbrain biobank, Geir Bråthen) until analysis. The sample will be analysed for risk genes and AD related biomarkers. Some portion of the sample will be stored for future analysis. Spinal puncture will be performed at baseline, week 24, week 52, after 2 years, and offered 5 years after baseline (Figure 1). All sample collection, handling and analysis will be performed in accordance with hospital/laboratory standard procedures.

Sample size and statistics

We do not expect that ExPlas will cause more adverse effects than Octaplasma, but have made the following considerations about power calculations related to safety. The most common reaction to transfusion of Octaplasma is allergy and occurs in up to 1% of the elderly. If we consider that ExPlas would cause a dramatic increase in allergic reactions, of e.g. 35% vs. 1% after Octaplasma treatment, 19 participants will be needed in each group to show significant differences (with alpha 0.05 and power of 0.8). There are substantial uncertainties in the assumptions for this power calculation. But considering that another study found that transfusing plasma from young donors to patients (n=18) with mild cognitive impairment or early AD was safe with no adverse events (39) we find it likely that 20 patients in each group will be enough to test safety in ExPlas.

The magnitude of a possible treatment effect of ExPlas is currently not established. The following information has been established: i) a difference of 2 points on the Mini-Mental State Examination Score (MMSE) primarily between those receiving ExPlas vs. Octaplasma will be clinically relevant after 1 and 2 years; ii) Based on several clinical studies in this population, we expect an average MMSE-NR-3 of about 24 in our population, iii) Based on previous studies, we expect a standard deviation of MMSE-NR-3 score of approximately 3. These assumptions suggest that 17 participants are needed in each group (with alpha 0.05, and power 0.8) to demonstrate a clinically relevant effect of ExPlas. Given potential dropouts, it is reasonable to include 20 in each group. The plan is therefore to include 60 patients in the study.

For the primary endpoints, counts will be reported and compared using recommended methods for analysis of contingency tables (57). Secondary and other endpoints will be analyzed using mixed models with the outcome variable as dependent variable, treatment group, time and their interaction as categorical covariates, and patient as random effect. In these analyses, we will adjust for the baseline value of the outcome variable, as recommended (65, 66).

Ethics

The study will be performed according to the Declaration of Helsinki. Written informed consent will be obtained from all participants by the treating neurologist, and participation is voluntary. Patients will be insured according to Norwegian regulations for patients involved in medical research (npe.no). The patients' abilities to keep track of the objectives of the project and assess its relevance will progressively deteriorate during the project period. In view of this, all participants are required to include a next of kin who will follow them throughout the study and represent the patient's interest. The burden from participation, number of tests and time points of conducting tests during the study have been planned in dialogue with the user group consisting of three next of kin of current and previous AD-patients. The study is approved by the Regional Committee for Medical and Health Research Ethics (REK 2018/702) and the Norwegian Medicines Agency (Statens Legemiddelverk, EudraCT No. 2018-000148-24).

Organization

The steering committee of ExPlas has developed the study protocol and is responsible for overall study management, data collection, analyses, publications, and the final data set.

A safety committee consisting of two clinicians (one neurologist and one specialist in Transfusion Medicine) has been appointed to ensure the safety of study participants. In case of adverse events, the safety committee will evaluate whether treatments can continue or must be stopped. A study nurse will observe the patients during and for one hour after infusion and a physician will evaluate the patients in case of adverse effects. Neither the safety committee nor the attending physician responsible for each infusion are involved in other parts of the study and they will not be blinded for the treatment given.

Study monitors

The primary goal of the study monitors is to ensure that the site follows the standardized operation procedures described for the trial, and to report and manage any deviations that may occur from the investigational plan. The ExPlas Study has been appointed two study monitors by the Unit for Applied Clinical research at NTNU, one who has the overall overview of the study, and is blinded to the treatment randomization, and one who is unblinded. A study monitoring plan has been developed and includes regular visits by the Clinical Study Monitors (headed by Sigve Nyvik Aas, sigve.n.aas@ntnu.no), who will check the following:

- Informed consent process
- Reporting of adverse events and all other safety data
- Adherence to protocol
- Maintenance of required regulatory documents
- Study Supply accountability
- Facilities and equipments (treatment storage and manufacturing at the Blood Bank)
- Data completion on the CRFs including source data verification (SDV).

The monitor will review the relevant CRFs for accuracy and completeness and will ask the site staff to adjust any discrepancies as required. Sponsor's representatives (e.g., monitors, auditors) and/or competent authorities will be allowed access to source data for source data verification in which case a review of those parts of the hospital records relevant to the study will be required.

Data management

The Clinical Data Management System (CDMS) used for the electronic case report forms (eCRF) in this study is The Unit for Applied Clinical Research at NTNU. The setup of the study specific eCRF in the CDMS will be performed by The Unit for Applied Clinical Research at NTNU. The eCRF system will be FDA Code of Federal Regulations 21 Part 11 compliant. The designated investigator staff will enter the data required by the protocol into the eCRF. The Investigator is responsible for assuring that data entered into the eCRF is complete, accurate, and that entry is performed in a timely manner. The signature of the investigator will attest the accuracy of the data on each eCRF. If any assessments are omitted, the reason for such omissions will be noted on the eCRFs. Corrections, with the reason for the corrections will also be recorded. After database lock, the investigator will receive a digital copy of the subject data for archiving at the investigational site.

The medical records of each patient will clearly describe at least:

- That the patient is participating in the study, by including the enrollment number and the study code or other study identification;
- Date when Informed Consent was obtained from the patient and statement that patient received a copy of the signed and dated Informed Consent;
- Results of all assessments confirming a patient's eligibility for the study;
- Diseases (past and current; both the disease studied and others, as relevant);
- Surgical history, as relevant;
- Treatments withdrawn/withheld due to participation in the study;
- Results of assessments performed during the study;
- Treatments given, changes in treatments during the study and the time points for the changes;
- Visits to the clinic / telephone contacts during the study, including those for study purposes only;
- Non-Serious Adverse Events and Serious Adverse Events (if any) including causality assessments;
- Date of, and reason for, discontinuation from study treatment;
- Date of, and reason for, withdrawal from study;
- Date of death and cause of death, if available;

- Additional information according to local regulations and practice.

Data management will be performed by the Unit for Applied Clinical Research at NTNU (Berit Bjelkåsen). The Data management procedures will be performed in accordance with the department's SOPs and ICH guidelines. The data management process will be described in the study specific data handling plan and the study specific data handling report after database closure. Data entered into the eCRF will be validated as defined in the data validation plan. Validation includes, but is not limited to, validity checks (e.g. range checks), consistency checks and customised checks (logical checks between variables to ensure that study data are accurately reported) for eCRF data and external data (e.g. laboratory data). A majority of edit checks will be triggered during data entry and will therefore facilitate efficient 'point of entry' data cleaning. Data management personnel will perform both manual eCRF review and review of additional electronic edit checks to ensure that the data are complete, consistent and reasonable. The electronic edit checks will run continually throughout the course of the study and the issues will be reviewed manually online to determine what action needs to be taken. Manual queries may be added to the system by clinical data management or study monitor. Clinical data managers and study monitors are able to remotely and proactively monitor the patient eCRFs to improve data quality. All updates to queried data will be made by authorised study centre personnel only and all modifications to the database will be recorded in an audit trail. Once the queries have been resolved, eCRFs will be signed by electronic signature Any changes to signed eCRFs will be approved and resigned by the Investigator. Once the full set of eCRFs have been completed and locked, the Sponsor will authorise database lock and all electronic data will be sent to the designated statistician for analysis. Subsequent changes to the database will then be made only by written agreement. The data will be stored in a dedicated and secured area at NTNU. Data will be stored in a de-identified manner, where each study participant is recognisable by his/her unique trial subject number. The data will be stored until 15 years after database lock.

Patients and public involvement

To ensure a high study quality and relevance a user board consisting of three next kin of present and past AD patients has been established. As we conduct research on a patient group that is considered vulnerable, this board is particularly important. We have met the user group on several occasions while working on the study protocol (first meeting in Feb 2017) and have received input on several matters, such as how to ensure a tolerable load of participation for the individual patient/relatives. The board will continue to consult the study team twice annually throughout the study period, on implementation, results, and future developments. They are encouraged to give their opinions regarding the project as a whole and particularly on the patients' well-being. The study group has already made changes and adjustments in the planned protocols based on feedback from the user group. The study has a user representative who participates in meetings and presentations of the study to the general public. On initiative from the ExPlas user group, we are currently making three information videos about "AD and participation in research studies", for AD patients and their families, where patients and their next of kin tell their story to help new patients and their next of kin in the coming process. These videos will also be used in the recruitment phase of the study to inform and motivate to take part in ExPlas.

Dissemination

Direct communication with users and patient organisations: ExPlas Study group regularly present at various meetings of patient organizations (such as the National Association for Public Health) and for senior citizens' societies. This type of contact with the public, patients and relatives has proven mutually useful. We plan to intensify participation in such meetings to inform about current knowledge about prevention and treatment of AD, particularly via the established user groups. Communication via Internet: One of the most important media for spreading the news and awareness will be the Internet. The results and information (including videos) about the studies will be presented on CERG's webpage (ntnu.edu/cerg), one of the most visited sites at Scandinavian universities; and ExPlas's own

Norweian webpage (ntnu.no/cerg/explas). Scientific and non-scientific communication: General communication activities include publication in open access peer reviewed journals, non-scientific journals and at national- and international meetings, to reach the general public, patients, scientists, and policy makers. Importantly, our group is closely linked and active partners in the Norwegian Research School in Neuroscience, Physical Activity and Health (master program) and medical education where we actively will present our research to the next generation of health care personnel and scientists. We also have a journalist at CERG (Anders Revdal), available for ExPlas, who will be responsible for communication.

DISCUSSION

To our knowledge, ExPlas will be the first study with the aim of studying safety and efficacy of transfusion of plasma from well endurance trained donors to patients in the early symptomatic phase of AD. Even if prevention probably will be the most effective way to reduce numbers of patients with AD worldwide, we need a cure for this devastating disease which impacts the lives for both patients and their families substantially. There is also a need to understand the mechanisms behind the beneficial effect of physical exercise on the brain, and it seems logically to try to exploit this effect in treatment of the early phase of symptomatic AD.

On June 7th, 2021, the U.S. Food and Drug Administration, approved aducanumab (marketed as Aduhelm) for use in treatment of AD (67), due to its ability to reduce amyloid plaques in the brain, under an accelerated approval pathway (68). Confirmation of the clinical benefit is still required to be confirmed for continued approval (67). Independent of the usefulness of aducanumab in AD therapeutics, other interventions capable of delaying the clinical onset of AD dementia should continue to be studied. The findings from preclinical AD models (36-38, 69), and a small clinical trial (39) clearly indicate that there is communication between the systemic environment and the hippocampus. Systemic factors are capable of inducing changes, and even therapeutic effects, in the brain without hindrance by the blood-brain barrier; potentially an effective strategy to reverse neurodegeneration in the AD-brain (30). There are myriads of factors and processes that are set in motion during and after exercise training, and much of this is reflected in the composition of the blood (70). Thus, it is not likely that it is a single factor is orchestrating the beneficial effects of exercise, but rather an interplay between several molecular factors that need to be discovered and understood to develop the first-generation of exercise-mimicking drugs. This is a promising idea as a large population of patients are simply unable to exercise, such as patients in the intensive care unit, the critically ill, patients recovering from accidents, the morbidly obese and paralyzed patients. For these patients, innovative exercise-mimicking therapies could be of benefit.

However, development of exercise-mimicking therapies is a very complex and time-consuming undertaking, that should not delay the testing of a potential benefit of exercise trained plasma, with most of its natural components, on safety and therapeutic effect in patients with AD. In the context of lack of disease-modifying treatments, ExPlas is innovative and potentially groundbreaking if it is found to be safe with few side effects and with similar promising results as seen in preclinical AD models (35-38).

Another key question is at what stage of AD interventions such as ExPlas treatment can be expected to have an effect. Today we know that AD-related changes in the brain are present 10-30 years before symptoms develop. The optimal time window for treatment is probably as early as possible during this period, to decelerate or stop the progressive loss of neurons before functions are lost. As in all diseases, prevention will always be the optimal path. Depending upon outcomes in the ExPlas Study, a natural next step may be treatment with ExPlas of patients in the preclinical phase of the disease (61).

A small clinical trial found that plasma from young donors (young blood) transfused to patients with mild to moderate AD dementia (MMSE score ranging from 12-24) was safe with no adverse events and possibly beneficial with improvement in functional activity. In this study, 9 patients were randomized to a cross-over cohort, receiving 4 once-weekly infusions of either 250 mL of plasma from male donors (aged 18-30 years) or 250 mL of saline, followed by a 6-week washout and crossover to 4 once-weekly infusions of the alternate treatment. In addition, 9 patients were included in an open-label design in which patients received 4 once-weekly infusions of only young plasma. Considering the low number of patients, short follow-up period and promising findings in the study by Sha *et al.* (39) there is reason to believe that transfusion of exercise-trained plasma also is safe. With increased treatment periods and extended follow-up, we believe the ExPlas Study is well designed also to evaluate the potential therapeutic effect of exercise trained plasma. The relatively large number of patients will also likely enable us to assess whether endpoints become differentially affected by *APOE* 4 status. As the ExPlas Study is the first of its kind it is not straightforward to undertake power calculations, and the results of our study may be useful for planning of an appropriately sample sized study in the future.

We expect the ExPlas study to give new knowledge about whether transfusion of plasma from exercise-trained donors is safe and indications on whether it has therapeutic effects. ExPlas will also contribute to pioneering the discovery of molecular targets to potentially treat AD and lay the foundation for first-generation exercise-mimicking drugs, by capturing the molecular signature of high-fitness and molecular mechanisms provided by exercise.

Contributor statement

Atefe R. Tari Conception and design of the study, obtained funding, drafting the manuscript and applications to ethical committee and Norwegian Medicine Agency for study approval

Helene H. Berg Design of the study, drafting the manuscript, critical review of manuscript

Vibeke Videm Conception and design of the study, critical review of the manuscript, supervision, obtained funding, applications to ethical committee and Norwegian Medicine Agency for study approval

Geir Bråthen Conception and design of the study, critical review of the manuscript, supervision, obtained funding, applications to ethical committee and Norwegian Medicine Agency for study approval

Linda R. White Conception and design of the study, critical review of the manuscript, supervision, obtained funding, applications to ethical committee and Norwegian Medicine Agency for study approval

Ragnhild Røsbjørgen Critical review of the manuscript, supervision, established methods and operating procedures for the study

Katja Scheffler Design of the study, critical review of the manuscript, established procedures for AD biomarkers in blood and CSF for ExPlas

Håvard Dalen Critical review of the manuscript, established echocardiographic procedures for AD patients in ExPlas

Espen Holte Critical review of the manuscript, established echocardiographic procedures for AD patients in ExPlas

Asta Håberg Design of the study, critical review of the manuscript, obtained funding, established structural and functional-MRI procedures for AD patients in ExPlas, applications to ethical committee for study approval

Geir Selbæk Design of the study, critical review of the manuscript, obtained funding

Stian Lydersen Design of the study, critical review of the manuscript, making the statistical plan and analyses in ExPlas

Emrah Duzel Critical review of the manuscript, established methods and operating procedures for the study

Sverre Bergh Critical review of the manuscript, obtained funding

Kjell Rune Halvorsrud Design of the study, critical review of the manuscript, supervision, established methods and operating procedures for the blood-donor part of ExPlas, supervision, obtained funding

Sigrid B. Sando Conception and design of the study, critical review of the manuscript, supervision, obtained funding, applications to ethical committee and Norwegian Medicine Agency for study approval, Co-PI of ExPlas

Ulrik Wisløff Conception and design of the study, critical review of the manuscript, supervision, obtained funding, applications to ethical committee and Norwegian Medicine Agency for study approval, Co-PI of ExPlas

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Competing interests

None of the authors reports any competing interests or had financial relationships with any organisations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work. All authors will complete the ICMJE uniform disclosure form in due time.

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Authors reports no financial relationships with any organisations that might have an interest in the submitted work in the previous three years, or any other relationships or activities that could appear to have influenced the submitted work.

Country of Recruitment

Norway

Trial sponsor

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Data sharing

We are not permitted to share individual data from the current trial, but we are open to collaborative research with researchers worldwide, who can have access to analysed data from our university. We have also established a biobank of blood and genetic material that we plan to share with researchers worldwide, but individual data must be analysed within our university only.

References

1. World Health Organization. Aging and Life Course 2015 [Available from: http://www.who.int/ageing/en/.
2. Associaton As. Alzheimer's Disease Facts and Figures. Report. 2018 2018.
3. Andrieu S, Coley N, Lovestone S, Aisen PS, Vellas B. Prevention of sporadic Alzheimer's disease: lessons learned from clinical trials and future directions. Lancet Neurol. 2015;14(9):926-44.
4. Helsedirektoratet d.
5. GjØra L, Strand BH, Bergh S, Borza T, Brækhus A, Engedal K, et al. Current and Future Prevalence Estimates of Mild Cognitive Impairment, Dementia, and Its Subtypes in a Population-Based Sample of People 70 Years and Older in Norway: The HUNT Study. J Alzheimers Dis. 2021;79(3):1213-26.
6. Kvello-Alme M, Bråthen G, White LR, Sando SB. The Prevalence and Subtypes of Young Onset Dementia in Central Norway: A Population-Based Study. J Alzheimers Dis. 2019;69(2):479-87.
7. Association As. 2021 Alzheimer's Disease Facts and Figures. 2021.
8. Potter PE. Investigational medications for treatment of patients with Alzheimer disease. J Am Osteopath Assoc. 2010;110(9 Suppl 8):S27-36.
9. Mullane K, Williams M. Alzheimer's therapeutics: continued clinical failures question the validity of the amyloid hypothesis-but what lies beyond? Biochem Pharmacol. 2013;85(3):289-305.
10. Cummings JL, Morstorf T, Zhong K. Alzheimer's disease drug-development pipeline: few candidates, frequent failures. Alzheimers Res Ther. 2014;6(4):37.
11. Livingston G, Huntley J, Sommerlad A, Ames D, Ballard C, Banerjee S, et al. Dementia prevention, intervention, and care: 2020 report of the Lancet Commission. Lancet. 2020;396(10248):413-46.
12. Barnes DE, Yaffe K. The projected effect of risk factor reduction on Alzheimer's disease prevalence. Lancet Neurol. 2011;10(9):819-28.
13. Colcombe SJ, Erickson KI, Raz N, Webb AG, Cohen NJ, McAuley E, et al. Aerobic fitness reduces brain tissue loss in aging humans. The journals of gerontology. 2003;58(2):176-80.
14. de Bruijn RF, Ikram MA. Cardiovascular risk factors and future risk of Alzheimer's disease. BMC Med. 2014;12:130.
15. Levine DA, Langa KM. Vascular cognitive impairment: disease mechanisms and therapeutic implications. Neurotherapeutics. 2011;8(3):361-73.
16. Rovio S, Kareholt I, Helkala EL, Viitanen M, Winblad B, Tuomilehto J, et al. Leisure-time physical activity at midlife and the risk of dementia and Alzheimer's disease. The Lancet Neurology. 2005;4(11):705-11.
17. Qiu C, Xu W, Fratiglioni L. Vascular and psychosocial factors in Alzheimer's disease: epidemiological evidence toward intervention. J Alzheimers Dis. 2010;20(3):689-97.
18. Qiu C. Preventing Alzheimer's disease by targeting vascular risk factors: hope and gap. J Alzheimers Dis. 2012;32(3):721-31.
19. Exalto LG, Quesenberry CP, Barnes D, Kivipelto M, Biessels GJ, Whitmer RA. Midlife risk score for the prediction of dementia four decades later. Alzheimer's & dementia : the journal of the Alzheimer's Association. 2014;10(5):562-70.

20. Tolppanen AM, Solomon A, Soininen H, Kivipelto M. Midlife vascular risk factors and Alzheimer's disease: evidence from epidemiological studies. J Alzheimers Dis. 2012;32(3):531-40.
21. Norton S, Matthews FE, Barnes DE, Yaffe K, Brayne C. Potential for primary prevention of Alzheimer's disease: an analysis of population-based data. Lancet Neurol. 2014;13(8):788-94.
22. Kaminsky LA, Arena R, Beckie TM, Brubaker PH, Church TS, Forman DE, et al. The importance of cardiorespiratory fitness in the United States: the need for a national registry: a policy statement from the American Heart Association. Circulation. 2013;127(5):652-62.
23. Larson EB, Wang L, Bowen JD, McCormick WC, Teri L, Crane P, et al. Exercise is associated with reduced risk for incident dementia among persons 65 years of age and older. Ann Intern Med. 2006;144(2):73-81.
24. Tari AR, Nauman J, Zisko N, Skjellegrind HK, Bosnes I, Bergh S, et al. Temporal changes in cardiorespiratory fitness and risk of dementia incidence and mortality: a population-based prospective cohort study. The Lancet Public Health. 2019;4(11):e565-e74.
25. Hoffmann K, Frederiksen KS, Sobol NA, Beyer N, Vogel A, Simonsen AH, et al. Preserving cognition, quality of life, physical health and functional ability in Alzheimer's disease: the effect of physical exercise (ADEX trial): rationale and design. Neuroepidemiology. 2013;41(3-4):198-207.
26. Lautenschlager NT, Cox KL, Flicker L, Foster JK, van Bockxmeer FM, Xiao J, et al. Effect of physical activity on cognitive function in older adults at risk for Alzheimer disease: a randomized trial. Jama. 2008;300(9):1027-37.
27. van Praag H, Shubert T, Zhao C, Gage FH. Exercise enhances learning and hippocampal neurogenesis in aged mice. J Neurosci. 2005;25(38):8680-5.
28. van Praag H, Christie BR, Sejnowski TJ, Gage FH. Running enhances neurogenesis, learning, and long-term potentiation in mice. Proc Natl Acad Sci U S A. 1999;96(23):13427-31.
29. Maass A, Duzel S, Goerke M, Becke A, Sobieray U, Neumann K, et al. Vascular hippocampal plasticity after aerobic exercise in older adults. Molecular psychiatry. 2015;20(5):585-93.
30. Bouchard J, Villeda SA. Aging and brain rejuvenation as systemic events. J Neurochem. 2015;132(1):5-19.
31. Pereira AC, Huddleston DE, Brickman AM, Sosunov AA, Hen R, McKhann GM, et al. An in vivo correlate of exercise-induced neurogenesis in the adult dentate gyrus. Proc Natl Acad Sci U S A. 2007;104(13):5638-43.
32. Fabel K, Fabel K, Tam B, Kaufer D, Baiker A, Simmons N, et al. VEGF is necessary for exercise-induced adult hippocampal neurogenesis. The European journal of neuroscience. 2003;18(10):2803-12.
33. Castellano JM, Mosher KI, Abbey RJ, McBride AA, James ML, Berdnik D, et al. Human umbilical cord plasma proteins revitalize hippocampal function in aged mice. Nature. 2017;544(7651):488-92.
34. Tari AR, Norevik CS, Scrimgeour NR, Kobro-Flatmoen A, Storm-Mathisen J, Bergersen LH, et al. Are the neuroprotective effects of exercise training systemically mediated? Progress in cardiovascular diseases. 2019.
35. Villeda SA, Plambeck KE, Middeldorp J, Castellano JM, Mosher KI, Luo J, et al. Young blood reverses age-related impairments in cognitive function and synaptic plasticity in mice. Nat Med. 2014;20(6):659-63.

36. Katsimpardi L, Litterman NK, Schein PA, Miller CM, Loffredo FS, Wojtkiewicz GR, et al. Vascular and neurogenic rejuvenation of the aging mouse brain by young systemic factors. Science. 2014;344(6184):630-4.
37. Xia E, Xu F, Hu C, Kumal JPP, Tang X, Mao D, et al. Young Blood Rescues the Cognition of Alzheimer's Model Mice by Restoring the Hippocampal Cholinergic Circuit. Neuroscience. 2019;417:57-69.
38. Zhao Y, Qian R, Zhang J, Liu F, Iqbal K, Dai CL, et al. Young blood plasma reduces Alzheimer's disease-like brain pathologies and ameliorates cognitive impairment in 3×Tg-AD mice. Alzheimers Res Ther. 2020;12(1):70.
39. Sha SJ, Deutsch GK, Tian L, Richardson K, Coburn M, Gaudioso JL, et al. Safety, Tolerability, and Feasibility of Young Plasma Infusion in the Plasma for Alzheimer Symptom Amelioration Study: A Randomized Clinical Trial. JAMA Neurol. 2018.
40. Horowitz AM, Fan X, Bieri G, Smith LK, Sanchez-Diaz CI, Schroer AB, et al. Blood factors transfer beneficial effects of exercise on neurogenesis and cognition to the aged brain. Science. 2020;369(6500):167-73.
41. Aspenes ST, Nilsen TI, Skaug EA, Bertheussen GF, Ellingsen Ø, Vatten L, et al. Peak oxygen uptake and cardiovascular risk factors in 4631 healthy women and men. Med Sci Sports Exerc. 2011;43(8):1465-73.
42. Nauman J, Nes BM, Zisko N, Revdal A, Myers J, Kaminsky LA, et al. Personal Activity Intelligence (PAI): A new standard in activity tracking for obtaining a healthy cardiorespiratory fitness level and low cardiovascular risk. Progress in cardiovascular diseases. 2019;62(2):179-85.
43. Dubois B, Feldman HH, Jacova C, Hampel H, Molinuevo JL, Blennow K, et al. Advancing research diagnostic criteria for Alzheimer's disease: the IWG-2 criteria. Lancet Neurol. 2014;13(6):614-29.
44. Morris JC, Heyman A, Mohs RC, Hughes JP, van Belle G, Fillenbaum G, et al. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part I. Clinical and neuropsychological assessment of Alzheimer's disease. Neurology. 1989;39(9):1159-65.
45. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res. 1975;12(3):189-98.
46. McDougall F, Edgar C, Mertes M, Delmar P, Fontoura P, Abi-Saab D, et al. Psychometric Properties of the Clinical Dementia Rating - Sum of Boxes and Other Cognitive and Functional Outcomes in a Prodromal Alzheimer's Disease Population. J Prev Alzheimers Dis. 2021;8(2):151-60.
47. Reitan RM. Validity of the Trail Making Test as an indication of organic brain damage. Perceptual and motor skills. 1958;8(3):271-6.
48. Espenes J, Hessen E, Eliassen IV, Waterloo K, Eckerström M, Sando SB, et al. Demographically adjusted trail making test norms in a Scandinavian sample from 41 to 84 years. Clin Neuropsychol. 2020;34(sup1):110-26.
49. Brodaty H, Moore CM. The Clock Drawing Test for dementia of the Alzheimer's type: A comparison of three scoring methods in a memory disorders clinic. Int J Geriatr Psychiatry. 1997;12(6):619-27.
50. Nutter-Upham KE, Saykin AJ, Rabin LA, Roth RM, Wishart HA, Pare N, et al. Verbal fluency performance in amnestic MCI and older adults with cognitive complaints. Arch Clin Neuropsychol. 2008;23(3):229-41.
51. Patterson C. World Alzheimer report 2018. Alzheimer's Disease International; 2018.
52. Elizabeth K Warrington MJ. Visual Object and Space Perception Battery (VOSP)1991.

53. Binetti T. Disorders of visual and spatial perception in the early stage of Alzheimers disease1996.
54. Hughes CP, Berg L, Danziger WL, Coben LA, Martin RL. A new clinical scale for the staging of dementia. Br J Psychiatry. 1982;140:566-72.
55. O'Bryant SE, Humphreys JD, Smith GE, Ivnik RJ, Graff-Radford NR, Petersen RC, et al. Detecting dementia with the mini-mental state examination in highly educated individuals. Arch Neurol. 2008;65(7):963-7.
56. Lawton MP, Brody EM. Assessment of older people: self-maintaining and instrumental activities of daily living. Gerontologist. 1969;9(3):179-86.
57. Fagerland M, Lydersen, S., & Laake, P. Statistical Analysis of Contingency Tables. Chapman and Hall/CRC2017.
58. Ries JD, Echternach JL, Nof L, Gagnon Blodgett M. Test-retest reliability and minimal detectable change scores for the timed "up & go" test, the six-minute walk test, and gait speed in people with Alzheimer disease. Phys Ther. 2009;89(6):569-79.
59. Geschke K, Fellgiebel A, Laux N, Schermuly I, Scheurich A. Quality of life in dementia: impact of cognition and insight on applicability of the SF-36. Am J Geriatr Psychiatry. 2013;21(7):646-54.
60. Ware JE, Jr., Sherbourne CD. The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. Med Care. 1992;30(6):473-83.
61. Dubois B, Hampel H, Feldman HH, Scheltens P, Aisen P, Andrieu S, et al. Preclinical Alzheimer's disease: Definition, natural history, and diagnostic criteria. Alzheimers Dement. 2016;12(3):292-323.
62. Bateman RJ, Xiong C, Benzinger TL, Fagan AM, Goate A, Fox NC, et al. Clinical and biomarker changes in dominantly inherited Alzheimer's disease. N Engl J Med. 2012;367(9):795-804.
63. Dubois B, Hampel H, Feldman HH, Scheltens P, Aisen P, Andrieu S, et al. Preclinical Alzheimer's disease: Definition, natural history, and diagnostic criteria. Alzheimers Dement. 2016;12(3):292-323.
64. Bye A, Røsjø H, Aspenes ST, Condorelli G, Omland T, Wisløff U. Circulating microRNAs and aerobic fitness--the HUNT-Study. PLoS One. 2013;8(2):e57496.
65. J T, L B, T H, J R, M W, M H. Different ways to estimate treatment effects in randomised controlled trials. Contemp Clin Trials Commun. 2018;10:80-5.
66. Coffman CJ, Edelman D, Woolson RF. To condition or not condition? Analysing 'change' in longitudinal randomised controlled trials. BMJ Open. 2016;6(12):e013096.
67. Cavazzoni P. FDA's Decision to Approve New Treatment for Alzheimer's Disease: fda.gov; 06/07/2021 [Available from: https://www.fda.gov/drugs/news-events-human-drugs/fdas-decision-approve-new-treatment-alzheimers-disease.
68. Sevigny J, Chiao P, Bussière T, Weinreb PH, Williams L, Maier M, et al. The antibody aducanumab reduces Aβ plaques in Alzheimer's disease. Nature. 2016;537(7618):50-6.
69. Villeda SA, Plambeck KE, Middeldorp J, Castellano JM, Mosher KI, Luo J, et al. Young blood reverses age-related impairments in cognitive function and synaptic plasticity in mice. Nature Medicine. 2014;20:659.
70. Tari AR, Norevik CS, Scrimgeour NR, Kobro-Flatmoen A, Storm-Mathisen J, Bergersen LH, et al. Are the neuroprotective effects of exercise training systemically mediated? Progress in cardiovascular diseases. 2019;62(2):94-101.

Figure text

Figure 1. Flow chart of the ExPlas Study. Infusion solution, Sodium Chloride 0.9% (Saline); US, ultrasound of the heart; fMRI, functional magnetic resonance imaging.

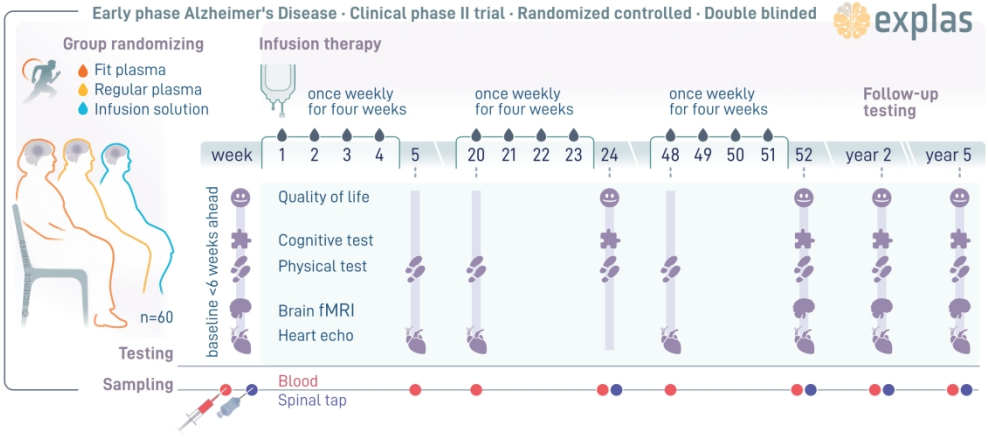

Figure 1. Flow chart of the ExPlas Study. Infusion solution, Sodium Chloride 0.9% (Saline); US, ultrasound of the heart; fMRI, functional magnetic resonance imaging.

533x239mm (118 x 118 DPI)

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	Page /Line No
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	p. 1/ 1-2
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	p. 1/ 49
	2b	All items from the World Health Organization Trial Registration Data Set	p. 1-16
Protocol version	3	Date and version identifier	p. 1/ 44
Funding	4	Sources and types of financial, material, and other support	p.16/ 610 -617
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	p. 1 and 14-15
	5b	Name and contact information for the trial sponsor	p. 17/ 625-629
	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	p. 16/ 618-620
	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)	p. 11/ 375 -377
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	p. 2-4
	6b	Explanation for choice of comparators	p. 4-5, 8
Objectives	7	Specific objectives or hypotheses	p. 4

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)	p. 4/ 135 -140
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	p. 4/ 142 -160
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)	p. 5/ 187 -189
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	p. 4 /135 -141 + fig.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)	p. 11 /378 -384
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	p. 5/ 187 -189
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	p. 4 / 99-124 + fig.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)	Fig. 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	p. 11/ 336 -360
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	p. 13/ 460 -475

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	p. 4/ 140 -145
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	p. 4/ 140 -145
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	p. 4/ 140 -145
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	p. 4/ 140 -145
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	p. 11/ 387 -384
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	p. 8-10/ 190 -334
	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	p. 13/ 460 -475
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	p. 12/ 407 -458
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	p. 11/ 336 -360
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	p. 11/ 336 -360

	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)	p. 11/ 336 -360
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	NA. The project has an independent safety committee
	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	p. 11/ 379 -385
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	p. 11/ 379 -385
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	p. 12/ 387 -406
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	p. 11/ 362 -372
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)	p. 11-12/ 387-459
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	p. 11/ 363 -364
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	
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	p. 12/ 407 -417
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	p. 16/ 618 -620
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	p. 12/ 407 -417

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	
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	p. 13-14/ 477-493
	31b	Authorship eligibility guidelines and any intended use of professional writers	p. 15/ 559 -598
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	p. 17/ 631 -635
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Attachment
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	p. 17/ 631 -635

*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

Safety and efficacy of plasma transfusion from exercise-trained donors in patients with early Alzheimer's disease: protocol for the ExPlas Study

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Safety and efficacy of plasma transfusion from exercise-trained donors in patients with early Alzheimer's disease: protocol for the ExPlas Study

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Protocol version no. 3.0 – 05.01.2021.

Study start: first donor included 01.03.2021, first patient screened 19.08.2021, first patient randomized 15.09.2021, first infusion given for 22.09.2021.

Trial registration: EudraCT No. 2018-000148-24. ClinicalTrials.gov, NCT05068830

ABSTRACT

Introduction

Given that exercise training reduces the risk of developing Alzheimer's disease (AD), induces changes in the blood composition and has widespread systemic benefits, it is reasonable to hypothesize that exercised plasma may have rejuvenative properties. The main objective is to test safety and tolerability of transfusing exercised plasma (ExPlas) from young, healthy, fit adults to patients with mild cognitive impairment or early AD. The study is a pilot for a future efficacy study. The key secondary objectives are examining the effect of plasma transfusions on cognitive function, fitness level, vascular risk profile, assessment of cerebral blood flow and hippocampal volume, quality of life, functional connectivity assessed by resting state functional MRI and biomarkers in blood and cerebrospinal fluid.

Methods and analysis

ExPlas is a double-blinded, randomized controlled clinical single center trial. Patients aged 50-75 years with diagnosis mild cognitive impairment or early AD will be recruited from two Norwegian hospitals. ExPlas is plasma drawn by plasmapheresis once a month for 4 months, from a total of 30 donors (aged 18-40, BMI ≤27 kg/m2 and VO2max >55 mL/kg/min). All units will be virus inactivated by the Intercept method in accordance with procedures at St. Olavs Hospital. Comparison with isotonic saline allows differentiation from a non-blood product. The main study consists of 6 rounds of examinations in addition to 12 plasma transfusions divided over three 4-weeks periods during study year-1. Follow-up examinations after 2 and 5 years after baseline is also planned.

Ethics and dissemination

Written informed consent will be obtained from all participants and participation is voluntary. All participants have a next of kin who will follow them throughout the study and represent the patient's interest. The study is approved by the Regional Committee for Medical and Health Research Ethics (REK 2018/702) and the Norwegian Medicines Agency (EudraCT No. 2018-000148-24). The study will be published in a leading clinical journal and results will be presented at numerous national and international meetings as well as on social media platforms.

STRENGTH AND LIMITATIONS OF THIS STUDY

- First double blinded, randomized controlled clinical phase II trial to examine safety and explore therapeutic effects of "exercised blood" in 60 AD patients.
- Relatively long follow-up (up to 5 years) in patients diagnosed with AD according to the IWG-2 criteria, making the study group homogenous.
- We have an active user board, consisting of next kin of present and past AD patients, that has taken part in study design, recruitment and dissemination. We have received input on several matters, such as how to ensure a tolerable load of participation for the individual patient/relatives and made changes and adjustments in the planned protocols based on feedback from the user group. The board will continue to consult the study team throughout the study period, on implementation, results, and future developments.
- ExPlas is innovative and potentially groundbreaking if it is found to be safe with few side effects and with similar promising results as seen in preclinical AD models.
- Uncertainties in the assumptions for power calculation and in addition to being a safety study, ExPlas must be regarded as a pilot for a future efficacy study.

BACKGROUND

The forecast of about 2 billion people being above the age of 60 by the year 2050 (1) implies an expected increased prevalence of Alzheimer's disease (AD) from today's 36 million to 108 (2-4). New estimates from Norway show a higher prevalence of dementia and mild cognitive impairment (MCI) in both younger adults and elderly than previous calculations (5, 6). Recent American data show that deaths from AD increased by 145% during the last two decades (7); for comparison, deaths from heart disease decreased by 7.3% (7). During the Covid-19 pandemic, deaths from AD or other dementias have additionally increased by 16% from that expected based on previous years (7). As of 2021 there is no proven cure for AD (8-10) and the World Health Organization has stated that AD is a global crisis that requires a global solution. Without intervention, the expected rise in AD adds a major burden to public health and health care costs globally.

It is hypothesized that around 40-50% of dementia cases worldwide are caused by modifiable risk factors (11, 12), and that many factors associated with higher risk of cardiovascular disease, such as obesity, hyperlipidaemia, hypertension, smoking, diabetes and physical inactivity are also associated with increased risk of AD (12-20). These factors have in common that they can be substantially modified through physical activity that secures above average age- and sex-specific levels of cardiorespiratory fitness (CRF) (12, 19, 21-23) measured as peak oxygen uptake ($PeakVO_2$). In line with this we demonstrated in a prospective cohort study of 30 695 adults that participants who increased or sustained high $PeakVO_2$ over time (10 years apart) had 40-50% reduced risk of incident dementia, 30-40% reduced risk of dementia-related mortality, approximately 2.0 years delayed dementia onset, and 2-3 years of life gained when compared to persistently unfit individuals (24). Thus, at present exercise training leading to a high age-relative $PeakVO_2$ may be the most promising preventive "AD-medicine" (25, 26).

Although it is well established that exercise positively influences brain neurogenesis, plasticity (27, 28), and cognition (21, 27, 29) it is not well understood how these effects are mediated. The beneficial effects of exercise on the brain have traditionally been thought not to be mediated through systemic changes (30). However, a number of studies in both rodents and humans (31-33) demonstrates direct effects on the brain of exercised induced blood-born molecules crossing the the blood-brain barrier (34). For instance, systemic administration of blood from young mice into old mice counteracts age-related changes in the brain (35, 36). Furthermore, direct evidence of beneficial effects of young blood treatment for preserving brain health has been provided in two different mouse models of AD (37, 38), suggesting beneficial effects also in the AD brain. Of particular interest, a small clinical trial (39) reported that plasma from young donors transfused to patients with mild cognitive impairment or early AD is safe and possibly beneficial. An exploratory endpoint analysis showed improvements on functional abilities, although no changes were found in global cognition, mood, or functional connectivity.

Although evidence suggests beneficial effects of *young blood* treatment in *aged* animals, less is known about the effects of *exercised blood* treatment in the aging or *diseased* brain. A recent study demonstrated that administration of blood from exercised, *aged* donor mice into sedentary, *aged* mice conferred beneficial effects of exercise on hippocampal neurogenesis and cognition (40). Given that exercise training reduces the risk of AD development, induces changes in the blood composition and has widespread systemic benefits, it is reasonable to hypothesize that exercised plasma may have rejuvenative properties similar to, or stronger than young blood. In this study, we aim to test the safety profile of transfusing exercised plasma from young, healthy adults in AD patients, and perform a pilot test for potential therapeutic effects.

ENDPOINTS

The purpose of this study is to explore the safety of transfusion of plasma from exercise trained donors (ExPlas) compared to Octaplasma®, a commercially available virus inactivated plasma product pooled from approximately 1000 donors, and Sodium Chloride 0.9% (saline) in patients in the early symptomatic phase of AD and provide pilot data regarding efficacy. An additional aim is to provide advancements to the field by exploring therapeutical effects on AD of blood-borne factors.

Primary endpoint of ExPlas

Proportion of patients with adverse events after 1 year as a measure of safety and tolerability, and number of subjects who comply with the research protocol as a measure of feasibility.

Secondary endpoints of ExPlas after 1, 2 and 5 years

- Change in performance in the CERAD (The Consortium to Establish a Registry for Alzheimer's Disease) Ten word Test
- Change in the Mini-Mental State Examination Score
- Change in performance in Trail-Making test A and B
- Change in scores in other cognitive tests: the Clock Drawing Test, Controlled Oral Word Association Test (COWAT)-FAS, Visual Object and Space Perception (VOSP) Silhouettes
- Change in Clinical Dementia Rating Scale Global score and Sum of Boxes, and The Lawton Instrumental Activities of Daily Living Scale (IADL)
- Change in performance in the 6-minutes walk-test
- Change in/Reduced hippocampal atrophy and preservation of functional connectivity assessed by resting state functional MRI
- Change in score of quality-of-Life SF-36 Questionnaire
- Change in biomarkers in blood and cerebrospinal fluid
- Change in cardiac dimensions, volumes and functional indices

Hypothesis, primary outcome:

I) ExPlas transfusions to patients in early symptomatic phase of AD is safe

Hypotheses secondary outcome:

II) ExPlas transfusions to patients in early symptomatic phase of AD ameliorates the biomarker-profile in blood and cerebrospinal fluid, structural- and functional MRI, cognitive function, functional capacity, fitness, and quality of life

METHODS

Design

The study is a double blinded, randomized controlled clinical phase II trial recruiting at a single study site at St. Olavs Hospital in Norway . The first patient was enrolled and randomized on September 15th, 2021. The anticipated recruitment period is 36 months and the estimated date of last patient enrolled is September 1th, 2024. The treatment duration is 1 year, follow-up period: 61 months, including screening. There are 3 study arms with patient ratio 1:1:1 (ExPlas, Octaplasma, saline), stratified by *APOE* genotype. Electronic randomization, provided by the Unit for Applied Clinical Research at NTNU, ensures that allocation of patients to a treatment group is random. Electronic randomization is conducted by the blinded ExPlas study nurse. The results of the randomization is not visible to any member of the study group. An automated message on each patient allocation is sent directly to the study nurses who undertake transfusions. The flow chart of the ExPlas Study is given in Figure 1.

Settings and participants – Plasmapheresis, Cardiopulmonary testing and physical (donors)

Exercised plasma collected by plasmapheresis from male donors who have not themselves received plasma will be used in the study. The rationale for these selection criteria is that women may have developed antibodies during pregnancies and men may have developed antibodies during plasma transfusions. Thus, selection reduces the risk of antibody-induced transfusion complications. All donors have been recruited from the existing donor corps at St. Olavs Hospital Blood Bank. Donors must fulfill all requirements in the Norwegian laws and guidelines for blood donors. Potential donors will perform a cardiopulmonary exercise test to voluntary exhaustion on a treadmill (PPS55 Med, Woodway GmbH, Germany) with continuous gas and minute ventilation analysis using the Cortex MetaMax II (Cortex Biophysik Gmbh, Leipzig, Germany). The individualized steady-state test protocol starts at a speed and inclination that will be defined during a 15-minute warm-up. The first stage of the test will be held for three minutes, or longer until steady state is reached. Thereafter, speed is increased by 1 km/h every minute until maximal oxygen uptake is reached, defined as flattening of oxygen uptake despite increased workload. Test procedure has been described in detail previously (41). The first plasma donation will be performed within one month after the cardiopulmonary test. To ensure that the donors sustained a high physical activity level in between the 4 donations (within 4 months from first donation) they were equipped with a wristworn heart rate monitor (Huami GTS2, Huami North America Inc, Irvine, CA, USA) and required to have a physical activity level above 100 weekly Personalized Activity Intelligence (PAI) points, to sustain a high maximal oxygen uptake, as described in detail elsewhere (42), using the Zepp mobile Application downloaded from Apple Store or Google Play.

Donor inclusion criteria:

- Healthy male donors
- Age 18-40 years
- BMI ≤27 kg/m^2
- Maximal oxygen uptake ≥55 mL/kg/min
- Already an approved donor at the St. Olav's Hospital Blood Bank

Donor exclusion criteria:

- Injury or other incident preventing regular exercise during the last month
- Previous recipient of blood transfusion
- PeakVO2 ≤55 mL/kg/min

Several aspects regarding donors were considered in the planning of the study. Particularly age and required fitness level have been up for thorough discussions. Our idea is that we believe, although not ever studied, that "exercised plasma" may confer additional benefit to the brain compared to young plasma only. Thus, the point of departure was that we wanted the donors to have a fitness level (maximal oxygen uptake) that is regarded as "fit", and not be "classified" as old. We regard 40 years of age to be a relatively young age, and we, therefore, chose to require that donors were below the age of 40 and have a maximal oxygen uptake above 55 mL/kg/min that is above the average for a 20-year-old man in Norway (43).

Settings and participants - Patients

Patients will be recruited from the Department of Neurology or Geriatrics out-patient clinics at St. Olavs Hospital, and from the Department of Geriatric psychiatry, Levanger Hospital, both Norway. The diagnostic work up of the patient will decide if the patient is eligible for inclusion. Eligible patients who sign informed consent to join the study go through a further screening and are evaluated regarding the defined inclusion and exclusion criteria.

Patient inclusion criteria:

Patients will be included in the study if they meet **all the following criteria**:

- Signed informed consent
- Age 50-75 years
- Diagnosis AD in early phase according to the IWG-2 criteria (44)
- In-vivo evidence of Alzheimer´s pathology (one of the following):
- Decreased Aβ42 together with increased t-tau or p-tau in CSF
- Increased tracer retention on amyloid PET
- Mini-Mental State Examination (MMSE) Score ≥20
- Availability of a next of kin who knows the patient well and is willing to accompany the subject to all trial visits and give information about the patient's functional level
- The patient is judged fit for the study and capable to cooperate in treatment and follow-up.
- Ability to communicate in Norwegian or another Scandinavian language

Patient exclusion criteria:

Patients will be excluded from the study if they meet **any of the following criteria**:

- Pregnancy or unwilling to use adequate birth control for the duration of and 6 months beyond study participation. Defined according to Clinical Trial Facilitation Group document "Recommendations related to contraception and pregnancy testing in clinical trials"
- Positive for Hepatitis B, Hepatitis C or HIV at screening
- Not qualified to give consent at inclusionAny other condition judged to interfere with the safety of the patient or the intent and conduct of the study

Related to medical history:

- Stroke
- Anaphylaxis
- Prior adverse reaction to any human blood product
- Any history of a blood coagulation disorder or hypercoagulability
- Congestive heart failure, defined as any previous heart failure hospitalization, or current symptomatic heart failure in New York heart Association class ≥II with reduced, mid-range or preserved ejection fraction
- Coagulation defect or hypercoagulopathy
- Uncontrolled hypertension
- Renal failure
- Prior intolerance to intravenous fluids
- Recent history of uncontrolled atrial fibrillation
- Bone marrow transplant
- IgA deficiency
- Severe protein S deficiency
- Thrombocytopenia (platelets < 40 x 10^9/L)
- Contraindication for Octaplasma

Related to medications or other treatments:

- Any concurrent use of anticoagulant therapy, clopidogrel or acetylsalicylic acid/dipyridamole in combination
- Initiation or change in the dosage of a acetylcholine esterase inhibitor (AChEI) or memantine during the trial (week 0-52). Participants will be urged to start on AChEI when diagnosis is communicated, and must be on a stable dose for at least one month prior to screening
- Concurrent participation in another treatment trial for AD. If there was prior participation, the last dose of the investigational agent must have been given at least 6 months prior to screening, except if the patient received placebo medication
- Prior or concurrent participation in amyloid antibody trials, except if the patient received placebo medication

- Treatment with any human blood product, including intravenous immunoglobulin, during the 6 months prior to screening or during the trial
- Concurrent daily treatment with benzodiazepines, typical or atypical antipsychotics, long-acting opioids, or other medications that are judged to interfere with cognition. Intermittent treatment with short-acting benzodiazepines or atypical antipsychotics may be permitted, provided that no dose is administered within 72 hours prior to cognitive assessment

Related to magnetic resonance imaging:

- Claustrophobia
- Any metallic surgical implant, like a pacemaker or clip incompatible with MRI
  Certain metallic implants like joint prostheses may be permitted, provided that specific manufacturer specifications are available, and that the device is known to be safe for 7T MRI. In case a patient is not eligible for the 7T scanner, the 3T scanner will be used

198

Treatment and examinations

The main study consists of 6 rounds of examinations in addition to plasma transfusions, mainly during the time span of one year, and once 2 years after baseline. A follow-up visit is also planned 5 years after baseline.

Treatment

For this study, ExPlas (plasma from fit donors) and Octaplasma are defined as Investigational10 Medicinal Products. ExPlas is plasma drawn by plasmapheresis once a month for 4 months, from a total of 30 donors (aged 18-40, BMI ≤27 kg/m^2 and VO_{2max} >55 mL/kg/min). All units will be virus inactivated by the Intercept method (CERUS corporation, US), in accordance with the instructions from the manufacturer and the procedures at the Blood Bank at St. Olavs Hospital.

Octaplasma is human pooled plasma produced by Octapharma (Lachen, Switzerland). The rationale for including Octaplasma is to separate the effect of ExPlas from the "general untrained" plasma pooled from thousands of donors (relatively young men). Placebo for this study is isotonic saline (0.9% sodium chloride) (Fresenius Kabi, Halden, Norway). Comparison with isotonic saline allows differentiation from a non-blood product. ExPlas and Octaplasma are stored at ≤-18°C until the time of transfusion. The transfusion volume will be 200 mL at every time point for all three treatments.

Cognitive test battery

All tests will be undertaken at baseline, weeks 24, 52 and 104 after inclusion, and performance at all time points will be evaluated against baseline values.

CERAD Ten-word test will be used as a measure of objective evidence of an amnestic syndrome of the hippocampal type (45).

Mini-Mental State Examination Score – (MMSE-NR-3) will be used as a screening tool for cognitive function (46). The test consists of standardized questions within five areas: orientation for time and place, short-term memory, attention, short-term recall and language. The test may help to evaluate degree of cognitive impairment. The maximum score is 30 (47).

Trail-Making test A and B – (TMT-NR3) will be used to measure visual attention, processing speed and executive function (48, 49).

Clock Drawing Test is a cognitive screening tool and will be used as a supplement for examining visuospatial function and executive function (50).

COWAT-FAS. The Controlled Oral Word Association Test (COWAT)-FAS will be used to measure verbal fluency and executive function (51, 52).

VOSP - Visuospatial abilities will be evaluated with the silhouettes test from the Visual Object and Space Perception Battery. The test also assesses semantic memory and name retrieval (53, 54).

Clinical Dementia Rating Scale (CDR) is a clinical scale for the staging of dementia. The participant is rated from 0-3 on six cognitive and behavioral categories: memory, orientation, judgement and problem solving, community affairs, home and hobbies, and personal care. The Global score is calculated according to an established algorithm, where memory is considered the primary category and all others are secondary categories. A global score of 0 equals no dementia, 0,5 questionable dementia, 1 mild dementia, 2 moderate dementia and 3 severe dementia (55, 56). The Sum of Boxes score is a continuous measure of dementia severity and ranges from 0-18. The CDR Sum of Boxes are found to be adequate for use in prodromal AD and continued use is warranted and recommended in

clinical trials because it is continuous and provides a greater variation in values (47). Both the CDR-Global score and Sum of Boxes will be calculated.

The Lawton Instrumental Activities of Daily Living Scale (IADL). This IADL scale evaluate eight items, related to complex everyday activities, and each can be scored 0 that equals "dependent" and 1 that reflect "completely independent". Change from 0 to 1 in any of the eight items is considered a "clinically relevant change" (57).

Unified Parkinson's Disease Rating Scale (UPDRS). The motor examination part of UPDRS will be used to detect development of parkinsonian motor signs, such as tremor, rigidity, bradykinesia, and postural-gait abnormalities (58).

The 6-minute walk test

Fitness level will be measured using the 6 minute walk-test which is a good alternative to direct measurement of PeakVO_2 (59). The 6-minute walk test is considered safer for the current patient group than a treadmill test.

Structural- and functional MRI

For increased sensitivity we will use multiparameric MRI at 7T MRI to assess brain structure and function to uncover both neurodegenerative and cerbrovasular changes from baseline to 1, 2 and 5 years. The 7T protocol consists of the following scans: whole brain 3D MP2RAGE, gradient echo SWI/Quantitative susceptibility mapping (QSM), and FLAIR, high resolution T2 weighted spin echo sequence of the medial temporal lobe, multishell DTI, asl FLAIR, and rs-fMRI.The primary outcomes are hippocampal and entorhinal cortex structure ((subfield)-volume/thickness, diffusion characteristics, T2*-mapping) and function (blood flow, connectivity and BOLD signal). Other quantitative measures include brain morphometry (e.g., parenchymal fraction, at-risk AD pattern volume loss), and diffusion metrics in white and grey matter. Cerebrovascular pathology (perfusion, white matter hyperintensities, perivascular spaces (quantitative from mulitshell DTI), microhaemorrhages, micro- and macro- infarction) will be evaluated. For participants where 7T is contraindicated, but not 3T, a similar examination will take place using 3T.

A secondary aim is to identify any effect of treatment group on MRI markers of both neurodegenerative and cerebrovascular disease. MRI at 7T allows for a stronger signal and better anatomical localization in less time, but with the stronger magnetic field there are also more contraindications.

Quality of Life

Quality of life will be evaluated by the questionnaire SF-36, which contains questions about physical and mental health, pain, vitality and general health perceptions (60, 61).

Echocardiography

All patients will undergo echocardiography examination at screening and four times during the first year, and potentially at 2 and 5-years follow up. Screening echo is performed to ensure safety of transfusions for patients included in the study. Patients with reduced cardiac function will be excluded due to risk of developing heart failure triggered by fluid overload. Screening echocardiography serve as baseline for included patients. Following echocardiography will be performed at week 5 (one week after 4 weeks of transfusion treatment), week 20 (before treatment period two), week 48 (before treatment period three), week 52 (one week after 4 weeks of transfusion treatment), and assumedly at 2 and 5-years follow up. The echocardiography examination will be a complete examitation of cardiac structure and function, including ultrahigh framerate recordings at each time point.

Biomarkers in blood and spinal fluid

Although no single ideal biomarker yet exists for AD, there are substances currently considered to be 'core' biomarkers for AD. According to a landmark paper published in 2017 from the joint proceedings of the International Working Group (IWG) and the American Alzheimer's Association, the most important early indicators of incipient AD are changes in two key proteins: reduced amyloid-beta 1-42 (Aβ42) and increased total tau protein and hyperphosphorylated tau measured biochemically in cerebrospinal fluid (CSF), or increased deposition of amyloid plaque and neurofibrillary tangles of tau protein in brain as shown by PET(62-64). Since PET is exceptionally expensive, we plan to analyse these substances in CSF. Collected cerebrospinal fluid and blood will be analysed for established AD risk markers (including APOE genotyping in Blood and Amyloid Beta 1-42, Amyloid Beta 1-40, phosphor tau and total tau in spinal fluid). Individuals apparently without clinical symptoms of cognitive decline but with pathological levels of both these biomarkers are considered to have "preclinical AD". If only one of the biomarkers is found to have a pathological level, the individual is considered to be "at risk of AD" (the difference being that the individual is only at risk of AD, whereas preclinical AD is considered to be an early manifestation of the disease itself). In order to better understand the potential link between the cardiovascular system and the brain, the collected blood will also be analysed with respect to cardiovascular profile (Albumin, Ferritin, Natrium, Kalium, Kreatinin, Glukose, ALAT, GT, Kolesterol, Triglyserider, HDL, Hs-CRP, NT-proBNP, Troponin, Leukocytter, Trombocytter, HB, HbA1c). Some of the biological material will be stored for future analysis in the search for new biomarkers. For instance, the study group has previously identified potential "fitness-microRNAs" that could distinguish high- and low-fitness individuals (65). In the ExPlas Study we aim (as a start) to detect microRNAs that show a significant change in expression concomitant with ExPlas treatment and examine changes in level in relation to speed of AD progression. Unbiased screenings of microRNAs will be performed at the NTNU Genomics Core Facility and analysed together with the NTNU Bioinformatics Core Facility. Identification of other circulating factors will be performed by gel-free shotgun proteomics analysis. Proteomic RNA analyses will be performed at the NTNU Proteomics and Modomics Experimental Core Facility and analysed together with the NTNU Bioinformatics Core Facility.

Blood sampling procedures

All blood samples will be taken by trained biomedical engineer or nurse. Serum and plasma samples are collected with venous puncture using sterile disposable equipment in serum-gel, EDTA plasma and litsium heparine tubes. The tubes are centrifuged and stored on ice while shipped to further handling and analysis. If not analysed right away, samples are to be stored at -80ºC (in the established Trønderbrain biobank, Director of the biobank is Geir Bråthen; geir.brathen@ntnu.no, tel. 72575077). Blood tests will be taken on 7 occasions in addition to screening and after 2 years as well as offered 5 years after baseline (Figure 1).

Spinal puncture procedures

Lumbar puncture will be performed by neuorolgist. A thin needle is inserted into the spinal canal in the lowerback, while the patient is lying down on the side. The procedure is done using sterile technique (the area is cleaned with chlorhexidine and air dried before inserting the needle). The sample is collected directly into polypropylene tubes (used for dementia markers), and stored on ice until shipped to further handling. The samples are to be stored at -80ºC (Trønderbrain biobank, Geir Bråthen) until analysis. The sample will be analysed for risk genes and AD related biomarkers. Some portion of the sample will be stored for future analysis. Spinal puncture will be performed at baseline, week 24, week 52, after 2 years, and offered 5 years after baseline (Figure 1). All sample collection, handling and analysis will be performed in accordance with hospital/laboratory standard procedures.

Sample size and statistics

We do not expect that ExPlas will cause more adverse effects than Octaplasma, but have made the following considerations about power calculations related to safety. The most common reaction to transfusion of Octaplasma is allergy and occurs in up to 1% of the elderly. If we consider that ExPlas would cause a dramatic increase in allergic reactions, of e.g. 35% vs. 1% after Octaplasma treatment, 19 participants will be needed in each group to show significant differences (with alpha 0.05 and power of 0.8). There are substantial uncertainties in the assumptions for this power calculation. But considering that another study found that transfusing plasma from young donors to patients (n=18) with mild cognitive impairment or early AD was safe with no adverse events (39) we find it likely that 20 patients in each group will be enough to test safety in ExPlas.

The magnitude of a possible treatment effect of ExPlas is currently not established. The following information has been established: i) a difference of 2 points on the Mini-Mental State Examination Score (MMSE) primarily between those receiving ExPlas vs. Octaplasma will be clinically relevant after 1 and 2 years; ii) Based on several clinical studies in this population, we expect an average MMSE-NR-3 of about 24 in our population, iii) Based on previous studies, we expect a standard deviation of MMSE-NR-3 score of approximately 3. These assumptions suggest that 17 participants are needed in each group (with alpha 0.05, and power 0.8) to demonstrate a clinically relevant effect of ExPlas. Given potential dropouts, it is reasonable to include 20 in each group. The plan is therefore to include 60 patients in the study.

For the primary endpoints, counts will be reported and compared using recommended methods for analysis of contingency tables (58). Secondary and other endpoints will be analyzed using mixed models with the outcome variable as dependent variable, treatment group, time and their interaction as categorical covariates, and patient as random effect. In these analyses, we will adjust for the baseline value of the outcome variable, as recommended (66, 67).

Ethics

The study will be performed according to the Declaration of Helsinki. Written informed consent will be obtained from all participants by the treating neurologist, and participation is voluntary. Patients will be insured according to Norwegian regulations for patients involved in medical research (npe.no). The patients' abilities to keep track of the objectives of the project and assess its relevance will progressively deteriorate during the project period. In view of this, all participants are required to include a next of kin who will follow them throughout the study and represent the patient's interest. The burden from participation, number of tests and time points of conducting tests during the study have been planned in dialogue with the user group consisting of three next of kin of current and previous AD-patients. The study is approved by the Regional Committee for Medical and Health Research Ethics (REK 2018/702) and the Norwegian Medicines Agency (Statens Legemiddelverk, EudraCT No. 2018-000148-24).

Organization

The steering committee of ExPlas has developed the study protocol and is responsible for overall study management, data collection, analyses, publications, and the final data set.

A safety committee consisting of two clinicians (one neurologist and one specialist in Transfusion Medicine) has been appointed to ensure the safety of study participants. In case of adverse events, the safety committee will evaluate whether treatments can continue or must be stopped. A study nurse will observe the patients during and for one hour after infusion and a physician will evaluate the patients in case of adverse effects. Neither the safety committee nor the attending physician responsible for each infusion are involved in other parts of the study and they will not be blinded for the treatment given.

Study monitors

The primary goal of the study monitors is to ensure that the site follows the standardized operation procedures described for the trial, and to report and manage any deviations that may occur from the investigational plan. The ExPlas Study has been appointed two study monitors by the Unit for Applied Clinical research at NTNU, one who has the overall overview of the study, and is blinded to the treatment randomization, and one who is unblinded. A study monitoring plan has been developed and includes regular visits by the Clinical Study Monitors (headed by Sigve Nyvik Aas, sigve.n.aas@ntnu.no), who will check the following:

- Informed consent process
- Reporting of adverse events and all other safety data
- Adherence to protocol
- Maintenance of required regulatory documents
- Study Supply accountability
- Facilities and equipments (treatment storage and manufacturing at the Blood Bank)
- Data completion on the CRFs including source data verification (SDV).

The monitor will review the relevant CRFs for accuracy and completeness and will ask the site staff to adjust any discrepancies as required. Sponsor's representatives (e.g., monitors, auditors) and/or competent authorities will be allowed access to source data for source data verification in which case a review of those parts of the hospital records relevant to the study will be required.

Data management

The Clinical Data Management System (CDMS) used for the electronic case report forms (eCRF) in this study is The Unit for Applied Clinical Research at NTNU. The setup of the study specific eCRF in the CDMS will be performed by The Unit for Applied Clinical Research at NTNU. The eCRF system will be FDA Code of Federal Regulations 21 Part 11 compliant. The designated investigator staff will enter the data required by the protocol into the eCRF. The Investigator is responsible for assuring that data entered into the eCRF is complete, accurate, and that entry is performed in a timely manner. The signature of the investigator will attest the accuracy of the data on each eCRF. If any assessments are omitted, the reason for such omissions will be noted on the eCRFs. Corrections, with the reason for the corrections will also be recorded. After database lock, the investigator will receive a digital copy of the subject data for archiving at the investigational site.

The medical records of each patient will clearly describe at least:

- That the patient is participating in the study, by including the enrollment number and the study code or other study identification;
- Date when Informed Consent was obtained from the patient and statement that patient received a copy of the signed and dated Informed Consent;
- Results of all assessments confirming a patient's eligibility for the study;
- Diseases (past and current; both the disease studied and others, as relevant);
- Surgical history, as relevant;
- Treatments withdrawn/withheld due to participation in the study;
- Results of assessments performed during the study;
- Treatments given, changes in treatments during the study and the time points for the changes;
- Visits to the clinic / telephone contacts during the study, including those for study purposes only;
- Non-Serious Adverse Events and Serious Adverse Events (if any) including causality assessments;
- Date of, and reason for, discontinuation from study treatment;
- Date of, and reason for, withdrawal from study;
- Date of death and cause of death, if available;

- Additional information according to local regulations and practice.

Data management will be performed by the Unit for Applied Clinical Research at NTNU (Berit Bjelkåsen). The Data management procedures will be performed in accordance with the department's SOPs and ICH guidelines. The data management process will be described in the study specific data handling plan and the study specific data handling report after database closure. Data entered into the eCRF will be validated as defined in the data validation plan. Validation includes, but is not limited to, validity checks (e.g. range checks), consistency checks and customised checks (logical checks between variables to ensure that study data are accurately reported) for eCRF data and external data (e.g. laboratory data). A majority of edit checks will be triggered during data entry and will therefore facilitate efficient 'point of entry' data cleaning. Data management personnel will perform both manual eCRF review and review of additional electronic edit checks to ensure that the data are complete, consistent and reasonable. The electronic edit checks will run continually throughout the course of the study and the issues will be reviewed manually online to determine what action needs to be taken. Manual queries may be added to the system by clinical data management or study monitor. Clinical data managers and study monitors are able to remotely and proactively monitor the patient eCRFs to improve data quality. All updates to queried data will be made by authorised study centre personnel only and all modifications to the database will be recorded in an audit trail. Once the queries have been resolved, eCRFs will be signed by electronic signature Any changes to signed eCRFs will be approved and resigned by the Investigator. Once the full set of eCRFs have been completed and locked, the Sponsor will authorise database lock and all electronic data will be sent to the designated statistician for analysis. Subsequent changes to the database will then be made only by written agreement. The data will be stored in a dedicated and secured area at NTNU. Data will be stored in a de-identified manner, where each study participant is recognisable by his/her unique trial subject number. The data will be stored until 15 years after database lock.

Patients and public involvement

To ensure a high study quality and relevance a user board consisting of three next kin of present and past AD patients has been established. As we conduct research on a patient group that is considered vulnerable, this board is particularly important. We have met the user group on several occasions while working on the study protocol (first meeting in Feb 2017) and have received input on several matters, such as how to ensure a tolerable load of participation for the individual patient/relatives. The board will continue to consult the study team twice annually throughout the study period, on implementation, results, and future developments. They are encouraged to give their opinions regarding the project as a whole and particularly on the patients' well-being. The study group has already made changes and adjustments in the planned protocols based on feedback from the user group. The study has a user representative who participates in meetings and presentations of the study to the general public. On initiative from the ExPlas user group, we are currently making three information videos about "AD and participation in research studies", for AD patients and their families, where patients and their next of kin tell their story to help new patients and their next of kin in the coming process. These videos will also be used in the recruitment phase of the study to inform and motivate to take part in ExPlas.

Dissemination

Direct communication with users and patient organisations: ExPlas Study group regularly present at various meetings of patient organizations (such as the National Association for Public Health) and for senior citizens' societies. This type of contact with the public, patients and relatives has proven mutually useful. We plan to intensify participation in such meetings to inform about current knowledge about prevention and treatment of AD, particularly via the established user groups. Communication via Internet: One of the most important media for spreading the news and awareness will be the Internet. The results and information (including videos) about the studies will be presented on CERG's webpage (ntnu.edu/cerg), one of the most visited sites at Scandinavian universities; and ExPlas's own

Norweian webpage (ntnu.no/cerg/explas). Scientific and non-scientific communication: General communication activities include publication in open access peer reviewed journals, non-scientific journals and at national- and international meetings, to reach the general public, patients, scientists, and policy makers. Importantly, our group is closely linked and active partners in the Norwegian Research School in Neuroscience, Physical Activity and Health (master program) and medical education where we actively will present our research to the next generation of health care personnel and scientists. We also have a journalist at CERG (Anders Revdal), available for ExPlas, who will be responsible for communication.

DISCUSSION

To our knowledge, ExPlas will be the first study with the aim of studying safety and efficacy of transfusion of plasma from well endurance trained donors to patients in the early symptomatic phase of AD. Even if prevention probably will be the most effective way to reduce numbers of patients with AD worldwide, we need a cure for this devastating disease which impacts the lives for both patients and their families substantially. There is also a need to understand the mechanisms behind the beneficial effect of physical exercise on the brain, and it seems logically to try to exploit this effect in treatment of the early phase of symptomatic AD.

On June 7th, 2021, the U.S. Food and Drug Administration, approved aducanumab (marketed as Aduhelm) for use in treatment of AD (68), due to its ability to reduce amyloid plaques in the brain, under an accelerated approval pathway (69). Confirmation of the clinical benefit is still required to be confirmed for continued approval (68). Independent of the usefulness of aducanumab in AD therapeutics, other interventions capable of delaying the clinical onset of AD dementia should continue to be studied. The findings from preclinical AD models (36-38, 70), and a small clinical trial (39) clearly indicate that there is communication between the systemic environment and the hippocampus. Systemic factors are capable of inducing changes, and even therapeutic effects, in the brain without hindrance by the blood-brain barrier; potentially an effective strategy to reverse neurodegeneration in the AD-brain (30). There are myriads of factors and processes that are set in motion during and after exercise training, and much of this is reflected in the composition of the blood (71). Thus, it is not likely that it is a single factor is orchestrating the beneficial effects of exercise, but rather an interplay between several molecular factors that need to be discovered and understood to develop the first-generation of exercise-mimicking drugs. This is a promising idea as a large population of patients are simply unable to exercise, such as patients in the intensive care unit, the critically ill, patients recovering from accidents, the morbidly obese and paralyzed patients. For these patients, innovative exercise-mimicking therapies could be of benefit.

However, development of exercise-mimicking therapies is a very complex and time-consuming undertaking, that should not delay the testing of a potential benefit of exercise trained plasma, with most of its natural components, on safety and therapeutic effect in patients with AD. In the context of lack of disease-modifying treatments, ExPlas is innovative and potentially groundbreaking if it is found to be safe with few side effects and with similar promising results as seen in preclinical AD models (35-38).

Another key question is at what stage of AD interventions such as ExPlas treatment can be expected to have an effect. Today we know that AD-related changes in the brain are present 10-30 years before symptoms develop. The optimal time window for treatment is probably as early as possible during this period, to decelerate or stop the progressive loss of neurons before functions are lost. As in all diseases, prevention will always be the optimal path. Depending upon outcomes in the ExPlas Study, a natural next step may be treatment with ExPlas of patients in the preclinical phase of the disease (62).

A small clinical trial found that plasma from young donors (young blood) transfused to patients with mild to moderate AD dementia (MMSE score ranging from 12-24) was safe with no adverse events and possibly beneficial with improvement in functional activity. In this study, 9 patients were randomized to a cross-over cohort, receiving 4 once-weekly infusions of either 250 mL of plasma from male donors (aged 18-30 years) or 250 mL of saline, followed by a 6-week washout and crossover to 4 once-weekly infusions of the alternate treatment. In addition, 9 patients were included in an open-label design in which patients received 4 once-weekly infusions of only young plasma. Considering the low number of patients, short follow-up period and promising findings in the study by Sha *et al.* (39) there is reason to believe that transfusion of exercise-trained plasma also is safe. With increased treatment periods and extended follow-up, we believe the ExPlas Study is well designed also to evaluate the potential therapeutic effect of exercise trained plasma. The relatively large number of patients will also likely enable us to assess whether endpoints become differentially affected by *APOE* 4 status. As the ExPlas Study is the first of its kind it is not straightforward to undertake power calculations, and the results of our study may be useful for planning of an appropriately sample sized study in the future.

We expect the ExPlas study to give new knowledge about whether transfusion of plasma from exercise-trained donors is safe and indications on whether it has therapeutic effects. ExPlas will also contribute to pioneering the discovery of molecular targets to potentially treat AD and lay the foundation for first-generation exercise-mimicking drugs, by capturing the molecular signature of high-fitness and molecular mechanisms provided by exercise.

Contributor statement

Atefe R. Tari Conception and design of the study, obtained funding, drafting the manuscript and applications to ethical committee and Norwegian Medicine Agency for study approval

Helene H. Berg Design of the study, drafting the manuscript, critical review of manuscript

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Geir Selbæk Design of the study, critical review of the manuscript, obtained funding

Stian Lydersen Design of the study, critical review of the manuscript, making the statistical plan and analyses in ExPlas

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Kjell Rune Halvorsrud Design of the study, critical review of the manuscript, supervision, established methods and operating procedures for the blood-donor part of ExPlas, supervision, obtained funding

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Competing interests

None of the authors reports any competing interests or had financial relationships with any organisations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work. All authors will complete the ICMJE uniform disclosure form in due time.

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Authors reports no financial relationships with any organisations that might have an interest in the submitted work in the previous three years, or any other relationships or activities that could appear to have influenced the submitted work.

Country of Recruitment

Norway

Trial sponsor

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Data sharing

We are not permitted to share individual data from the current trial, but we are open to collaborative research with researchers worldwide, who can have access to analysed data from our university. We have also established a biobank of blood and genetic material that we plan to share with researchers worldwide, but individual data must be analysed within our university only.

References

1. World Health Organization. Aging and Life Course 2015 [Available from: http://www.who.int/ageing/en/.
2. alz.org. Alzheimer's Disease Facts and Figures [Report]. 2018 [updated 2018. 5,8]. Available from: http://www.alz.org.
3. Andrieu S, Coley N, Lovestone S, Aisen PS, Vellas B. Prevention of sporadic Alzheimer's disease: lessons learned from clinical trials and future directions. Lancet Neurol. 2015;14(9):926-44.
4. Helsedirektoratet d. Demensplan 2025.
5. GjØra L, Strand BH, Bergh S, Borza T, Brækhus A, Engedal K, et al. Current and Future Prevalence Estimates of Mild Cognitive Impairment, Dementia, and Its Subtypes in a Population-Based Sample of People 70 Years and Older in Norway: The HUNT Study. J Alzheimers Dis. 2021;79(3):1213-26.
6. Kvello-Alme M, Bråthen G, White LR, Sando SB. The Prevalence and Subtypes of Young Onset Dementia in Central Norway: A Population-Based Study. J Alzheimers Dis. 2019;69(2):479-87.
7. Association As. 2021 Alzheimer's Disease Facts and Figures. 2021.
8. Potter PE. Investigational medications for treatment of patients with Alzheimer disease. J Am Osteopath Assoc. 2010;110(9 Suppl 8):S27-36.
9. Mullane K, Williams M. Alzheimer's therapeutics: continued clinical failures question the validity of the amyloid hypothesis-but what lies beyond? Biochem Pharmacol. 2013;85(3):289-305.
10. Cummings JL, Morstorf T, Zhong K. Alzheimer's disease drug-development pipeline: few candidates, frequent failures. Alzheimers Res Ther. 2014;6(4):37.
11. Livingston G, Huntley J, Sommerlad A, Ames D, Ballard C, Banerjee S, et al. Dementia prevention, intervention, and care: 2020 report of the Lancet Commission. Lancet. 2020;396(10248):413-46.
12. Barnes DE, Yaffe K. The projected effect of risk factor reduction on Alzheimer's disease prevalence. Lancet Neurol. 2011;10(9):819-28.
13. Colcombe SJ, Erickson KI, Raz N, Webb AG, Cohen NJ, McAuley E, et al. Aerobic fitness reduces brain tissue loss in aging humans. The journals of gerontology. 2003;58(2):176-80.
14. de Bruijn RF, Ikram MA. Cardiovascular risk factors and future risk of Alzheimer's disease. BMC Med. 2014;12:130.
15. Levine DA, Langa KM. Vascular cognitive impairment: disease mechanisms and therapeutic implications. Neurotherapeutics. 2011;8(3):361-73.
16. Rovio S, Kareholt I, Helkala EL, Viitanen M, Winblad B, Tuomilehto J, et al. Leisure-time physical activity at midlife and the risk of dementia and Alzheimer's disease. The Lancet Neurology. 2005;4(11):705-11.
17. Qiu C, Xu W, Fratiglioni L. Vascular and psychosocial factors in Alzheimer's disease: epidemiological evidence toward intervention. J Alzheimers Dis. 2010;20(3):689-97.
18. Qiu C. Preventing Alzheimer's disease by targeting vascular risk factors: hope and gap. J Alzheimers Dis. 2012;32(3):721-31.
19. Exalto LG, Quesenberry CP, Barnes D, Kivipelto M, Biessels GJ, Whitmer RA. Midlife risk score for the prediction of dementia four decades later. Alzheimer's & dementia : the journal of the Alzheimer's Association. 2014;10(5):562-70.

20. Tolppanen AM, Solomon A, Soininen H, Kivipelto M. Midlife vascular risk factors and Alzheimer's disease: evidence from epidemiological studies. J Alzheimers Dis. 2012;32(3):531-40.
21. Norton S, Matthews FE, Barnes DE, Yaffe K, Brayne C. Potential for primary prevention of Alzheimer's disease: an analysis of population-based data. Lancet Neurol. 2014;13(8):788-94.
22. Kaminsky LA, Arena R, Beckie TM, Brubaker PH, Church TS, Forman DE, et al. The importance of cardiorespiratory fitness in the United States: the need for a national registry: a policy statement from the American Heart Association. Circulation. 2013;127(5):652-62.
23. Larson EB, Wang L, Bowen JD, McCormick WC, Teri L, Crane P, et al. Exercise is associated with reduced risk for incident dementia among persons 65 years of age and older. Ann Intern Med. 2006;144(2):73-81.
24. Tari AR, Nauman J, Zisko N, Skjellegrind HK, Bosnes I, Bergh S, et al. Temporal changes in cardiorespiratory fitness and risk of dementia incidence and mortality: a population-based prospective cohort study. The Lancet Public Health. 2019;4(11):e565-e74.
25. Hoffmann K, Frederiksen KS, Sobol NA, Beyer N, Vogel A, Simonsen AH, et al. Preserving cognition, quality of life, physical health and functional ability in Alzheimer's disease: the effect of physical exercise (ADEX trial): rationale and design. Neuroepidemiology. 2013;41(3-4):198-207.
26. Lautenschlager NT, Cox KL, Flicker L, Foster JK, van Bockxmeer FM, Xiao J, et al. Effect of physical activity on cognitive function in older adults at risk for Alzheimer disease: a randomized trial. Jama. 2008;300(9):1027-37.
27. van Praag H, Shubert T, Zhao C, Gage FH. Exercise enhances learning and hippocampal neurogenesis in aged mice. J Neurosci. 2005;25(38):8680-5.
28. van Praag H, Christie BR, Sejnowski TJ, Gage FH. Running enhances neurogenesis, learning, and long-term potentiation in mice. Proc Natl Acad Sci U S A. 1999;96(23):13427-31.
29. Maass A, Duzel S, Goerke M, Becke A, Sobieray U, Neumann K, et al. Vascular hippocampal plasticity after aerobic exercise in older adults. Molecular psychiatry. 2015;20(5):585-93.
30. Bouchard J, Villeda SA. Aging and brain rejuvenation as systemic events. J Neurochem. 2015;132(1):5-19.
31. Pereira AC, Huddleston DE, Brickman AM, Sosunov AA, Hen R, McKhann GM, et al. An in vivo correlate of exercise-induced neurogenesis in the adult dentate gyrus. Proc Natl Acad Sci U S A. 2007;104(13):5638-43.
32. Fabel K, Fabel K, Tam B, Kaufer D, Baiker A, Simmons N, et al. VEGF is necessary for exercise-induced adult hippocampal neurogenesis. The European journal of neuroscience. 2003;18(10):2803-12.
33. Castellano JM, Mosher KI, Abbey RJ, McBride AA, James ML, Berdnik D, et al. Human umbilical cord plasma proteins revitalize hippocampal function in aged mice. Nature. 2017;544(7651):488-92.
34. Tari AR, Norevik CS, Scrimgeour NR, Kobro-Flatmoen A, Storm-Mathisen J, Bergersen LH, et al. Are the neuroprotective effects of exercise training systemically mediated? Progress in cardiovascular diseases. 2019.
35. Villeda SA, Plambeck KE, Middeldorp J, Castellano JM, Mosher KI, Luo J, et al. Young blood reverses age-related impairments in cognitive function and synaptic plasticity in mice. Nat Med. 2014;20(6):659-63.

36. Katsimpardi L, Litterman NK, Schein PA, Miller CM, Loffredo FS, Wojtkiewicz GR, et al. Vascular and neurogenic rejuvenation of the aging mouse brain by young systemic factors. Science. 2014;344(6184):630-4.

37. Xia E, Xu F, Hu C, Kumal JPP, Tang X, Mao D, et al. Young Blood Rescues the Cognition of Alzheimer's Model Mice by Restoring the Hippocampal Cholinergic Circuit. Neuroscience. 2019;417:57-69.

38. Zhao Y, Qian R, Zhang J, Liu F, Iqbal K, Dai CL, et al. Young blood plasma reduces Alzheimer's disease-like brain pathologies and ameliorates cognitive impairment in 3×Tg-AD mice. Alzheimers Res Ther. 2020;12(1):70.

39. Sha SJ, Deutsch GK, Tian L, Richardson K, Coburn M, Gaudioso JL, et al. Safety, Tolerability, and Feasibility of Young Plasma Infusion in the Plasma for Alzheimer Symptom Amelioration Study: A Randomized Clinical Trial. JAMA Neurol. 2018.

40. Horowitz AM, Fan X, Bieri G, Smith LK, Sanchez-Diaz CI, Schroer AB, et al. Blood factors transfer beneficial effects of exercise on neurogenesis and cognition to the aged brain. Science. 2020;369(6500):167-73.

41. Aspenes ST, Nilsen TI, Skaug EA, Bertheussen GF, Ellingsen Ø, Vatten L, et al. Peak oxygen uptake and cardiovascular risk factors in 4631 healthy women and men. Med Sci Sports Exerc. 2011;43(8):1465-73.

42. Nauman J, Nes BM, Zisko N, Revdal A, Myers J, Kaminsky LA, et al. Personal Activity Intelligence (PAI): A new standard in activity tracking for obtaining a healthy cardiorespiratory fitness level and low cardiovascular risk. Progress in cardiovascular diseases. 2019;62(2):179-85.

43. Aspenes ST, Nilsen TI, Skaug EA, Bertheussen GF, Ellingsen O, Vatten L, et al. Peak oxygen uptake and cardiovascular risk factors in 4631 healthy women and men. Med Sci Sports Exerc. 2011;43(8):1465-73.

44. Dubois B, Feldman HH, Jacova C, Hampel H, Molinuevo JL, Blennow K, et al. Advancing research diagnostic criteria for Alzheimer's disease: the IWG-2 criteria. Lancet Neurol. 2014;13(6):614-29.

45. Morris JC, Heyman A, Mohs RC, Hughes JP, van Belle G, Fillenbaum G, et al. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part I. Clinical and neuropsychological assessment of Alzheimer's disease. Neurology. 1989;39(9):1159-65.

46. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res. 1975;12(3):189-98.

47. McDougall F, Edgar C, Mertes M, Delmar P, Fontoura P, Abi-Saab D, et al. Psychometric Properties of the Clinical Dementia Rating - Sum of Boxes and Other Cognitive and Functional Outcomes in a Prodromal Alzheimer's Disease Population. J Prev Alzheimers Dis. 2021;8(2):151-60.

48. Reitan RM. Validity of the Trail Making Test as an indication of organic brain damage. Perceptual and motor skills. 1958;8(3):271-6.

49. Espenes J, Hessen E, Eliassen IV, Waterloo K, Eckerström M, Sando SB, et al. Demographically adjusted trail making test norms in a Scandinavian sample from 41 to 84 years. Clin Neuropsychol. 2020;34(sup1):110-26.

50. Brodaty H, Moore CM. The Clock Drawing Test for dementia of the Alzheimer's type: A comparison of three scoring methods in a memory disorders clinic. Int J Geriatr Psychiatry. 1997;12(6):619-27.

51. Nutter-Upham KE, Saykin AJ, Rabin LA, Roth RM, Wishart HA, Pare N, et al. Verbal fluency performance in amnestic MCI and older adults with cognitive complaints. Arch Clin Neuropsychol. 2008;23(3):229-41.
52. Patterson C. World Alzheimer report 2018. Alzheimer's Disease International; 2018.
53. Elizabeth K Warrington MJ. Visual Object and Space Perception Battery (VOSP)1991.
54. Binetti T. Disorders of visual and spatial perception in the early stage of Alzheimers disease1996.
55. Hughes CP, Berg L, Danziger WL, Coben LA, Martin RL. A new clinical scale for the staging of dementia. Br J Psychiatry. 1982;140:566-72.
56. O'Bryant SE, Humphreys JD, Smith GE, Ivnik RJ, Graff-Radford NR, Petersen RC, et al. Detecting dementia with the mini-mental state examination in highly educated individuals. Arch Neurol. 2008;65(7):963-7.
57. Lawton MP, Brody EM. Assessment of older people: self-maintaining and instrumental activities of daily living. Gerontologist. 1969;9(3):179-86.
58. Fagerland M, Lydersen, S., & Laake, P. Statistical Analysis of Contingency Tables. Chapman and Hall/CRC2017.
59. Ries JD, Echternach JL, Nof L, Gagnon Blodgett M. Test-retest reliability and minimal detectable change scores for the timed "up & go" test, the six-minute walk test, and gait speed in people with Alzheimer disease. Phys Ther. 2009;89(6):569-79.
60. Geschke K, Fellgiebel A, Laux N, Schermuly I, Scheurich A. Quality of life in dementia: impact of cognition and insight on applicability of the SF-36. Am J Geriatr Psychiatry. 2013;21(7):646-54.
61. Ware JE, Jr., Sherbourne CD. The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. Med Care. 1992;30(6):473-83.
62. Dubois B, Hampel H, Feldman HH, Scheltens P, Aisen P, Andrieu S, et al. Preclinical Alzheimer's disease: Definition, natural history, and diagnostic criteria. Alzheimers Dement. 2016;12(3):292-323.
63. Bateman RJ, Xiong C, Benzinger TL, Fagan AM, Goate A, Fox NC, et al. Clinical and biomarker changes in dominantly inherited Alzheimer's disease. N Engl J Med. 2012;367(9):795-804.
64. Dubois B, Hampel H, Feldman HH, Scheltens P, Aisen P, Andrieu S, et al. Preclinical Alzheimer's disease: Definition, natural history, and diagnostic criteria. Alzheimers Dement. 2016;12(3):292-323.
65. Bye A, Røsjø H, Aspenes ST, Condorelli G, Omland T, Wisløff U. Circulating microRNAs and aerobic fitness--the HUNT-Study. PLoS One. 2013;8(2):e57496.
66. J T, L B, T H, J R, M W, M H. Different ways to estimate treatment effects in randomised controlled trials. Contemp Clin Trials Commun. 2018;10:80-5.
67. Coffman CJ, Edelman D, Woolson RF. To condition or not condition? Analysing 'change' in longitudinal randomised controlled trials. BMJ Open. 2016;6(12):e013096.
68. Cavazzoni P. FDA's Decision to Approve New Treatment for Alzheimer's Disease: fda.gov; 06/07/2021 [Available from: https://www.fda.gov/drugs/news-events-human-drugs/fdas-decision-approve-new-treatment-alzheimers-disease.
69. Sevigny J, Chiao P, Bussière T, Weinreb PH, Williams L, Maier M, et al. The antibody aducanumab reduces Aβ plaques in Alzheimer's disease. Nature. 2016;537(7618):50-6.
70. Villeda SA, Plambeck KE, Middeldorp J, Castellano JM, Mosher KI, Luo J, et al. Young blood reverses age-related impairments in cognitive function and synaptic plasticity in mice. Nature Medicine. 2014;20:659.

71. Tari AR, Norevik CS, Scrimgeour NR, Kobro-Flatmoen A, Storm-Mathisen J, Bergersen LH, et al. Are the neuroprotective effects of exercise training systemically mediated? Progress in cardiovascular diseases. 2019;62(2):94-101.

Figure text

Figure 1. Flow chart of the ExPlas Study. Infusion solution, Sodium Chloride 0.9% (Saline); US, ultrasound of the heart; fMRI, functional magnetic resonance imaging.

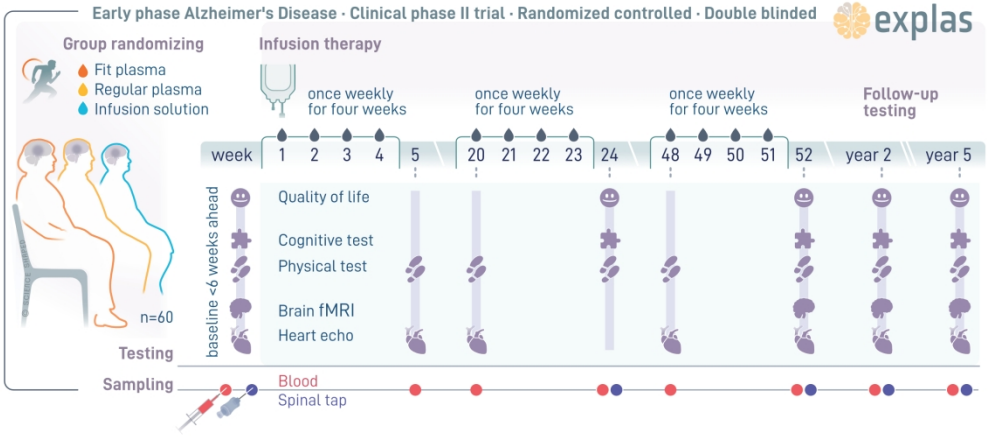

Figure 1. Flow chart of the ExPlas Study. Infusion solution, Sodium Chloride 0.9% (Saline); US, ultrasound of the heart; fMRI, functional magnetic resonance imaging.

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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	Page /Line No
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	p. 1/ 1-2
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	p. 1/ 49
	2b	All items from the World Health Organization Trial Registration Data Set	p. 1-16
Protocol version	3	Date and version identifier	p. 1/ 44
Funding	4	Sources and types of financial, material, and other support	p.16/ 610 -617
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	p. 1 and 14-15
	5b	Name and contact information for the trial sponsor	p. 17/ 625-629
	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	p. 16/ 618-620
	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)	p. 11/ 375 -377
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	p. 2-4
	6b	Explanation for choice of comparators	p. 4-5, 8
Objectives	7	Specific objectives or hypotheses	p. 4

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)	p. 4/ 135 -140
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	p. 4/ 142 -160
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)	p. 5/ 187 -189
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	p. 4 /135 -141 + fig.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)	p. 11 /378 -384
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	p. 5/ 187 -189
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	p. 4 / 99-124 + fig.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)	Fig. 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	p. 11/ 336 -360
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	p. 13/ 460 -475

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	p. 4/ 140 -145
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	p. 4/ 140 -145
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	p. 4/ 140 -145
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	p. 4/ 140 -145
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	p. 11/ 387 -384

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	p. 8-10/ 190 -334
	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	p. 13/ 460 -475
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	p. 12/ 407 -458
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	p. 11/ 336 -360
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	p. 11/ 336 -360

	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)	p. 11/ 336 -360
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	NA. The project has an independent safety committee
	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	p. 11/ 379 -385
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	p. 11/ 379 -385
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	p. 12/ 387 -406
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	p. 11/ 362 -372
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)	p. 11-12/ 387-459
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	p. 11/ 363 -364
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	
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	p. 12/ 407 -417
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	p. 16/ 618 -620
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	p. 12/ 407 -417

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	
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	p. 13-14/ 477-493
	31b	Authorship eligibility guidelines and any intended use of professional writers	p. 15/ 559 -598
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	p. 17/ 631 -635
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Attachment
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	p. 17/ 631 -635

*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.