

BMJ Open

BMJ Open is committed to open peer review. As part of this commitment we make the peer review history of every article we publish publicly available.

When an article is published we post the peer reviewers' comments and the authors' responses online. We also post the versions of the paper that were used during peer review. These are the versions that the peer review comments apply to.

The versions of the paper that follow are the versions that were submitted during the peer review process. They are not the versions of record or the final published versions. They should not be cited or distributed as the published version of this manuscript.

BMJ Open is an open access journal and the full, final, typeset and author-corrected version of record of the manuscript is available on our site with no access controls, subscription charges or pay-per-view fees (<http://bmjopen.bmj.com>).

If you have any questions on BMJ Open's open peer review process please email info.bmjopen@bmj.com

BMJ Open

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

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2021-056964
Article Type:	Protocol
Date Submitted by the Author:	01-Sep-2021
Complete List of Authors:	<p>Tari, Atefe R.; Norwegian University of Science and Technology Faculty of Medicine and Health Sciences; St Olavs Hospital Trondheim University Hospital, Department of Neurology and Clinical Neurophysiology Berg, Helene Haugen; Norwegian University of Science and Technology Faculty of Medicine and Health Sciences; St Olavs Hospital Trondheim University Hospital, Department of Neurology and Clinical Neurophysiology Videm, Vibeke; Norges teknisk-naturvitenskapelige universitet Bråthen, Geir; St Olavs Hospital Trondheim University Hospital Neurology Clinic, Department of Neurology and Clinical Neurophysiology; Norwegian University of Science and Technology, Department of Neuromedicine and Movement Science White, Linda R.; Norwegian University of Science and Technology Faculty of Medicine and Health Sciences, Department of Neuromedicine and Movement Science Røsbjørgen, Ragnhild Nyhus; Norwegian University of Science and Technology Faculty of Medicine and Health Sciences Scheffler, Katja; St Olavs Hospital Trondheim University Hospital, Department of Neurology and Clinical Neurophysiology; Norwegian University of Science and Technology Faculty of Medicine and Health Sciences, Department of Neuromedicine and Movement Science Dalen, Havard; Nord-Trøndelag Hospital Trust, Medicine; Norwegian University of Science and Technology Faculty of Medicine and Health Sciences, MI Lab and Department of Circulation and Medical Imaging Holte, Espen; St Olavs Hospital Trondheim University Hospital Haberg, Asta; Norges Teknisk Naturvitenskapelige Universitet Institutt for biologi Selbaek, Geir; Norwegian National Advisory Unit on Ageing and Health, Centre for Old Age Psychiatric Research Lydersen, Stian; Norwegian University of Science and Technology Faculty of Medicine and Health Sciences, Regional Centre for Child and Youth Mental Health and Child Welfare Duezal, Emrah; German Centre for Neurodegenerative Diseases Site Magdeburg Bergh, Sverre; Innlandet Hospital Trust Hamar Hospital Logan-Halvorsrud, Kjell Rune; St Olavs Hospital Trondheim University Hospital, Department of Immunology and Transfusion Medicine Sando, Sigrid Botne; St Olavs Hospital Trondheim University Hospital, Department of Neurology and Clinical Neurophysiology; Norwegian</p>

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

	University of Science and Technology Faculty of Medicine and Health Sciences, Department of Neuromedicine and Movement Science Wisløff, Ulrik; Norwegian University of Science and Technology Faculty of Medicine and Health Sciences
Keywords:	Neurophysiology < NEUROLOGY, PREVENTIVE MEDICINE, Dementia < NEUROLOGY



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

Atefe R. Tari^{1,2}, Helene Haugen Berg^{1,2}, Vibeke Videm^{3,4}, Geir Bråthen^{2,5}, Linda R. White⁵, Ragnhild Nyhus RøsbjØrger¹, Katja Scheffler^{2,5}, Håvard Dalen^{1,6,7}, Espen Holte⁶, Asta Håberg^{5,8}, Geir Selbæk^{9,10,11}, Stian Lydersen¹², Emrah Duzel^{13,14,15}, Sverre Bergh^{9,16}, Kjell Rune Logan-Halvorsrud⁴, Sigrild Botne Sando^{2,5}, Ulrik Wisløff^{1,17}

1. Cardiac Exercise Research Group at the Department of Circulation and Medical Imaging, NTNU - Norwegian University of Science and Technology, Trondheim, Norway
2. Department of Neurology and Clinical Neurophysiology, St. Olavs University Hospital, Trondheim, Norway
3. Department of Clinical and Molecular Medicine, NTNU – Norwegian University of Science and Technology, Trondheim, Norway
4. Department of Immunology and Transfusion Medicine, St. Olavs University Hospital, Trondheim, Norway.
5. Department of Neuromedicine and Movement Science, NTNU – Norwegian University of Science and Technology, Trondheim, Norway
6. Clinic of Cardiology, St. Olavs University Hospital, Trondheim, Norway
7. Department of Medicine, Levanger Hospital, Nord-Trøndelag Hospital Trust, Levanger, Norway
8. Department of Radiology and Nuclear Medicine, St. Olavs University Hospital, Trondheim, Norway
9. Norwegian National Advisory Unit on Ageing and Health, Vestfold Hospital Trust, Tønsberg, Norway
10. Department of Geriatric Medicine, Oslo University Hospital-Ullevål, Oslo, Norway
11. Faculty of Medicine, University of Oslo, Oslo, Norway
12. Department of Mental Health, Regional Centre for Child and Youth Mental Health and Child Welfare, NTNU - Norwegian University of Science and Technology, Trondheim, Norway
13. German Center for Neurodegenerative Diseases (DZNE), Magdeburg, Germany
14. Institute of Cognitive Neurology and Dementia Research, Otto-von-Guericke University Magdeburg, Magdeburg, Germany
15. Center for Behavioral Brain Sciences (CBBS), Magdeburg, Germany
16. Research Centre for Age-related Functional Decline and Disease, Innlandet Hospital Trust, Ottestad, Norway
17. School of Human Movement and Nutrition Science, University of Queensland, Queensland, Australia

Address for correspondence:

Ulrik Wisløff, email: ulrik.wisloff@ntnu.no

Cardiac Exercise Research Group at the Department of Circulation and Medical Imaging, NTNU - Norwegian University of Science and Technology, Olav Kyrres gt. 9, 7489 Trondheim, Norway.

Protocol version no. 3.0 – 05.01.2021

Study start: first donor included 01.03.2021, first patient screened 19.08.2021, first infusion planned for 22.08.2021

Trial registration: EudraCT No. 2018-000148-24

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.

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
- Active participation in study design, recruitment and dissemination 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 (PeakVO₂). In line with this we demonstrated in a prospective cohort study of 30 695 adults that participants who increased or sustained high PeakVO₂ 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 PeakVO₂ 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-borne 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/m²
- 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 A β 42 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

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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 \geq 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 $< 40 \times 10^9/L$)
- 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/m² 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 $\leq -18^{\circ}\text{C}$ 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 amnesic 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

1
2
3 clinical trials because it is continuous and provides a greater variation in values (45). Both the CDR-
4 Global score and Sum of Boxes will be calculated.
5

6 **The Lawton Instrumental Activities of Daily Living Scale (IADL).** This IADL scale evaluate eight items,
7 related to complex everyday activities, and each can be scored 0 that equals “dependent” and 1 that
8 reflect “completely independent”. Change from 0 to 1 in any of the eight items is considered a
9 “clinically relevant change” (55).
10

11
12 **Unified Parkinson’s Disease Rating Scale (UPDRS).** The motor examination part of UPDRS will be used
13 to detect development of parkinsonian motor signs, such as tremor, rigidity, bradykinesia, and
14 postural-gait abnormalities (56).
15

16 The 6-minute walk test

17 Fitness level will be measured using the 6 minute walk-test which is a good alternative to direct
18 measurement of PeakVO₂ (57). The 6-minute walk test is considered safer for the current patient group
19 than a treadmill test.
20
21

22 Structural- and functional MRI

23 For increased sensitivity we will use multiparameric MRI at 7T MRI to assess brain structure and
24 function to uncover both neurodegenerative and cerebrovascular changes from baseline to 1, 2 and 5
25 years. The 7T protocol consists of the following scans: whole brain 3D MP2RAGE, gradient echo
26 SWI/Quantitative susceptibility mapping (QSM), and FLAIR, high resolution T2 weighted spin echo
27 sequence of the medial temporal lobe, multishell DTI, asl FLAIR, and rs-fMRI. The primary outcomes
28 are hippocampal and entorhinal cortex structure ((subfield)-volume/thickness, diffusion
29 characteristics, T2*-mapping) and function (blood flow, connectivity and BOLD signal). Other
30 quantitative measures include brain morphometry (e.g., parenchymal fraction, at-risk AD pattern
31 volume loss), and diffusion metrics in white and grey matter. Cerebrovascular pathology (perfusion,
32 white matter hyperintensities, perivascular spaces (quantitative from multishell DTI),
33 microhaemorrhages, micro- and macro- infarction) will be evaluated. For participants where 7T is
34 contraindicated, but not 3T, a similar examination will take place using 3T.
35
36
37

38 A secondary aim is to identify any effect of treatment group on MRI markers of both
39 neurodegenerative and cerebrovascular disease. MRI at 7T allows for a stronger signal and better
40 anatomical localization in less time, but with the stronger magnetic field there are also more
41 contraindications.
42
43

44 Quality of Life

45 Quality of life will be evaluated by the questionnaire SF-36, which contains questions about physical
46 and mental health, pain, vitality and general health perceptions (58, 59).
47
48

49 Echocardiography

50 All patients will undergo echocardiography examination at screening and four times during the first
51 year, and potentially at 2 and 5-years follow up. Screening echo is performed to ensure safety of
52 transfusions for patients included in the study. Patients with reduced cardiac function will be excluded
53 due to risk of developing heart failure triggered by fluid overload. Screening echocardiography serve
54 as baseline for included patients. Following echocardiography will be performed at week 5 (one week
55 after 4 weeks of transfusion treatment), week 20 (before treatment period two), week 48 (before
56 treatment period three), week 52 (one week after 4 weeks of transfusion treatment), and assumedly
57 at 2 and 5-years follow up. The echocardiography examination will be a complete examination of
58 cardiac structure and function, including ultrahigh framerate recordings at each time point.
59
60

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 (A β 42) 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 lithium 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 -80°C (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 neurologist. 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 -80°C (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 Øvreneess, Torbjorn.Ovreneess@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.

1
2
3 Data management will be performed by the Unit for Applied Clinical Research at NTNU (Berit
4 Bjelkåsen). The Data management procedures will be performed in accordance with the department's
5 SOPs and ICH guidelines. The data management process will be described in the study specific data
6 handling plan and the study specific data handling report after database closure. Data entered into the
7 eCRF will be validated as defined in the data validation plan. Validation includes, but is not limited to,
8 validity checks (e.g. range checks), consistency checks and customised checks (logical checks between
9 variables to ensure that study data are accurately reported) for eCRF data and external data (e.g.
10 laboratory data). A majority of edit checks will be triggered during data entry and will therefore
11 facilitate efficient 'point of entry' data cleaning. Data management personnel will perform both
12 manual eCRF review and review of additional electronic edit checks to ensure that the data are
13 complete, consistent and reasonable. The electronic edit checks will run continually throughout the
14 course of the study and the issues will be reviewed manually online to determine what action needs
15 to be taken. Manual queries may be added to the system by clinical data management or study
16 monitor. Clinical data managers and study monitors are able to remotely and proactively monitor the
17 patient eCRFs to improve data quality. All updates to queried data will be made by authorised study
18 centre personnel only and all modifications to the database will be recorded in an audit trail. Once the
19 queries have been resolved, eCRFs will be signed by electronic signature Any changes to signed eCRFs
20 will be approved and resigned by the Investigator. Once the full set of eCRFs have been completed and
21 locked, the Sponsor will authorise database lock and all electronic data will be sent to the designated
22 statistician for analysis. Subsequent changes to the database will then be made only by written
23 agreement. The data will be stored in a dedicated and secured area at NTNU. Data will be stored in a
24 de-identified manner, where each study participant is recognisable by his/her unique trial subject
25 number. The data will be stored until 15 years after database lock.
26
27
28
29

30 Patients and public involvement

31 To ensure a high study quality and relevance a user board consisting of three next kin of present and
32 past AD patients has been established. As we conduct research on a patient group that is considered
33 vulnerable, this board is particularly important. We have met the user group on several occasions while
34 working on the study protocol (first meeting in Feb 2017) and have received input on several matters,
35 such as how to ensure a tolerable load of participation for the individual patient/relatives. The board
36 will continue to consult the study team twice annually throughout the study period, on
37 implementation, results, and future developments. They are encouraged to give their opinions
38 regarding the project as a whole and particularly on the patients' well-being. The study group has
39 already made changes and adjustments in the planned protocols based on feedback from the user
40 group. The study has a user representative who participates in meetings and presentations of the study
41 to the general public. On initiative from the ExPlas user group, we are currently making three
42 information videos about "AD and participation in research studies", for AD patients and their families,
43 where patients and their next of kin tell their story to help new patients and their next of kin in the
44 coming process. These videos will also be used in the recruitment phase of the study to inform and
45 motivate to take part in ExPlas.
46
47
48

49 Dissemination

50 Direct communication with users and patient organisations: ExPlas Study group regularly present at
51 various meetings of patient organizations (such as the National Association for Public Health) and for
52 senior citizens' societies. This type of contact with the public, patients and relatives has proven
53 mutually useful. We plan to intensify participation in such meetings to inform about current knowledge
54 about prevention and treatment of AD, particularly via the established user groups. Communication
55 via Internet: One of the most important media for spreading the news and awareness will be the
56 Internet. The results and information (including videos) about the studies will be presented on CERG's
57 webpage (ntnu.edu/cerg), one of the most visited sites at Scandinavian universities; and ExPlas's own
58 Norwegian webpage (ntnu.no/cerg/expas). Scientific and non-scientific communication: General
59
60

1
2
3 communication activities include publication in open access peer reviewed journals, non-scientific
4 journals and at national- and international meetings, to reach the general public, patients, scientists,
5 and policy makers. Importantly, our group is closely linked and active partners in the Norwegian
6 Research School in Neuroscience, Physical Activity and Health (master program) and medical education
7 where we actively will present our research to the next generation of health care personnel and
8 scientists. We also have a journalist at CERG (Anders Revdal), available for ExPlas, who will be
9 responsible for communication.
10

11 DISCUSSION

12
13
14 To our knowledge, ExPlas will be the first study with the aim of studying safety and efficacy of
15 transfusion of plasma from well endurance trained donors to patients in the early symptomatic phase
16 of AD. Even if prevention probably will be the most effective way to reduce numbers of patients with
17 AD worldwide, we need a cure for this devastating disease which impacts the lives for both patients
18 and their families substantially. There is also a need to understand the mechanisms behind the
19 beneficial effect of physical exercise on the brain, and it seems logically to try to exploit this effect in
20 treatment of the early phase of symptomatic AD.
21
22

23
24 On June 7th, 2021, the U.S. Food and Drug Administration, approved aducanumab (marketed as
25 Aduhelm) for use in treatment of AD (63), due to its ability to reduce amyloid plaques in the brain,
26 under an accelerated approval pathway (64). Confirmation of the clinical benefit is still required to be
27 confirmed for continued approval (63). Independent of the usefulness of aducanumab in AD
28 therapeutics, other interventions capable of delaying the clinical onset of AD dementia should
29 continue to be studied. The findings from preclinical AD models (36-38, 65), and a small clinical trial
30 (39) clearly indicate that there is communication between the systemic environment and the
31 hippocampus. Systemic factors are capable of inducing changes, and even therapeutic effects, in the
32 brain without hindrance by the blood-brain barrier; potentially an effective strategy to reverse
33 neurodegeneration in the AD-brain (30). There are myriads of factors and processes that are set in
34 motion during and after exercise training, and much of this is reflected in the composition of the blood
35 (66). Thus, it is not likely that it is a single factor is orchestrating the beneficial effects of exercise, but
36 rather an interplay between several molecular factors that need to be discovered and understood to
37 develop the first-generation of exercise-mimicking drugs. This is a promising idea as a large population
38 of patients are simply unable to exercise, such as patients in the intensive care unit, the critically ill,
39 patients recovering from accidents, the morbidly obese and paralyzed patients. For these patients,
40 innovative exercise-mimicking therapies could be of benefit.
41
42

43
44 However, development of exercise-mimicking therapies is a very complex and time-consuming
45 undertaking, that should not delay the testing of a potential benefit of exercise trained plasma, with
46 most of its natural components, on safety and therapeutic effect in patients with AD. In the context of
47 lack of disease-modifying treatments, ExPlas is innovative and potentially groundbreaking if it is found
48 to be safe with few side effects and with similar promising results as seen in preclinical AD models (35-
49 38).
50

51
52 Another key question is at what stage of AD interventions such as ExPlas treatment can be expected
53 to have an effect. Today we know that AD-related changes in the brain are present 10-30 years before
54 symptoms develop. The optimal time window for treatment is probably as early as possible during this
55 period, to decelerate or stop the progressive loss of neurons before functions are lost. As in all
56 diseases, prevention will always be the optimal path. Depending upon outcomes in the ExPlas Study,
57 a natural next step may be treatment with ExPlas of patients in the preclinical phase of the disease
58 (67).
59
60

1
2
3 A small clinical trial found that plasma from young donors (young blood) transfused to patients with
4 mild to moderate AD dementia (MMSE score ranging from 12-24) was safe with no adverse events and
5 possibly beneficial with improvement in functional activity. In this study, 9 patients were randomized
6 to a cross-over cohort, receiving 4 once-weekly infusions of either 250 mL of plasma from male donors
7 (aged 18-30 years) or 250 mL of saline, followed by a 6-week washout and crossover to 4 once-weekly
8 infusions of the alternate treatment. In addition, 9 patients were included in an open-label design in
9 which patients received 4 once-weekly infusions of only young plasma. Considering the low number of
10 patients, short follow-up period and promising findings in the study by Sha *et al.* (39) there is reason
11 to believe that transfusion of exercise-trained plasma also is safe. With increased treatment periods
12 and extended follow-up, we believe the ExPlas Study is well designed also to evaluate the potential
13 therapeutic effect of exercise trained plasma. The relatively large number of patients will also likely
14 enable us to assess whether endpoints become differentially affected by *APOE 4* status. As the ExPlas
15 Study is the first of its kind it is not straightforward to undertake power calculations, and the results of
16 our study may be useful for planning of an appropriately sample sized study in the future.
17
18

19
20 In conclusion, we expect the ExPlas study to give new knowledge about whether transfusion of plasma
21 from exercise-trained donors is safe and indications on whether it has therapeutic effects. ExPlas will
22 also contribute to pioneering the discovery of molecular targets to potentially treat AD and lay the
23 foundation for first-generation exercise-mimicking drugs, by capturing the molecular signature of high-
24 fitness and molecular mechanisms provided by exercise.
25

26 27 **Contributor statement**

28 **Atefe R. Tari** Conception and design of the study, obtained funding, drafting the
29 manuscript and applications to ethical committee and Norwegian Medicine Agency for study
30 approval
31

32 **Helene H. Berg** Conception of the study, drafting the manuscript, critical review of
33 manuscript, applications to ethical committee and Norwegian Medicine Agency for study approval
34

35 **Vibeke Videm** Conception and design of the study, critical review of the manuscript,
36 supervision, obtained funding, applications to ethical committee and Norwegian Medicine Agency
37 for study approval
38

39 **Geir Bråthen** Conception and design of the study, critical review of the manuscript,
40 supervision, obtained funding, applications to ethical committee and Norwegian Medicine Agency
41 for study approval
42

43 **Linda R. White** Critical review of the manuscript, supervision, obtained funding, applications
44 to ethical committee and Norwegian Medicine Agency for study approval
45

46 **Ragnhild RøsbjØrgen** Critical review of the manuscript, supervision, established methods and
47 operating procedures for the study
48

49 **Katja Scheffler** Conception and design of the study, critical review of the manuscript,
50 supervision, obtained funding, applications to ethical committee and Norwegian Medicine Agency
51 for study approval
52

53 **Håvard Dalen** Critical review of the manuscript, established echocardiographic procedures
54 for AD patients in ExPlas, supervision, obtained funding
55

56 **Espen Holte** Critical review of the manuscript, established echocardiographic procedures
57 for AD patients in ExPlas, supervision, obtained funding
58

59 **Asta Håberg** Conception and design of the study, critical review of the manuscript,
60 supervision, obtained funding, applications to ethical committee and Norwegian Medicine Agency
for study approval

1
2
3 **Geir Selbæk** Conception and design of the study, critical review of the manuscript,
4 supervision, obtained funding, applications to ethical committee and Norwegian Medicine Agency
5 for study approval
6

7 **Stian Lydersen** Conception and design of the study, critical review of the manuscript,
8 supervision, obtained funding, applications to ethical committee and Norwegian Medicine Agency
9 for study approval, making the statistical plan and analyses in ExPlas
10

11 **Emrah Duzel** Critical review of the manuscript, supervision, established methods and
12 operating procedures for the study, supervision, obtained funding
13

14 **Sverre Bergh** Conception and design of the study, critical review of the manuscript,
15 supervision, obtained funding, applications to ethical committee and Norwegian Medicine Agency
16 for study approval
17

18 **Kjell Rune Halvorsrud** Critical review of the manuscript, supervision, established methods and
19 operating procedures for the blood-donor part of ExPlas, supervision, obtained funding
20

21 **Sigrid B. Sando** Conception and design of the study, critical review of the manuscript,
22 supervision, obtained funding, applications to ethical committee and Norwegian Medicine Agency
23 for study approval, Co-PI of ExPlas
24

25 **Ulrik Wisløff** Conception and design of the study, critical review of the manuscript,
26 supervision, obtained funding, applications to ethical committee and Norwegian Medicine Agency
27 for study approval, Co-PI of ExPlas
28

29 **Acknowledgement**

30
31 ExPlas study group acknowledge Randi Granbo, Tone Stav and Geir Suul for their active role in ExPlas
32 user group.
33

34 **Competing interests**

35
36
37 None of the authors reports any competing interests or had financial relationships with any
38 organisations that might have an interest in the submitted work in the previous three years; no other
39 relationships or activities that could appear to have influenced the submitted work. All authors will
40 complete the ICMJE uniform disclosure form in due time.
41
42

43 **Funding**

- 44
45 ■ Research Council of Norway;
- 46
47 ■ The K.G. Jebsen Foundation for Medical Research, Norway;
- 48
49 ■ Norwegian University of Science and Technology (NTNU);
- 50
51 ■ Central Norway Regional Health Authority;
- 52
53 ■ St Olavs hospital, Trondheim, Norway;
- 54
55 ■ The National Association for Public Health, Norway
- 56
57 ■ The Liaison Committee for Central Norway Regional Health Authority

58
59 Authors reports no financial relationships with any organisations that might have an interest in the
60 submitted work in the previous three years, or any other relationships or activities that could appear
to have influenced the submitted work.

Trial sponsor

Øystein Risa, Head of Department

Department of Circulation and Medical Imaging, NTNU - Norwegian University of Science and Technology, Box 8905 MTF5, 7491 Trondheim, Norway. Tel: (+47) 92613734

E-mail: oystein.risa@ntnu.no

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. Helseidirektoratet 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.

- 1
- 2
- 3
- 4 20. Tolppanen AM, Solomon A, Soininen H, Kivipelto M. Midlife vascular risk factors and
- 5 Alzheimer's disease: evidence from epidemiological studies. *J Alzheimers Dis.*
- 6 2012;32(3):531-40.
- 7 21. Norton S, Matthews FE, Barnes DE, Yaffe K, Brayne C. Potential for primary
- 8 prevention of Alzheimer's disease: an analysis of population-based data. *Lancet Neurol.*
- 9 2014;13(8):788-94.
- 10 22. Kaminsky LA, Arena R, Beckie TM, Brubaker PH, Church TS, Forman DE, et al. The
- 11 importance of cardiorespiratory fitness in the United States: the need for a national registry:
- 12 a policy statement from the American Heart Association. *Circulation.* 2013;127(5):652-62.
- 13 23. Larson EB, Wang L, Bowen JD, McCormick WC, Teri L, Crane P, et al. Exercise is
- 14 associated with reduced risk for incident dementia among persons 65 years of age and older.
- 15 *Ann Intern Med.* 2006;144(2):73-81.
- 16 24. Tari AR, Nauman J, Zisko N, Skjellegrind HK, Bosnes I, Bergh S, et al. Temporal
- 17 changes in cardiorespiratory fitness and risk of dementia incidence and mortality: a
- 18 population-based prospective cohort study. *The Lancet Public Health.* 2019;4(11):e565-e74.
- 19 25. Hoffmann K, Frederiksen KS, Sobol NA, Beyer N, Vogel A, Simonsen AH, et al.
- 20 Preserving cognition, quality of life, physical health and functional ability in Alzheimer's
- 21 disease: the effect of physical exercise (ADEX trial): rationale and design.
- 22 *Neuroepidemiology.* 2013;41(3-4):198-207.
- 23 26. Lautenschlager NT, Cox KL, Flicker L, Foster JK, van Bockxmeer FM, Xiao J, et al. Effect
- 24 of physical activity on cognitive function in older adults at risk for Alzheimer disease: a
- 25 randomized trial. *Jama.* 2008;300(9):1027-37.
- 26 27. van Praag H, Shubert T, Zhao C, Gage FH. Exercise enhances learning and
- 27 hippocampal neurogenesis in aged mice. *J Neurosci.* 2005;25(38):8680-5.
- 28 28. van Praag H, Christie BR, Sejnowski TJ, Gage FH. Running enhances neurogenesis,
- 29 learning, and long-term potentiation in mice. *Proc Natl Acad Sci U S A.* 1999;96(23):13427-
- 30 31.
- 31 29. Maass A, Duzel S, Goerke M, Becke A, Sobieray U, Neumann K, et al. Vascular
- 32 hippocampal plasticity after aerobic exercise in older adults. *Molecular psychiatry.*
- 33 2015;20(5):585-93.
- 34 30. Bouchard J, Villeda SA. Aging and brain rejuvenation as systemic events. *J*
- 35 *Neurochem.* 2015;132(1):5-19.
- 36 31. Pereira AC, Huddleston DE, Brickman AM, Sosunov AA, Hen R, McKhann GM, et al. An
- 37 in vivo correlate of exercise-induced neurogenesis in the adult dentate gyrus. *Proc Natl Acad*
- 38 *Sci U S A.* 2007;104(13):5638-43.
- 39 32. Fabel K, Fabel K, Tam B, Kaufer D, Baiker A, Simmons N, et al. VEGF is necessary for
- 40 exercise-induced adult hippocampal neurogenesis. *The European journal of neuroscience.*
- 41 2003;18(10):2803-12.
- 42 33. Castellano JM, Mosher KI, Abbey RJ, McBride AA, James ML, Berdnik D, et al. Human
- 43 umbilical cord plasma proteins revitalize hippocampal function in aged mice. *Nature.*
- 44 2017;544(7651):488-92.
- 45 34. Tari AR, Norevik CS, Scrimgeour NR, Kobro-Flatmoen A, Storm-Mathisen J, Bergersen
- 46 LH, et al. Are the neuroprotective effects of exercise training systemically mediated?
- 47 *Progress in cardiovascular diseases.* 2019.
- 48 35. Villeda SA, Plambeck KE, Middeldorp J, Castellano JM, Mosher KI, Luo J, et al. Young
- 49 blood reverses age-related impairments in cognitive function and synaptic plasticity in mice.
- 50 *Nat Med.* 2014;20(6):659-63.
- 51
- 52
- 53
- 54
- 55
- 56
- 57
- 58
- 59
- 60

- 1
- 2
- 3 36. Katsimpardi L, Litterman NK, Schein PA, Miller CM, Loffredo FS, Wojtkiewicz GR, et al.
- 4 Vascular and neurogenic rejuvenation of the aging mouse brain by young systemic factors.
- 5 *Science*. 2014;344(6184):630-4.
- 6
- 7 37. Xia E, Xu F, Hu C, Kumal JPP, Tang X, Mao D, et al. Young Blood Rescues the Cognition
- 8 of Alzheimer's Model Mice by Restoring the Hippocampal Cholinergic Circuit. *Neuroscience*.
- 9 2019;417:57-69.
- 10
- 11 38. Zhao Y, Qian R, Zhang J, Liu F, Iqbal K, Dai CL, et al. Young blood plasma reduces
- 12 Alzheimer's disease-like brain pathologies and ameliorates cognitive impairment in 3xTg-AD
- 13 mice. *Alzheimers Res Ther*. 2020;12(1):70.
- 14
- 15 39. Sha SJ, Deutsch GK, Tian L, Richardson K, Coburn M, Gaudioso JL, et al. Safety,
- 16 Tolerability, and Feasibility of Young Plasma Infusion in the Plasma for Alzheimer Symptom
- 17 Amelioration Study: A Randomized Clinical Trial. *JAMA Neurol*. 2018.
- 18
- 19 40. Horowitz AM, Fan X, Bieri G, Smith LK, Sanchez-Diaz CI, Schroer AB, et al. Blood
- 20 factors transfer beneficial effects of exercise on neurogenesis and cognition to the aged
- 21 brain. *Science*. 2020;369(6500):167-73.
- 22
- 23 41. Aspenes ST, Nilsen TI, Skaug EA, Bertheussen GF, Ellingsen Ø, Vatten L, et al. Peak
- 24 oxygen uptake and cardiovascular risk factors in 4631 healthy women and men. *Med Sci*
- 25 *Sports Exerc*. 2011;43(8):1465-73.
- 26
- 27 42. Dubois B, Feldman HH, Jacova C, Hampel H, Molinuevo JL, Blennow K, et al.
- 28 Advancing research diagnostic criteria for Alzheimer's disease: the IWG-2 criteria. *Lancet*
- 29 *Neurol*. 2014;13(6):614-29.
- 30
- 31 43. Morris JC, Heyman A, Mohs RC, Hughes JP, van Belle G, Fillenbaum G, et al. The
- 32 Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part I. Clinical and
- 33 neuropsychological assessment of Alzheimer's disease. *Neurology*. 1989;39(9):1159-65.
- 34
- 35 44. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for
- 36 grading the cognitive state of patients for the clinician. *J Psychiatr Res*. 1975;12(3):189-98.
- 37
- 38 45. McDougall F, Edgar C, Mertes M, Delmar P, Fontoura P, Abi-Saab D, et al.
- 39 Psychometric Properties of the Clinical Dementia Rating - Sum of Boxes and Other Cognitive
- 40 and Functional Outcomes in a Prodromal Alzheimer's Disease Population. *J Prev Alzheimers*
- 41 *Dis*. 2021;8(2):151-60.
- 42
- 43 46. Reitan RM. Validity of the Trail Making Test as an indication of organic brain damage.
- 44 Perceptual and motor skills. 1958;8(3):271-6.
- 45
- 46 47. Espenes J, Hessen E, Eliassen IV, Waterloo K, Eckerström M, Sando SB, et al.
- 47 Demographically adjusted trail making test norms in a Scandinavian sample from 41 to 84
- 48 years. *Clin Neuropsychol*. 2020;34(sup1):110-26.
- 49
- 50 48. Brodaty H, Moore CM. The Clock Drawing Test for dementia of the Alzheimer's type:
- 51 A comparison of three scoring methods in a memory disorders clinic. *Int J Geriatr Psychiatry*.
- 52 1997;12(6):619-27.
- 53
- 54 49. Nutter-Upham KE, Saykin AJ, Rabin LA, Roth RM, Wishart HA, Pare N, et al. Verbal
- 55 fluency performance in amnesic MCI and older adults with cognitive complaints. *Arch Clin*
- 56 *Neuropsychol*. 2008;23(3):229-41.
- 57
- 58 50. Benton ALH, K. deS. *Multilingual Aphasia Examination*: AJA Associates; 1989.
- 59
- 60 51. Elizabeth K Warrington MJ. *Visual Object and Space Perception Battery (VOSP)1991*.
- 52 52. Binetti T. Disorders of visual and spatial perception in the early stage of Alzheimers
- 53 disease1996.
- 54 53. Hughes CP, Berg L, Danziger WL, Coben LA, Martin RL. A new clinical scale for the
- 55 staging of dementia. *Br J Psychiatry*. 1982;140:566-72.
- 56
- 57
- 58
- 59
- 60

- 1
 - 2
 - 3
 - 4
 - 5
 - 6
 - 7
 - 8
 - 9
 - 10
 - 11
 - 12
 - 13
 - 14
 - 15
 - 16
 - 17
 - 18
 - 19
 - 20
 - 21
 - 22
 - 23
 - 24
 - 25
 - 26
 - 27
 - 28
 - 29
 - 30
 - 31
 - 32
 - 33
 - 34
 - 35
 - 36
 - 37
 - 38
 - 39
 - 40
 - 41
 - 42
 - 43
 - 44
 - 45
 - 46
 - 47
 - 48
 - 49
 - 50
 - 51
 - 52
 - 53
 - 54
 - 55
 - 56
 - 57
 - 58
 - 59
 - 60
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](https://www.fda.gov/drugs/news-events-human-drugs/fdas-decision-approve-new-treatment-alzheimers-disease) [Available from: <https://www.fda.gov/drugs/news-events-human-drugs/fdas-decision-approve-new-treatment-alzheimers-disease>].
64. Sevigny J, Chiao P, Bussi re T, Weinreb PH, Williams L, Maier M, et al. The antibody aducanumab reduces A β 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.

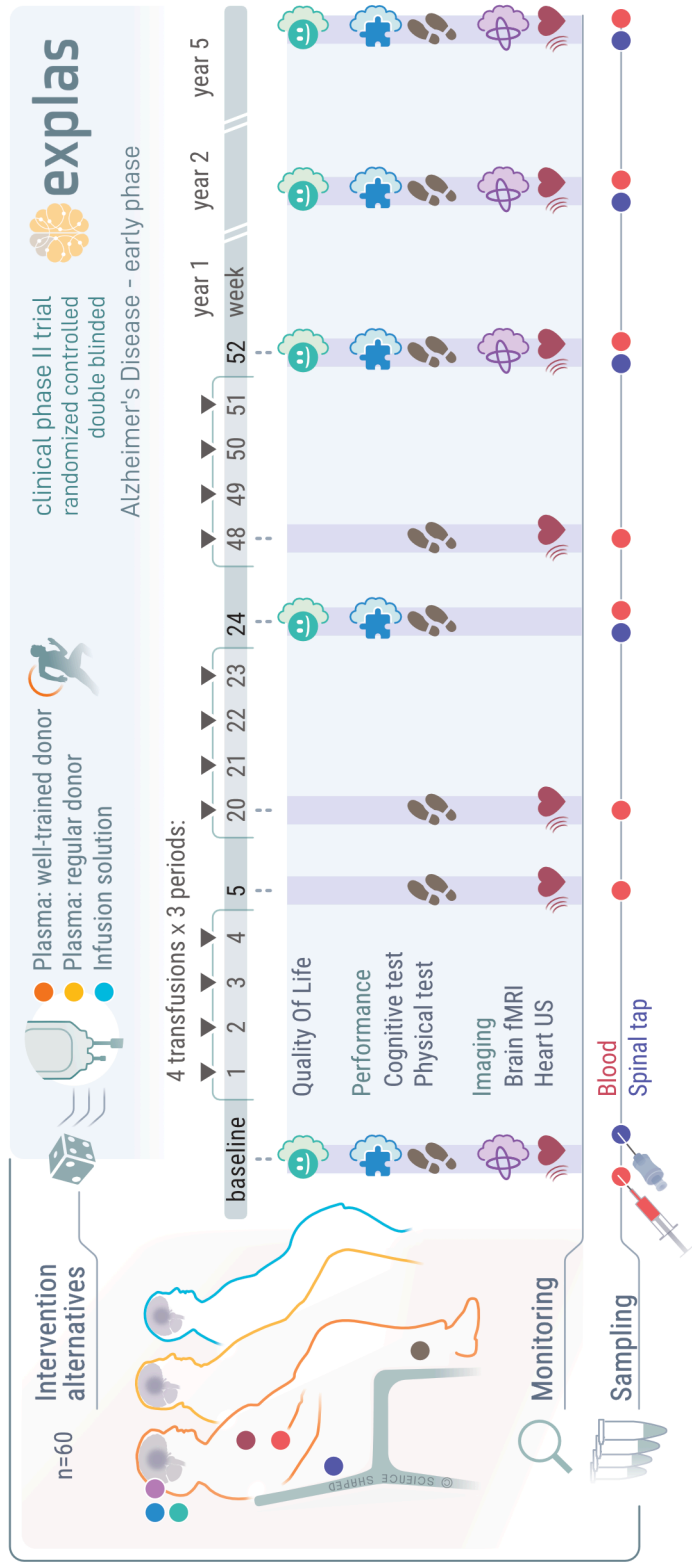
1
2
3 **Figure text**
4
5

6 **Figure 1.** Flow chart of the ExPlas Study. Infusion solution, Sodium Chloride 0.9% (Saline); US,
7 ultrasound of the heart; fMRI, functional magnetic resonance imaging.
8
9

10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For peer review only

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60



BMJ Open

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

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2021-056964.R1
Article Type:	Protocol
Date Submitted by the Author:	01-Mar-2022
Complete List of Authors:	<p>Tari, Atefe R.; Norwegian University of Science and Technology Faculty of Medicine and Health Sciences; St Olavs Hospital Trondheim University Hospital, Department of Neurology and Clinical Neurophysiology Berg, Helene Haugen; Norwegian University of Science and Technology Faculty of Medicine and Health Sciences; St Olavs Hospital Trondheim University Hospital, Department of Neurology and Clinical Neurophysiology Videm, Vibeke; Norges teknisk-naturvitenskapelige universitet Bråthen, Geir; St Olavs Hospital Trondheim University Hospital Neurology Clinic, Department of Neurology and Clinical Neurophysiology; Norwegian University of Science and Technology, Department of Neuromedicine and Movement Science White, Linda R.; Norwegian University of Science and Technology Faculty of Medicine and Health Sciences, Department of Neuromedicine and Movement Science Røsbjørgen, Ragnhild Nyhus; Norwegian University of Science and Technology Faculty of Medicine and Health Sciences Scheffler, Katja; St Olavs Hospital Trondheim University Hospital, Department of Neurology and Clinical Neurophysiology; Norwegian University of Science and Technology Faculty of Medicine and Health Sciences, Department of Neuromedicine and Movement Science Dalen, Havard; Nord-Trøndelag Hospital Trust, Medicine; Norwegian University of Science and Technology Faculty of Medicine and Health Sciences, MI Lab and Department of Circulation and Medical Imaging Holte, Espen; St Olavs Hospital Trondheim University Hospital Haberg, Asta; Norges Teknisk Naturvitenskapelige Universitet Institutt for biologi Selbaek, Geir; Norwegian National Advisory Unit on Ageing and Health, Centre for Old Age Psychiatric Research Lydersen, Stian; Norwegian University of Science and Technology Faculty of Medicine and Health Sciences, Regional Centre for Child and Youth Mental Health and Child Welfare Duezal, Emrah; German Centre for Neurodegenerative Diseases Site Magdeburg Bergh, Sverre; Innlandet Hospital Trust Hamar Hospital Logan-Halvorsrud, Kjell Rune; St Olavs Hospital Trondheim University Hospital, Department of Immunology and Transfusion Medicine Sando, Sigrid Botne; St Olavs Hospital Trondheim University Hospital, Department of Neurology and Clinical Neurophysiology; Norwegian</p>

	University of Science and Technology Faculty of Medicine and Health Sciences, Department of Neuromedicine and Movement Science Wisløff, Ulrik; Norwegian University of Science and Technology Faculty of Medicine and Health Sciences
Primary Subject Heading :	Neurology
Secondary Subject Heading:	Cardiovascular medicine, Patient-centred medicine, Pharmacology and therapeutics, Sports and exercise medicine
Keywords:	Neurophysiology < NEUROLOGY, PREVENTIVE MEDICINE, Dementia < NEUROLOGY, Echocardiography < CARDIOLOGY

SCHOLARONE™
Manuscripts

1 Safety and efficacy of plasma transfusion from exercise-trained donors in patients with early 2 Alzheimer's disease: protocol for the ExPlas Study

3
4 Atefe R. Tari^{1,2}, Helene Haugen Berg^{1,2}, Vibeke Videm^{3,4}, Geir Bråthen^{2,5}, Linda R. White⁵, Ragnhild
5 Nyhus Røsbjørgen¹, Katja Scheffler^{2,5}, Håvard Dalen^{1,6,7}, Espen Holte⁶, Asta Håberg^{5,8}, Geir Selbæk^{9,10,11},
6 Stian Lydersen¹², Emrah Duzel^{13,14,15}, Sverre Bergh^{9,16}, Kjell Rune Logan-Halvorsrud⁴, Sigrild Botne
7 Sando^{2,5}, Ulrik Wisløff^{1,17}

- 8
9 1. Cardiac Exercise Research Group at the Department of Circulation and Medical Imaging, NTNU
10 - Norwegian University of Science and Technology, Trondheim, Norway
- 11 2. Department of Neurology and Clinical Neurophysiology, St. Olavs University Hospital,
12 Trondheim, Norway
- 13 3. Department of Clinical and Molecular Medicine, NTNU – Norwegian University of Science and
14 Technology, Trondheim, Norway
- 15 4. Department of Immunology and Transfusion Medicine, St. Olavs University Hospital,
16 Trondheim, Norway.
- 17 5. Department of Neuromedicine and Movement Science, NTNU – Norwegian University of
18 Science and Technology, Trondheim, Norway
- 19 6. Clinic of Cardiology, St. Olavs University Hospital, Trondheim, Norway
- 20 7. Department of Medicine, Levanger Hospital, Nord-Trøndelag Hospital Trust, Levanger, Norway
- 21 8. Department of Radiology and Nuclear Medicine, St. Olavs University Hospital, Trondheim,
22 Norway
- 23 9. Norwegian National Advisory Unit on Ageing and Health, Vestfold Hospital Trust, Tønsberg,
24 Norway
- 25 10. Department of Geriatric Medicine, Oslo University Hospital-Ullevål, Oslo, Norway
- 26 11. Faculty of Medicine, University of Oslo, Oslo, Norway
- 27 12. Department of Mental Health, Regional Centre for Child and Youth Mental Health and Child
28 Welfare, NTNU - Norwegian University of Science and Technology, Trondheim, Norway
- 29 13. German Center for Neurodegenerative Diseases (DZNE), Magdeburg, Germany
- 30 14. Institute of Cognitive Neurology and Dementia Research, Otto-von-Guericke University
31 Magdeburg, Magdeburg, Germany
- 32 15. Center for Behavioral Brain Sciences (CBBS), Magdeburg, Germany
- 33 16. Research Centre for Age-related Functional Decline and Disease, Innlandet Hospital Trust,
34 Ottestad, Norway
- 35 17. School of Human Movement and Nutrition Science, University of Queensland, Queensland,
36 Australia

37 38 39 **Address for correspondence:**

40 Ulrik Wisløff, email: ulrik.wisloff@ntnu.no

41 Cardiac Exercise Research Group at the Department of Circulation and Medical Imaging, NTNU -
42 Norwegian University of Science and Technology, Olav Kyrres gt. 9, 7489 Trondheim, Norway.

43
44 **Protocol version** no. 3.0 – 05.01.2021.

45
46 **Study start:** first donor included 01.03.2021, first patient screened 19.08.2021, first patient
47 randomized 15.09.2021, first infusion given for 22.09.2021.

48
49 **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.

52 BACKGROUND

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

63 It is hypothesized that around 40-50% of dementia cases worldwide are caused by modifiable risk
64 factors (11, 12), and that many factors associated with higher risk of cardiovascular disease, such as
65 obesity, hyperlipidaemia, hypertension, smoking, diabetes and physical inactivity are also associated
66 with increased risk of AD (12-20). These factors have in common that they can be substantially
67 modified through physical activity that secures above average age- and sex-specific levels of
68 cardiorespiratory fitness (CRF) (12, 19, 21-23) measured as peak oxygen uptake (PeakVO₂). In line with
69 this we demonstrated in a prospective cohort study of 30 695 adults that participants who increased
70 or sustained high PeakVO₂ over time (10 years apart) had 40-50% reduced risk of incident dementia,
71 30-40% reduced risk of dementia-related mortality, approximately 2.0 years delayed dementia onset,
72 and 2-3 years of life gained when compared to persistently unfit individuals (24). Thus, at present
73 exercise training leading to a high age-relative PeakVO₂ may be the most promising preventive "AD-
74 medicine" (25, 26).

75
76 Although it is well established that exercise positively influences brain neurogenesis, plasticity (27, 28),
77 and cognition (21, 27, 29) it is not well understood how these effects are mediated. The beneficial
78 effects of exercise on the brain have traditionally been thought not to be mediated through systemic
79 changes (30). However, a number of studies in both rodents and humans (31-33) demonstrates direct
80 effects on the brain of exercised induced blood-borne molecules crossing the the blood-brain barrier
81 (34). For instance, systemic administration of blood from young mice into old mice counteracts age-
82 related changes in the brain (35, 36). Furthermore, direct evidence of beneficial effects of young blood
83 treatment for preserving brain health has been provided in two different mouse models of AD (37, 38),
84 suggesting beneficial effects also in the AD brain. Of particular interest, a small clinical trial (39)
85 reported that plasma from young donors transfused to patients with mild cognitive impairment or
86 early AD is safe and possibly beneficial. An exploratory endpoint analysis showed improvements on
87 functional abilities, although no changes were found in global cognition, mood, or functional
88 connectivity.

89
90 Although evidence suggests beneficial effects of *young blood* treatment in *aged* animals, less is known
91 about the effects of *exercised blood* treatment in the *aging* or *diseased* brain. A recent study
92 demonstrated that administration of blood from exercised, *aged* donor mice into sedentary, *aged* mice
93 conferred beneficial effects of exercise on hippocampal neurogenesis and cognition (40). Given that
94 exercise training reduces the risk of AD development, induces changes in the blood composition and
95 has widespread systemic benefits, it is reasonable to hypothesize that exercised plasma may have
96 rejuvenative properties similar to, or stronger than young blood. In this study, we aim to test the safety
97 profile of transfusing exercised plasma from young, healthy adults in AD patients, and perform a pilot
98 test for potential therapeutic effects.

99 ENDPOINTS

100 The purpose of this study is to explore the safety of transfusion of plasma from exercise trained donors
101 (ExPlas) compared to Octaplasma®, a commercially available virus inactivated plasma product pooled
102 from approximately 1000 donors, and Sodium Chloride 0.9% (saline) in patients in the early
103 symptomatic phase of AD and provide pilot data regarding efficacy. An additional aim is to provide
104 advancements to the field by exploring therapeutical effects on AD of blood-borne factors.
105

106 Primary endpoint of ExPlas

107 Proportion of patients with adverse events after 1 year as a measure of safety and tolerability, and
108 number of subjects who comply with the research protocol as a measure of feasibility.
109

110 Secondary endpoints of ExPlas after 1, 2 and 5 years

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

126 Hypothesis, primary outcome:

- 127 I) ExPlas transfusions to patients in early symptomatic phase of AD is safe
128

129 Hypotheses secondary outcome:

- 130 II) ExPlas transfusions to patients in early symptomatic phase of AD ameliorates the biomarker-
131 profile in blood and cerebrospinal fluid, structural- and functional MRI, cognitive function, functional
132 capacity, fitness, and quality of life
133

134 METHODS

135 Design

136 The study is a double blinded, randomized controlled clinical phase II trial recruiting at a single study
137 site at St. Olavs Hospital in Norway . The first patient was enrolled and randomized on September 15th,
138 2021. The anticipated recruitment period is 36 months and the estimated date of last patient enrolled
139 is September 1th, 2024. The treatment duration is 1 year, follow-up period: 61 months, including
140 screening. There are 3 study arms with patient ratio 1:1:1 (ExPlas, Octaplasma, saline), stratified by
141 *APOE* genotype. Electronic randomization, provided by the Unit for Applied Clinical Research at NTNU,
142 ensures that allocation of patients to a treatment group is random. Electronic randomization is
143 conducted by the blinded ExPlas study nurse. The results of the randomization is not visible to any
144 member of the study group. An automated message on each patient allocation is sent directly to the
145 study nurses who undertake transfusions. The flow chart of the ExPlas Study is given in Figure 1.
146

1
2
3 147 Settings and participants – Plasmapheresis, Cardiopulmonary testing and physical (donors)
4 148 Exercised plasma collected by plasmapheresis from male donors who have not themselves received
5 149 plasma will be used in the study. The rationale for these selection criteria is that women may have
6 150 developed antibodies during pregnancies and men may have developed antibodies during plasma
7 151 transfusions. Thus, selection reduces the risk of antibody-induced transfusion complications. All
8 152 donors have been recruited from the existing donor corps at St. Olavs Hospital Blood Bank. Donors
9 153 must fulfill all requirements in the Norwegian laws and guidelines for blood donors.
10 154 Potential donors will perform a cardiopulmonary exercise test to voluntary exhaustion on a treadmill
11 155 (PPS55 Med, Woodway GmbH, Germany) with continuous gas and minute ventilation analysis using
12 156 the Cortex MetaMax II (Cortex Biophysik GmbH, Leipzig, Germany). The individualized steady-state test
13 157 protocol starts at a speed and inclination that will be defined during a 15-minute warm-up. The first
14 158 stage of the test will be held for three minutes, or longer until steady state is reached. Thereafter,
15 159 speed is increased by 1 km/h every minute until maximal oxygen uptake is reached, defined as
16 160 flattening of oxygen uptake despite increased workload. Test procedure has been described in detail
17 161 previously (41). The first plasma donation will be performed within one month after the
18 162 cardiopulmonary test. To ensure that the donors sustained a high physical activity level in between the
19 163 4 donations (within 4 months from first donation) they were equipped with a wristworn heart rate
20 164 monitor (Huami GTS2, Huami North America Inc, Irvine, CA, USA) and required to have a physical
21 165 activity level above 100 weekly Personalized Activity Intelligence (PAI) points, to sustain a high maximal
22 166 oxygen uptake, as described in detail elsewhere (42), using the Zepp mobile Application downloaded
23 167 from Apple Store or Google Play.
24 168

25 169 **Donor inclusion criteria:**

- 26 170
27 171
28 172
29 173
30 174
- Healthy male donors
 - Age 18-40 years
 - BMI ≤ 27 kg/m²
 - Maximal oxygen uptake ≥ 55 mL/kg/min
 - Already an approved donor at the St. Olav's Hospital Blood Bank

31 175 **Donor exclusion criteria:**

- 32 176
33 177
34 178
35 179
- Injury or other incident preventing regular exercise during the last month
 - Previous recipient of blood transfusion
 - PeakVO₂ ≤ 55 mL/kg/min

36 180 Settings and participants - Patients

37 181 Patients will be recruited from the Department of Neurology or Geriatrics out-patient clinics at St.
38 182 Olavs Hospital, and from the Department of Geriatric psychiatry, Levanger Hospital, both Norway. The
39 183 diagnostic work up of the patient will decide if the patient is eligible for inclusion. Eligible patients who
40 184 sign informed consent to join the study go through a further screening and are evaluated regarding
41 185 the defined inclusion and exclusion criteria.
42 186

43 187 **Patient inclusion criteria:**

44 188 Patients will be included in the study if they meet all the following criteria:

- 1
 - 2
 - 3
 - 4
 - 5
 - 6
 - 7
 - 8
 - 9
 - 10
 - 11
 - 12
 - 13
 - 14
 - 15
 - 16
 - 17
 - 18
 - 19
 - 20
 - 21
 - 22
 - 23
 - 24
 - 25
 - 26
 - 27
 - 28
 - 29
 - 30
 - 31
 - 32
 - 33
 - 34
 - 35
 - 36
 - 37
 - 38
 - 39
 - 40
 - 41
 - 42
 - 43
 - 44
 - 45
 - 46
 - 47
 - 48
 - 49
 - 50
 - 51
 - 52
 - 53
 - 54
 - 55
 - 56
 - 57
 - 58
 - 59
 - 60
- 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 A β 42 together with increased t-tau or p-tau in CSF
 - Increased tracer retention on amyloid PET
 - Mini-Mental State Examination (MMSE) Score \geq 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 \geq 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 $<$ $40 \times 10^9/L$)
- 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

- 1
2
3
4
5
6
7
8
9
- 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

10
11
12

Related to magnetic resonance imaging:

- 13
14
15
16
17
18
19
20
- 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

21 189

190 **Treatment and examinations**

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

194

195 **Treatment**

196 For this study, ExPlas (plasma from fit donors) and Octaplasma are defined as Investigational
197 Medicinal Products. ExPlas is plasma drawn by plasmapheresis once a month for 4 months, from a
198 total of 30 donors (aged 18-40, BMI ≤ 27 kg/m² and VO_{2max} >55 mL/kg/min). All units will be virus
199 inactivated by the Intercept method (CERUS corporation, US), in accordance with the instructions from
200 the manufacturer and the procedures at the Blood Bank at St. Olavs Hospital.

201

202 Octaplasma is human pooled plasma produced by Octapharma (Lachen, Switzerland). The rationale
203 for including Octaplasma is to separate the effect of ExPlas from the “general untrained” plasma
204 pooled from thousands of donors (relatively young men). Placebo for this study is isotonic saline (0.9%
205 sodium chloride) (Fresenius Kabi, Halden, Norway). Comparison with isotonic saline allows
206 differentiation from a non-blood product. ExPlas and Octaplasma are stored at $\leq -18^{\circ}\text{C}$ until the time
207 of transfusion. The transfusion volume will be 200 mL at every time point for all three treatments.

208

209 **Cognitive test battery**

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

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

214

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

219

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

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

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

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

228 **Clinical Dementia Rating Scale (CDR)** is a clinical scale for the staging of dementia. The participant is
229 rated from 0-3 on six cognitive and behavioral categories: memory, orientation, judgement and
230 problem solving, community affairs, home and hobbies, and personal care. The Global score is
231 calculated according to an established algorithm, where memory is considered the primary category
232 and all others are secondary categories. A global score of 0 equals no dementia, 0.5 questionable
233 dementia, 1 mild dementia, 2 moderate dementia and 3 severe dementia (54, 55). The Sum of Boxes
234 score is a continuous measure of dementia severity and ranges from 0-18. The CDR Sum of Boxes are
235 found to be adequate for use in prodromal AD and continued use is warranted and recommended in

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

238
239 **The Lawton Instrumental Activities of Daily Living Scale (IADL).** This IADL scale evaluate eight items,
240 related to complex everyday activities, and each can be scored 0 that equals “dependent” and 1 that
241 reflect “completely independent”. Change from 0 to 1 in any of the eight items is considered a
242 “clinically relevant change” (56).

243
244 **Unified Parkinson’s Disease Rating Scale (UPDRS).** The motor examination part of UPDRS will be used
245 to detect development of parkinsonian motor signs, such as tremor, rigidity, bradykinesia, and
246 postural-gait abnormalities (57).

247 248 The 6-minute walk test

249 Fitness level will be measured using the 6 minute walk-test which is a good alternative to direct
250 measurement of PeakVO₂ (58). The 6-minute walk test is considered safer for the current patient group
251 than a treadmill test.

252 253 Structural- and functional MRI

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

266
267 A secondary aim is to identify any effect of treatment group on MRI markers of both
268 neurodegenerative and cerebrovascular disease. MRI at 7T allows for a stronger signal and better
269 anatomical localization in less time, but with the stronger magnetic field there are also more
270 contraindications.

271 272 Quality of Life

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

275 276 Echocardiography

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

286

287 Biomarkers in blood and spinal fluid

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

315

316 Blood sampling procedures

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

324

325 Spinal puncture procedures

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

335

336 Sample size and statistics

337 We do not expect that ExPlas will cause more adverse effects than Octaplasma, but have made the
338 following considerations about power calculations related to safety. The most common reaction to
339 transfusion of Octaplasma is allergy and occurs in up to 1% of the elderly. If we consider that ExPlas
340 would cause a dramatic increase in allergic reactions, of e.g. 35% vs. 1% after Octaplasma treatment,
341 19 participants will be needed in each group to show significant differences (with alpha 0.05 and power
342 of 0.8). There are substantial uncertainties in the assumptions for this power calculation. But
343 considering that another study found that transfusing plasma from young donors to patients (n=18)
344 with mild cognitive impairment or early AD was safe with no adverse events (39) we find it likely that
345 20 patients in each group will be enough to test safety in ExPlas.

346 The magnitude of a possible treatment effect of ExPlas is currently not established. The following
347 information has been established: i) a difference of 2 points on the Mini-Mental State Examination
348 Score (MMSE) primarily between those receiving ExPlas vs. Octaplasma will be clinically relevant after
349 1 and 2 years; ii) Based on several clinical studies in this population, we expect an average MMSE-NR-
350 3 of about 24 in our population, iii) Based on previous studies, we expect a standard deviation of
351 MMSE-NR-3 score of approximately 3. These assumptions suggest that 17 participants are needed in
352 each group (with alpha 0.05, and power 0.8) to demonstrate a clinically relevant effect of ExPlas. Given
353 potential dropouts, it is reasonable to include 20 in each group. The plan is therefore to include 60
354 patients in the study.

355
356 For the primary endpoints, counts will be reported and compared using recommended methods for
357 analysis of contingency tables (57). Secondary and other endpoints will be analyzed using mixed
358 models with the outcome variable as dependent variable, treatment group, time and their interaction
359 as categorical covariates, and patient as random effect. In these analyses, we will adjust for the
360 baseline value of the outcome variable, as recommended (65, 66).

362 Ethics

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

375 Organization

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

378
379 A safety committee consisting of two clinicians (one neurologist and one specialist in Transfusion
380 Medicine) has been appointed to ensure the safety of study participants. In case of adverse events,
381 the safety committee will evaluate whether treatments can continue or must be stopped. A study
382 nurse will observe the patients during and for one hour after infusion and a physician will evaluate the
383 patients in case of adverse effects. Neither the safety committee nor the attending physician
384 responsible for each infusion are involved in other parts of the study and they will not be blinded for
385 the treatment given.

386

387 Study monitors

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

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

402

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

407

408 Data management

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

419

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

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

1
2
3 436 • Additional information according to local regulations and practice.
4 437 Data management will be performed by the Unit for Applied Clinical Research at NTNU (Berit
5 438 Bjelkåsen). The Data management procedures will be performed in accordance with the department's
6 439 SOPs and ICH guidelines. The data management process will be described in the study specific data
7 440 handling plan and the study specific data handling report after database closure. Data entered into the
8 441 eCRF will be validated as defined in the data validation plan. Validation includes, but is not limited to,
9 442 validity checks (e.g. range checks), consistency checks and customised checks (logical checks between
10 443 variables to ensure that study data are accurately reported) for eCRF data and external data (e.g.
11 444 laboratory data). A majority of edit checks will be triggered during data entry and will therefore
12 445 facilitate efficient 'point of entry' data cleaning. Data management personnel will perform both
13 446 manual eCRF review and review of additional electronic edit checks to ensure that the data are
14 447 complete, consistent and reasonable. The electronic edit checks will run continually throughout the
15 448 course of the study and the issues will be reviewed manually online to determine what action needs
16 449 to be taken. Manual queries may be added to the system by clinical data management or study
17 450 monitor. Clinical data managers and study monitors are able to remotely and proactively monitor the
18 451 patient eCRFs to improve data quality. All updates to queried data will be made by authorised study
19 452 centre personnel only and all modifications to the database will be recorded in an audit trail. Once the
20 453 queries have been resolved, eCRFs will be signed by electronic signature Any changes to signed eCRFs
21 454 will be approved and resigned by the Investigator. Once the full set of eCRFs have been completed and
22 455 locked, the Sponsor will authorise database lock and all electronic data will be sent to the designated
23 456 statistician for analysis. Subsequent changes to the database will then be made only by written
24 457 agreement. The data will be stored in a dedicated and secured area at NTNU. Data will be stored in a
25 458 de-identified manner, where each study participant is recognisable by his/her unique trial subject
26 459 number. The data will be stored until 15 years after database lock.

30 460 31 461 Patients and public involvement

32 462 To ensure a high study quality and relevance a user board consisting of three next kin of present and
33 463 past AD patients has been established. As we conduct research on a patient group that is considered
34 464 vulnerable, this board is particularly important. We have met the user group on several occasions while
35 465 working on the study protocol (first meeting in Feb 2017) and have received input on several matters,
36 466 such as how to ensure a tolerable load of participation for the individual patient/relatives. The board
37 467 will continue to consult the study team twice annually throughout the study period, on
38 468 implementation, results, and future developments. They are encouraged to give their opinions
39 469 regarding the project as a whole and particularly on the patients' well-being. The study group has
40 470 already made changes and adjustments in the planned protocols based on feedback from the user
41 471 group. The study has a user representative who participates in meetings and presentations of the study
42 472 to the general public. On initiative from the ExPlas user group, we are currently making three
43 473 information videos about "AD and participation in research studies", for AD patients and their families,
44 474 where patients and their next of kin tell their story to help new patients and their next of kin in the
45 475 coming process. These videos will also be used in the recruitment phase of the study to inform and
46 476 motivate to take part in ExPlas.

49 477 50 478 Dissemination

51 479 Direct communication with users and patient organisations: ExPlas Study group regularly present at
52 480 various meetings of patient organizations (such as the National Association for Public Health) and for
53 481 senior citizens' societies. This type of contact with the public, patients and relatives has proven
54 482 mutually useful. We plan to intensify participation in such meetings to inform about current knowledge
55 483 about prevention and treatment of AD, particularly via the established user groups. Communication
56 484 via Internet: One of the most important media for spreading the news and awareness will be the
57 485 Internet. The results and information (including videos) about the studies will be presented on CERG's
58 486 webpage (ntnu.edu/cerg), one of the most visited sites at Scandinavian universities; and ExPlas's own

1
2
3 487 Norwegian webpage (ntnu.no/cerg/explas). Scientific and non-scientific communication: General
4 488 communication activities include publication in open access peer reviewed journals, non-scientific
5 489 journals and at national- and international meetings, to reach the general public, patients, scientists,
6 490 and policy makers. Importantly, our group is closely linked and active partners in the Norwegian
7 491 Research School in Neuroscience, Physical Activity and Health (master program) and medical education
8 492 where we actively will present our research to the next generation of health care personnel and
9 493 scientists. We also have a journalist at CERG (Anders Revdal), available for ExPlas, who will be
10 494 responsible for communication.
11 495

13 496 DISCUSSION

14 497
15 498 To our knowledge, ExPlas will be the first study with the aim of studying safety and efficacy of
16 499 transfusion of plasma from well endurance trained donors to patients in the early symptomatic phase
17 500 of AD. Even if prevention probably will be the most effective way to reduce numbers of patients with
18 501 AD worldwide, we need a cure for this devastating disease which impacts the lives for both patients
19 502 and their families substantially. There is also a need to understand the mechanisms behind the
20 503 beneficial effect of physical exercise on the brain, and it seems logically to try to exploit this effect in
21 504 treatment of the early phase of symptomatic AD.
22 505

23 506 On June 7th, 2021, the U.S. Food and Drug Administration, approved aducanumab (marketed as
24 507 Aduhelm) for use in treatment of AD (67), due to its ability to reduce amyloid plaques in the brain,
25 508 under an accelerated approval pathway (68). Confirmation of the clinical benefit is still required to be
26 509 confirmed for continued approval (67). Independent of the usefulness of aducanumab in AD
27 510 therapeutics, other interventions capable of delaying the clinical onset of AD dementia should
28 511 continue to be studied. The findings from preclinical AD models (36-38, 69), and a small clinical trial
29 512 (39) clearly indicate that there is communication between the systemic environment and the
30 513 hippocampus. Systemic factors are capable of inducing changes, and even therapeutic effects, in the
31 514 brain without hindrance by the blood-brain barrier; potentially an effective strategy to reverse
32 515 neurodegeneration in the AD-brain (30). There are myriads of factors and processes that are set in
33 516 motion during and after exercise training, and much of this is reflected in the composition of the blood
34 517 (70). Thus, it is not likely that it is a single factor is orchestrating the beneficial effects of exercise, but
35 518 rather an interplay between several molecular factors that need to be discovered and understood to
36 519 develop the first-generation of exercise-mimicking drugs. This is a promising idea as a large population
37 520 of patients are simply unable to exercise, such as patients in the intensive care unit, the critically ill,
38 521 patients recovering from accidents, the morbidly obese and paralyzed patients. For these patients,
39 522 innovative exercise-mimicking therapies could be of benefit.
40 523

41 524 However, development of exercise-mimicking therapies is a very complex and time-consuming
42 525 undertaking, that should not delay the testing of a potential benefit of exercise trained plasma, with
43 526 most of its natural components, on safety and therapeutic effect in patients with AD. In the context of
44 527 lack of disease-modifying treatments, ExPlas is innovative and potentially groundbreaking if it is found
45 528 to be safe with few side effects and with similar promising results as seen in preclinical AD models (35-
46 529 38).
47 530

48 531 Another key question is at what stage of AD interventions such as ExPlas treatment can be expected
49 532 to have an effect. Today we know that AD-related changes in the brain are present 10-30 years before
50 533 symptoms develop. The optimal time window for treatment is probably as early as possible during this
51 534 period, to decelerate or stop the progressive loss of neurons before functions are lost. As in all
52 535 diseases, prevention will always be the optimal path. Depending upon outcomes in the ExPlas Study,
53 536 a natural next step may be treatment with ExPlas of patients in the preclinical phase of the disease
54 537 (61).
55
56
57
58
59
60

538
 539 A small clinical trial found that plasma from young donors (young blood) transfused to patients with
 540 mild to moderate AD dementia (MMSE score ranging from 12-24) was safe with no adverse events and
 541 possibly beneficial with improvement in functional activity. In this study, 9 patients were randomized
 542 to a cross-over cohort, receiving 4 once-weekly infusions of either 250 mL of plasma from male donors
 543 (aged 18-30 years) or 250 mL of saline, followed by a 6-week washout and crossover to 4 once-weekly
 544 infusions of the alternate treatment. In addition, 9 patients were included in an open-label design in
 545 which patients received 4 once-weekly infusions of only young plasma. Considering the low number of
 546 patients, short follow-up period and promising findings in the study by Sha *et al.* (39) there is reason
 547 to believe that transfusion of exercise-trained plasma also is safe. With increased treatment periods
 548 and extended follow-up, we believe the ExPlas Study is well designed also to evaluate the potential
 549 therapeutic effect of exercise trained plasma. The relatively large number of patients will also likely
 550 enable us to assess whether endpoints become differentially affected by *APOE 4* status. As the ExPlas
 551 Study is the first of its kind it is not straightforward to undertake power calculations, and the results of
 552 our study may be useful for planning of an appropriately sample sized study in the future.

553
 554 We expect the ExPlas study to give new knowledge about whether transfusion of plasma from
 555 exercise-trained donors is safe and indications on whether it has therapeutic effects. ExPlas will also
 556 contribute to pioneering the discovery of molecular targets to potentially treat AD and lay the
 557 foundation for first-generation exercise-mimicking drugs, by capturing the molecular signature of high-
 558 fitness and molecular mechanisms provided by exercise.

559
 560 **Contributor statement**

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

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

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

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

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

574 **Ragnhild Røsbjorgen** Critical review of the manuscript, supervision, established methods and
 575 operating procedures for the study

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

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

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

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

- 1
2
3 585 **Geir Selbæk** Design of the study, critical review of the manuscript, obtained funding
4
5 586 **Stian Lydersen** Design of the study, critical review of the manuscript, making the statistical
6 587 plan and analyses in ExPlas
7
8 588 **Emrah Duzel** Critical review of the manuscript, established methods and operating
9 589 procedures for the study
10
11 590 **Sverre Bergh** Critical review of the manuscript, obtained funding
12
13 591 **Kjell Rune Halvorsrud** Design of the study, critical review of the manuscript, supervision,
14 592 established methods and operating procedures for the blood-donor part of ExPlas, supervision,
15 593 obtained funding
16
17 594 **Sigrid B. Sando** Conception and design of the study, critical review of the manuscript,
18 595 supervision, obtained funding, applications to ethical committee and Norwegian Medicine Agency for
19 596 study approval, Co-PI of ExPlas
20
21 597 **Ulrik Wisløff** Conception and design of the study, critical review of the manuscript,
22 598 supervision, obtained funding, applications to ethical committee and Norwegian Medicine Agency for
23 599 study approval, Co-PI of ExPlas

600

601 **Acknowledgement**

602 ExPlas study group acknowledge Randi Granbo, Tone Stav and Geir Suul for their active role in the
603 ExPlas user group.

604

605 **Competing interests**

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

610

611 **Funding**

- 612 ▪ Research Council of Norway;
- 613 ▪ The K.G. Jebsen Foundation for Medical Research, Norway;
- 614 ▪ Norwegian University of Science and Technology (NTNU);
- 615 ▪ Central Norway Regional Health Authority;
- 616 ▪ St Olavs hospital, Trondheim, Norway;
- 617 ▪ The National Association for Public Health, Norway
- 618 ▪ The Liaison Committee for Central Norway Regional Health Authority

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

622

623 **Country of Recruitment**

624 Norway

1
2
3 625 **Trial sponsor**

4 626 Øystein Risa, Head of Department

5 627 Department of Circulation and Medical Imaging, NTNU - Norwegian University of Science and

6 628 Technology, Box 8905 MTF, 7491 Trondheim, Norway. Tel: (+47) 92613734

7 629 E-mail: oystein.risa@ntnu.no

8
9 630

10
11
12 631 **Data sharing**

13 632 We are not permitted to share individual data from the current trial, but we are open to
14 633 collaborative research with researchers worldwide, who can have access to analysed data from our
15 634 university. We have also established a biobank of blood and genetic material that we plan to share
16 635 with researchers worldwide, but individual data must be analysed within our university only.

17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For peer review 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.

- 1
2
3 681 20. Tolppanen AM, Solomon A, Soininen H, Kivipelto M. Midlife vascular risk factors and
4 682 Alzheimer's disease: evidence from epidemiological studies. *J Alzheimers Dis.*
5 683 2012;32(3):531-40.
6
7 684 21. Norton S, Matthews FE, Barnes DE, Yaffe K, Brayne C. Potential for primary
8 685 prevention of Alzheimer's disease: an analysis of population-based data. *Lancet Neurol.*
9 686 2014;13(8):788-94.
10
11 687 22. Kaminsky LA, Arena R, Beckie TM, Brubaker PH, Church TS, Forman DE, et al. The
12 688 importance of cardiorespiratory fitness in the United States: the need for a national registry:
13 689 a policy statement from the American Heart Association. *Circulation.* 2013;127(5):652-62.
14 690 23. Larson EB, Wang L, Bowen JD, McCormick WC, Teri L, Crane P, et al. Exercise is
15 691 associated with reduced risk for incident dementia among persons 65 years of age and older.
16 692 *Ann Intern Med.* 2006;144(2):73-81.
17
18 693 24. Tari AR, Nauman J, Zisko N, Skjellegrind HK, Bosnes I, Bergh S, et al. Temporal
19 694 changes in cardiorespiratory fitness and risk of dementia incidence and mortality: a
20 695 population-based prospective cohort study. *The Lancet Public Health.* 2019;4(11):e565-e74.
21
22 696 25. Hoffmann K, Frederiksen KS, Sobol NA, Beyer N, Vogel A, Simonsen AH, et al.
23 697 Preserving cognition, quality of life, physical health and functional ability in Alzheimer's
24 698 disease: the effect of physical exercise (ADEX trial): rationale and design.
25 699 *Neuroepidemiology.* 2013;41(3-4):198-207.
26 700 26. Lautenschlager NT, Cox KL, Flicker L, Foster JK, van Bockxmeer FM, Xiao J, et al. Effect
27 701 of physical activity on cognitive function in older adults at risk for Alzheimer disease: a
28 702 randomized trial. *Jama.* 2008;300(9):1027-37.
29
30 703 27. van Praag H, Shubert T, Zhao C, Gage FH. Exercise enhances learning and
31 704 hippocampal neurogenesis in aged mice. *J Neurosci.* 2005;25(38):8680-5.
32
33 705 28. van Praag H, Christie BR, Sejnowski TJ, Gage FH. Running enhances neurogenesis,
34 706 learning, and long-term potentiation in mice. *Proc Natl Acad Sci U S A.* 1999;96(23):13427-
35 707 31.
36 708 29. Maass A, Duzel S, Goerke M, Becke A, Sobieray U, Neumann K, et al. Vascular
37 709 hippocampal plasticity after aerobic exercise in older adults. *Molecular psychiatry.*
38 710 2015;20(5):585-93.
39
40 711 30. Bouchard J, Villeda SA. Aging and brain rejuvenation as systemic events. *J*
41 712 *Neurochem.* 2015;132(1):5-19.
42
43 713 31. Pereira AC, Huddleston DE, Brickman AM, Sosunov AA, Hen R, McKhann GM, et al. An
44 714 in vivo correlate of exercise-induced neurogenesis in the adult dentate gyrus. *Proc Natl Acad*
45 715 *Sci U S A.* 2007;104(13):5638-43.
46 716 32. Fabel K, Fabel K, Tam B, Kaufer D, Baiker A, Simmons N, et al. VEGF is necessary for
47 717 exercise-induced adult hippocampal neurogenesis. *The European journal of neuroscience.*
48 718 2003;18(10):2803-12.
49
50 719 33. Castellano JM, Mosher KI, Abbey RJ, McBride AA, James ML, Berdnik D, et al. Human
51 720 umbilical cord plasma proteins revitalize hippocampal function in aged mice. *Nature.*
52 721 2017;544(7651):488-92.
53
54 722 34. Tari AR, Norevik CS, Scrimgeour NR, Kobro-Flatmoen A, Storm-Mathisen J, Bergersen
55 723 LH, et al. Are the neuroprotective effects of exercise training systemically mediated?
56 724 *Progress in cardiovascular diseases.* 2019.
57 725 35. Villeda SA, Plambeck KE, Middeldorp J, Castellano JM, Mosher KI, Luo J, et al. Young
58 726 blood reverses age-related impairments in cognitive function and synaptic plasticity in mice.
59 727 *Nat Med.* 2014;20(6):659-63.
60

- 1
2
3 728 36. Katsimpardi L, Litterman NK, Schein PA, Miller CM, Loffredo FS, Wojtkiewicz GR, et al.
4 729 Vascular and neurogenic rejuvenation of the aging mouse brain by young systemic factors.
5 730 Science. 2014;344(6184):630-4.
6
7 731 37. Xia E, Xu F, Hu C, Kumal JPP, Tang X, Mao D, et al. Young Blood Rescues the Cognition
8 732 of Alzheimer's Model Mice by Restoring the Hippocampal Cholinergic Circuit. Neuroscience.
9 733 2019;417:57-69.
10
11 734 38. Zhao Y, Qian R, Zhang J, Liu F, Iqbal K, Dai CL, et al. Young blood plasma reduces
12 735 Alzheimer's disease-like brain pathologies and ameliorates cognitive impairment in 3xTg-AD
13 736 mice. *Alzheimers Res Ther.* 2020;12(1):70.
14 737 39. Sha SJ, Deutsch GK, Tian L, Richardson K, Coburn M, Gaudioso JL, et al. Safety,
15 738 Tolerability, and Feasibility of Young Plasma Infusion in the Plasma for Alzheimer Symptom
16 739 Amelioration Study: A Randomized Clinical Trial. *JAMA Neurol.* 2018.
17 740 40. Horowitz AM, Fan X, Bieri G, Smith LK, Sanchez-Diaz CI, Schroer AB, et al. Blood
18 741 factors transfer beneficial effects of exercise on neurogenesis and cognition to the aged
19 742 brain. *Science.* 2020;369(6500):167-73.
20
21 743 41. Aspenes ST, Nilsen TI, Skaug EA, Bertheussen GF, Ellingsen Ø, Vatten L, et al. Peak
22 744 oxygen uptake and cardiovascular risk factors in 4631 healthy women and men. *Med Sci*
23 745 *Sports Exerc.* 2011;43(8):1465-73.
24
25 746 42. Nauman J, Nes BM, Zisko N, Revdal A, Myers J, Kaminsky LA, et al. Personal Activity
26 747 Intelligence (PAI): A new standard in activity tracking for obtaining a healthy
27 748 cardiorespiratory fitness level and low cardiovascular risk. *Progress in cardiovascular*
28 749 *diseases.* 2019;62(2):179-85.
29
30 750 43. Dubois B, Feldman HH, Jacova C, Hampel H, Molinuevo JL, Blennow K, et al.
31 751 Advancing research diagnostic criteria for Alzheimer's disease: the IWG-2 criteria. *Lancet*
32 752 *Neurol.* 2014;13(6):614-29.
33
34 753 44. Morris JC, Heyman A, Mohs RC, Hughes JP, van Belle G, Fillenbaum G, et al. The
35 754 Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part I. Clinical and
36 755 neuropsychological assessment of Alzheimer's disease. *Neurology.* 1989;39(9):1159-65.
37 756 45. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for
38 757 grading the cognitive state of patients for the clinician. *J Psychiatr Res.* 1975;12(3):189-98.
39
40 758 46. McDougall F, Edgar C, Mertes M, Delmar P, Fontoura P, Abi-Saab D, et al.
41 759 Psychometric Properties of the Clinical Dementia Rating - Sum of Boxes and Other Cognitive
42 760 and Functional Outcomes in a Prodromal Alzheimer's Disease Population. *J Prev Alzheimers*
43 761 *Dis.* 2021;8(2):151-60.
44
45 762 47. Reitan RM. Validity of the Trail Making Test as an indication of organic brain damage.
46 763 Perceptual and motor skills. 1958;8(3):271-6.
47 764 48. Espenes J, Hessen E, Eliassen IV, Waterloo K, Eckerström M, Sando SB, et al.
48 765 Demographically adjusted trail making test norms in a Scandinavian sample from 41 to 84
49 766 years. *Clin Neuropsychol.* 2020;34(sup1):110-26.
50
51 767 49. Brodaty H, Moore CM. The Clock Drawing Test for dementia of the Alzheimer's type:
52 768 A comparison of three scoring methods in a memory disorders clinic. *Int J Geriatr Psychiatry.*
53 769 1997;12(6):619-27.
54
55 770 50. Nutter-Upham KE, Saykin AJ, Rabin LA, Roth RM, Wishart HA, Pare N, et al. Verbal
56 771 fluency performance in amnesic MCI and older adults with cognitive complaints. *Arch Clin*
57 772 *Neuropsychol.* 2008;23(3):229-41.
58 773 51. Patterson C. World Alzheimer report 2018. *Alzheimer's Disease International;* 2018.
59 774 52. Elizabeth K Warrington MJ. Visual Object and Space Perception Battery (VOSP)1991.

- 1
2
3 775 53. Binetti T. Disorders of visual and spatial perception in the early stage of Alzheimers
4 776 disease1996.
5 777 54. Hughes CP, Berg L, Danziger WL, Coben LA, Martin RL. A new clinical scale for the
6 778 staging of dementia. *Br J Psychiatry*. 1982;140:566-72.
7 779 55. O'Bryant SE, Humphreys JD, Smith GE, Ivnik RJ, Graff-Radford NR, Petersen RC, et al.
8 780 Detecting dementia with the mini-mental state examination in highly educated individuals.
9 781 *Arch Neurol*. 2008;65(7):963-7.
10 782 56. Lawton MP, Brody EM. Assessment of older people: self-maintaining and
11 783 instrumental activities of daily living. *Gerontologist*. 1969;9(3):179-86.
12 784 57. Fagerland M, Lydersen, S., & Laake, P. *Statistical Analysis of Contingency Tables*.
13 785 Chapman and Hall/CRC2017.
14 786 58. Ries JD, Echternach JL, Nof L, Gagnon Blodgett M. Test-retest reliability and minimal
15 787 detectable change scores for the timed "up & go" test, the six-minute walk test, and gait
16 788 speed in people with Alzheimer disease. *Phys Ther*. 2009;89(6):569-79.
17 789 59. Geschke K, Fellgiebel A, Laux N, Schermuly I, Scheurich A. Quality of life in dementia:
18 790 impact of cognition and insight on applicability of the SF-36. *Am J Geriatr Psychiatry*.
19 791 2013;21(7):646-54.
20 792 60. Ware JE, Jr., Sherbourne CD. The MOS 36-item short-form health survey (SF-36). I.
21 793 Conceptual framework and item selection. *Med Care*. 1992;30(6):473-83.
22 794 61. Dubois B, Hampel H, Feldman HH, Scheltens P, Aisen P, Andrieu S, et al. Preclinical
23 795 Alzheimer's disease: Definition, natural history, and diagnostic criteria. *Alzheimers Dement*.
24 796 2016;12(3):292-323.
25 797 62. Bateman RJ, Xiong C, Benzinger TL, Fagan AM, Goate A, Fox NC, et al. Clinical and
26 798 biomarker changes in dominantly inherited Alzheimer's disease. *N Engl J Med*.
27 799 2012;367(9):795-804.
28 800 63. Dubois B, Hampel H, Feldman HH, Scheltens P, Aisen P, Andrieu S, et al. Preclinical
29 801 Alzheimer's disease: Definition, natural history, and diagnostic criteria. *Alzheimers Dement*.
30 802 2016;12(3):292-323.
31 803 64. Bye A, Røsjø H, Aspenes ST, Condorelli G, Omland T, Wisløff U. Circulating microRNAs
32 804 and aerobic fitness--the HUNT-Study. *PLoS One*. 2013;8(2):e57496.
33 805 65. J T, L B, T H, J R, M W, M H. Different ways to estimate treatment effects in
34 806 randomised controlled trials. *Contemp Clin Trials Commun*. 2018;10:80-5.
35 807 66. Coffman CJ, Edelman D, Woolson RF. To condition or not condition? Analysing
36 808 'change' in longitudinal randomised controlled trials. *BMJ Open*. 2016;6(12):e013096.
37 809 67. Cavazzoni P. FDA's Decision to Approve New Treatment for Alzheimer's Disease:
38 810 [fda.gov/06/07/2021](https://www.fda.gov/06/07/2021) [Available from: [https://www.fda.gov/drugs/news-events-human-](https://www.fda.gov/drugs/news-events-human-drugs/fdas-decision-approve-new-treatment-alzheimers-disease)
39 811 [drugs/fdas-decision-approve-new-treatment-alzheimers-disease](https://www.fda.gov/drugs/news-events-human-drugs/fdas-decision-approve-new-treatment-alzheimers-disease).
40 812 68. Sevigny J, Chiao P, Bussi re T, Weinreb PH, Williams L, Maier M, et al. The antibody
41 813 aducanumab reduces Aβ plaques in Alzheimer's disease. *Nature*. 2016;537(7618):50-6.
42 814 69. Villeda SA, Plambeck KE, Middeldorp J, Castellano JM, Mosher KI, Luo J, et al. Young
43 815 blood reverses age-related impairments in cognitive function and synaptic plasticity in mice.
44 816 *Nature Medicine*. 2014;20:659.
45 817 70. Tari AR, Norevik CS, Scrimgeour NR, Kobro-Flatmoen A, Storm-Mathisen J, Bergersen
46 818 LH, et al. Are the neuroprotective effects of exercise training systemically mediated?
47 819 *Progress in cardiovascular diseases*. 2019;62(2):94-101.
48 820
49 821
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

822 **Figure text**

823
824
825
826
827

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.

For peer review only

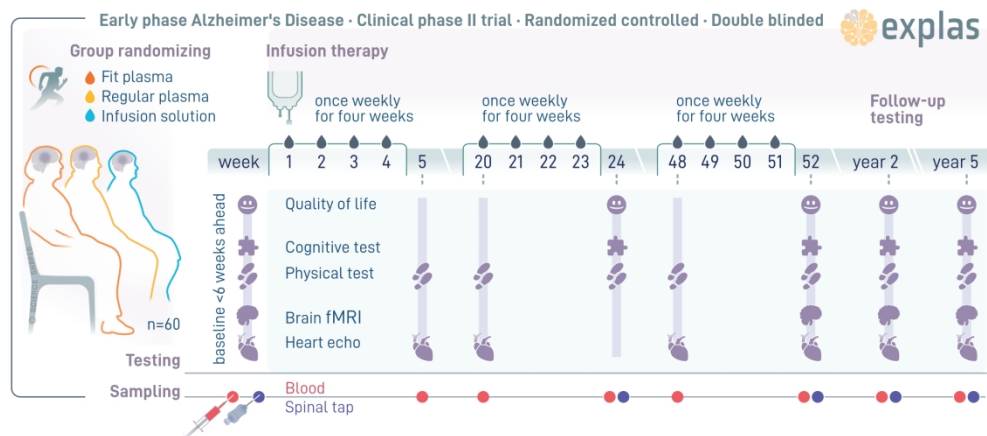


Figure 1. Flow chart of the ExPlas Study. Infusion solution, Sodium Chloride 0.9% (Saline); US, ultrasound of the heart; fMRI, functional magnetic resonance imaging.

533x239mm (118 x 118 DPI)



SPIRIT 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

1				
2	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
3				
4				
5				
6				
7				
8	Methods: Participants, interventions, and outcomes			
9				
10	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
11				
12				
13				
14	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
15				
16				
17				
18				
19	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
20				
21				
22		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
23				
24				
25				
26				
27		11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	
28				
29				
30				
31		11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	p. 5/ 187 -189
32				
33				
34	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
35				
36				
37				
38				
39				
40				
41				
42	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
43				
44				
45				
46				
47	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
48				
49				
50				
51	Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	p. 13/ 460 -475
52				
53				
54				
55				
56				
57				
58				
59				
60				

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

1				
2		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
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				
21				
22				
23				
24				
25				
26				
27				
28				
29				
30				
31				
32				
33				
34				
35				
36				
37				
38				
39				
40				
41				
42				
43				
44				
45				
46				
47				
48				
49				
50				
51				
52				
53				
54				
55				
56				
57				
58				
59				
60				

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

1				
2	Ancillary and	30	Provisions, if any, for ancillary and post-trial care, and for	
3	post-trial care		compensation to those who suffer harm from trial participation	
4				
5	Dissemination	31a	Plans for investigators and sponsor to communicate trial results to	p. 13-14/
6	policy		participants, healthcare professionals, the public, and other relevant	477-493
7			groups (eg, via publication, reporting in results databases, or other	
8			data sharing arrangements), including any publication restrictions	
9				
10				
11		31b	Authorship eligibility guidelines and any intended use of professional	p. 15/ 559
12			writers	-598
13				
14		31c	Plans, if any, for granting public access to the full protocol, participant-	p. 17/ 631
15			level dataset, and statistical code	-635
16				
17	Appendices			
18				
19	Informed consent	32	Model consent form and other related documentation given to	Attachment
20	materials		participants and authorised surrogates	
21				
22				
23	Biological	33	Plans for collection, laboratory evaluation, and storage of biological	p. 17/ 631
24	specimens		specimens for genetic or molecular analysis in the current trial and for	-635
25			future use in ancillary studies, if applicable	

*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](https://creativecommons.org/licenses/by-nc-nd/3.0/)" 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

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2021-056964.R2
Article Type:	Protocol
Date Submitted by the Author:	02-Jun-2022
Complete List of Authors:	<p>Tari, Atefe R.; Norwegian University of Science and Technology Faculty of Medicine and Health Sciences, Cardiac Exercise Research Group at the Department of Circulation and Medical Imaging; St Olavs Hospital Trondheim University Hospital, Department of Neurology and Clinical Neurophysiology</p> <p>Berg, Helene Haugen; Norwegian University of Science and Technology Faculty of Medicine and Health Sciences, Cardiac Exercise Research Group at the Department of Circulation and Medical Imaging; St. Olavs University Hospital, Department of Neurology and Clinical Neurophysiology</p> <p>Videm, Vibeke; Norwegian University of Science and Technology, Department of Clinical and Molecular Medicine; St Olavs University Hospital, Department of Immunology and Transfusion Medicine</p> <p>Bråthen, Geir; St. Olavs University Hospital, Department of Neurology and Clinical Neurophysiology; Norwegian University of Science and Technology, Department of Neuromedicine and Movement Science</p> <p>White, Linda R.; Norwegian University of Science and Technology Faculty of Medicine and Health Sciences, Department of Neuromedicine and Movement Science</p> <p>Røsbjørgen, Ragnhild Nyhus; Norwegian University of Science and Technology Faculty of Medicine and Health Sciences, Cardiac Exercise Research Group at the Department of Circulation and Medical Imaging</p> <p>Scheffler, Katja; St. Olavs University Hospital, Department of Neurology and Clinical Neurophysiology; Norwegian University of Science and Technology Faculty of Medicine and Health Sciences, Department of Neuromedicine and Movement Science</p> <p>Dalen, Havard; Nord-Trøndelag Hospital Trust, Department of Medicine, Levanger Hospital; Norwegian University of Science and Technology Faculty of Medicine and Health Sciences, MI Lab and Department of Circulation and Medical Imaging</p> <p>Holte, Espen; St Olavs Hospital Trondheim University Hospital</p> <p>Haberg, Asta; Norges Teknisk Naturvitenskapelige Universitet Institutt for biologi</p> <p>Selbaek, Geir; Norwegian National Advisory Unit on Ageing and Health, Centre for Old Age Psychiatric Research</p> <p>Lydersen, Stian; Norwegian University of Science and Technology Faculty of Medicine and Health Sciences, Regional Centre for Child and Youth Mental Health and Child Welfare</p> <p>Duezel, Emrah; German Centre for Neurodegenerative Diseases Site</p>

	Magdeburg Bergh, Sverre; Innlandet Hospital Trust Hamar Hospital Logan-Halvorsrud, Kjell Rune; St Olavs Hospital Trondheim University Hospital, Department of Immunology and Transfusion Medicine Sando, Sigrid Botne; St Olavs Hospital Trondheim University Hospital, Department of Neurology and Clinical Neurophysiology; Norwegian University of Science and Technology Faculty of Medicine and Health Sciences, Department of Neuromedicine and Movement Science Wisløff, Ulrik; Norwegian University of Science and Technology Faculty of Medicine and Health Sciences
Primary Subject Heading:	Neurology
Secondary Subject Heading:	Cardiovascular medicine, Patient-centred medicine, Pharmacology and therapeutics, Sports and exercise medicine
Keywords:	Neurophysiology < NEUROLOGY, PREVENTIVE MEDICINE, Dementia < NEUROLOGY, Echocardiography < CARDIOLOGY

SCHOLARONE™
Manuscripts

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4 **1 Safety and efficacy of plasma transfusion from exercise-trained donors in patients with early**
5 **2 Alzheimer's disease: protocol for the ExPlas Study**
6
7

8 4 Atefe R. Tari^{1,2}, Helene Haugen Berg^{1,2}, Vibeke Videm^{3,4}, Geir Bråthen^{2,5}, Linda R. White⁵, Ragnhild
9 5 Nyhus Røsbjørgen¹, Katja Scheffler^{2,5}, Håvard Dalen^{1,6,7}, Espen Holte⁶, Asta Håberg^{5,8}, Geir Selbæk^{9,10,11},
10 6 Stian Lydersen¹², Emrah Duezel^{13,14,15}, Sverre Bergh^{9,16}, Kjell Rune Logan-Halvorsrud⁴, Sigrid Botne
11 7 Sando^{2,5}, Ulrik Wisløff^{1,17}
12 8

- 13 9 1. Cardiac Exercise Research Group at the Department of Circulation and Medical Imaging, NTNU
14 10 - Norwegian University of Science and Technology, Trondheim, Norway
15 11 2. Department of Neurology and Clinical Neurophysiology, St. Olavs University Hospital,
16 12 Trondheim, Norway
17 13 3. Department of Clinical and Molecular Medicine, NTNU – Norwegian University of Science and
18 14 Technology, Trondheim, Norway
19 15 4. Department of Immunology and Transfusion Medicine, St. Olavs University Hospital,
20 16 Trondheim, Norway.
21 17 5. Department of Neuromedicine and Movement Science, NTNU – Norwegian University of
22 18 Science and Technology, Trondheim, Norway
23 19 6. Clinic of Cardiology, St. Olavs University Hospital, Trondheim, Norway
24 20 7. Department of Medicine, Levanger Hospital, Nord-Trøndelag Hospital Trust, Levanger, Norway
25 21 8. Department of Radiology and Nuclear Medicine, St. Olavs University Hospital, Trondheim,
26 22 Norway
27 23 9. Norwegian National Advisory Unit on Ageing and Health, Vestfold Hospital Trust, Tønsberg,
28 24 Norway
29 25 10. Department of Geriatric Medicine, Oslo University Hospital-Ullevål, Oslo, Norway
30 26 11. Faculty of Medicine, University of Oslo, Oslo, Norway
31 27 12. Department of Mental Health, Regional Centre for Child and Youth Mental Health and Child
32 28 Welfare, NTNU - Norwegian University of Science and Technology, Trondheim, Norway
33 29 13. German Center for Neurodegenerative Diseases (DZNE), Magdeburg, Germany
34 30 14. Institute of Cognitive Neurology and Dementia Research, Otto-von-Guericke University
35 31 Magdeburg, Magdeburg, Germany
36 32 15. Center for Behavioral Brain Sciences (CBBS), Magdeburg, Germany
37 33 16. Research Centre for Age-related Functional Decline and Disease, Innlandet Hospital Trust,
38 34 Ottestad, Norway
39 35 17. School of Human Movement and Nutrition Science, University of Queensland, Queensland,
40 36 Australia
41 37
42 38
43 39
44 40
45 41
46 42
47 43
48 44
49 45
50 46
51 47
52 48
53 49
54 50
55 51
56 52
57 53
58 54
59 55
60 56

39 **Address for correspondence:**

40 Ulrik Wisløff, email: ulrik.wisloff@ntnu.no

41 Cardiac Exercise Research Group at the Department of Circulation and Medical Imaging, NTNU -
42 Norwegian University of Science and Technology, Olav Kyrres gt. 9, 7489 Trondheim, Norway.
43

44 **Protocol version** no. 3.0 – 05.01.2021.
45

46 **Study start:** first donor included 01.03.2021, first patient screened 19.08.2021, first patient
47 randomized 15.09.2021, first infusion given for 22.09.2021.
48

49 **Trial registration:** EudraCT No. 2018-000148-24. ClinicalTrials.gov, NCT05068830
50
51
52
53
54
55
56
57
58
59
60

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.

52 BACKGROUND

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

63 It is hypothesized that around 40-50% of dementia cases worldwide are caused by modifiable risk
64 factors (11, 12), and that many factors associated with higher risk of cardiovascular disease, such as
65 obesity, hyperlipidaemia, hypertension, smoking, diabetes and physical inactivity are also associated
66 with increased risk of AD (12-20). These factors have in common that they can be substantially
67 modified through physical activity that secures above average age- and sex-specific levels of
68 cardiorespiratory fitness (CRF) (12, 19, 21-23) measured as peak oxygen uptake (PeakVO₂). In line with
69 this we demonstrated in a prospective cohort study of 30 695 adults that participants who increased
70 or sustained high PeakVO₂ over time (10 years apart) had 40-50% reduced risk of incident dementia,
71 30-40% reduced risk of dementia-related mortality, approximately 2.0 years delayed dementia onset,
72 and 2-3 years of life gained when compared to persistently unfit individuals (24). Thus, at present
73 exercise training leading to a high age-relative PeakVO₂ may be the most promising preventive "AD-
74 medicine" (25, 26).

75
76 Although it is well established that exercise positively influences brain neurogenesis, plasticity (27, 28),
77 and cognition (21, 27, 29) it is not well understood how these effects are mediated. The beneficial
78 effects of exercise on the brain have traditionally been thought not to be mediated through systemic
79 changes (30). However, a number of studies in both rodents and humans (31-33) demonstrates direct
80 effects on the brain of exercised induced blood-borne molecules crossing the the blood-brain barrier
81 (34). For instance, systemic administration of blood from young mice into old mice counteracts age-
82 related changes in the brain (35, 36). Furthermore, direct evidence of beneficial effects of young blood
83 treatment for preserving brain health has been provided in two different mouse models of AD (37, 38),
84 suggesting beneficial effects also in the AD brain. Of particular interest, a small clinical trial (39)
85 reported that plasma from young donors transfused to patients with mild cognitive impairment or
86 early AD is safe and possibly beneficial. An exploratory endpoint analysis showed improvements on
87 functional abilities, although no changes were found in global cognition, mood, or functional
88 connectivity.

89
90 Although evidence suggests beneficial effects of *young blood* treatment in *aged* animals, less is known
91 about the effects of *exercised blood* treatment in the *aging* or *diseased* brain. A recent study
92 demonstrated that administration of blood from exercised, *aged* donor mice into sedentary, *aged* mice
93 conferred beneficial effects of exercise on hippocampal neurogenesis and cognition (40). Given that
94 exercise training reduces the risk of AD development, induces changes in the blood composition and
95 has widespread systemic benefits, it is reasonable to hypothesize that exercised plasma may have
96 rejuvenative properties similar to, or stronger than young blood. In this study, we aim to test the safety
97 profile of transfusing exercised plasma from young, healthy adults in AD patients, and perform a pilot
98 test for potential therapeutic effects.

99 ENDPOINTS

100 The purpose of this study is to explore the safety of transfusion of plasma from exercise trained donors
101 (ExPlas) compared to Octaplasma®, a commercially available virus inactivated plasma product pooled
102 from approximately 1000 donors, and Sodium Chloride 0.9% (saline) in patients in the early
103 symptomatic phase of AD and provide pilot data regarding efficacy. An additional aim is to provide
104 advancements to the field by exploring therapeutical effects on AD of blood-borne factors.
105

106 Primary endpoint of ExPlas

107 Proportion of patients with adverse events after 1 year as a measure of safety and tolerability, and
108 number of subjects who comply with the research protocol as a measure of feasibility.
109

110 Secondary endpoints of ExPlas after 1, 2 and 5 years

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

126 Hypothesis, primary outcome:

- 127 I) ExPlas transfusions to patients in early symptomatic phase of AD is safe
128

129 Hypotheses secondary outcome:

- 130 II) ExPlas transfusions to patients in early symptomatic phase of AD ameliorates the biomarker-
131 profile in blood and cerebrospinal fluid, structural- and functional MRI, cognitive function, functional
132 capacity, fitness, and quality of life
133

134 METHODS

135 Design

136 The study is a double blinded, randomized controlled clinical phase II trial recruiting at a single study
137 site at St. Olavs Hospital in Norway . The first patient was enrolled and randomized on September 15th,
138 2021. The anticipated recruitment period is 36 months and the estimated date of last patient enrolled
139 is September 1th, 2024. The treatment duration is 1 year, follow-up period: 61 months, including
140 screening. There are 3 study arms with patient ratio 1:1:1 (ExPlas, Octaplasma, saline), stratified by
141 *APOE* genotype. Electronic randomization, provided by the Unit for Applied Clinical Research at NTNU,
142 ensures that allocation of patients to a treatment group is random. Electronic randomization is
143 conducted by the blinded ExPlas study nurse. The results of the randomization is not visible to any
144 member of the study group. An automated message on each patient allocation is sent directly to the
145 study nurses who undertake transfusions. The flow chart of the ExPlas Study is given in Figure 1.
146

1
2
3 147 Settings and participants – Plasmapheresis, Cardiopulmonary testing and physical (donors)
4 148 Exercised plasma collected by plasmapheresis from male donors who have not themselves received
5 149 plasma will be used in the study. The rationale for these selection criteria is that women may have
6 150 developed antibodies during pregnancies and men may have developed antibodies during plasma
7 151 transfusions. Thus, selection reduces the risk of antibody-induced transfusion complications. All
8 152 donors have been recruited from the existing donor corps at St. Olavs Hospital Blood Bank. Donors
9 153 must fulfill all requirements in the Norwegian laws and guidelines for blood donors. Potential donors
10 154 will perform a cardiopulmonary exercise test to voluntary exhaustion on a treadmill (PPS55 Med,
11 155 Woodway GmbH, Germany) with continuous gas and minute ventilation analysis using the Cortex
12 156 MetaMax II (Cortex Biophysik GmbH, Leipzig, Germany). The individualized steady-state test protocol
13 157 starts at a speed and inclination that will be defined during a 15-minute warm-up. The first stage of
14 158 the test will be held for three minutes, or longer until steady state is reached. Thereafter, speed is
15 159 increased by 1 km/h every minute until maximal oxygen uptake is reached, defined as flattening of
16 160 oxygen uptake despite increased workload. Test procedure has been described in detail previously
17 161 (41). The first plasma donation will be performed within one month after the cardiopulmonary test.
18 162 To ensure that the donors sustained a high physical activity level in between the 4 donations (within 4
19 163 months from first donation) they were equipped with a wristworn heart rate monitor (Huami GTS2,
20 164 Huami North America Inc, Irvine, CA, USA) and required to have a physical activity level above 100
21 165 weekly Personalized Activity Intelligence (PAI) points, to sustain a high maximal oxygen uptake, as
22 166 described in detail elsewhere (42), using the Zepp mobile Application downloaded from Apple Store
23 167 or Google Play.
24 168

25 169 **Donor inclusion criteria:**

- 26 170
27 171
28 172
29 173
30 174
- Healthy male donors
 - Age 18-40 years
 - BMI ≤ 27 kg/m²
 - Maximal oxygen uptake ≥ 55 mL/kg/min
 - Already an approved donor at the St. Olav's Hospital Blood Bank

31 175 **Donor exclusion criteria:**

- 32 176
33 177
34 178
35 179
- Injury or other incident preventing regular exercise during the last month
 - Previous recipient of blood transfusion
 - PeakVO₂ ≤ 55 mL/kg/min

36 180 Several aspects regarding donors were considered in the planning of the study. Particularly age and
37 181 required fitness level have been up for thorough discussions. Our idea is that we believe, although not
38 182 ever studied, that "exercised plasma" may confer additional benefit to the brain compared to young
39 183 plasma only. Thus, the point of departure was that we wanted the donors to have a fitness level
40 184 (maximal oxygen uptake) that is regarded as "fit", and not be "classified" as old. We regard 40 years
41 185 of age to be a relatively young age, and we, therefore, chose to require that donors were below the
42 186 age of 40 and have a maximal oxygen uptake above 55 mL/kg/min that is above the average for a 20-
43 187 year-old man in Norway (43).
44 188

45 189 Settings and participants - Patients

46 190 Patients will be recruited from the Department of Neurology or Geriatrics out-patient clinics at St.
47 191 Olavs Hospital, and from the Department of Geriatric psychiatry, Levanger Hospital, both Norway. The
48 192 diagnostic work up of the patient will decide if the patient is eligible for inclusion. Eligible patients who
49 193 sign informed consent to join the study go through a further screening and are evaluated regarding
50 194 the defined inclusion and exclusion criteria.
51 195

52 196 **Patient inclusion criteria:**

53 197 Patients will be included in the study if they meet all the following criteria:

- 1
 - 2
 - 3
 - 4
 - 5
 - 6
 - 7
 - 8
 - 9
 - 10
 - 11
 - 12
 - 13
 - 14
 - 15
 - 16
 - 17
 - 18
 - 19
 - 20
 - 21
 - 22
 - 23
 - 24
 - 25
 - 26
 - 27
 - 28
 - 29
 - 30
 - 31
 - 32
 - 33
 - 34
 - 35
 - 36
 - 37
 - 38
 - 39
 - 40
 - 41
 - 42
 - 43
 - 44
 - 45
 - 46
 - 47
 - 48
 - 49
 - 50
 - 51
 - 52
 - 53
 - 54
 - 55
 - 56
 - 57
 - 58
 - 59
 - 60
- 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 A β 42 together with increased t-tau or p-tau in CSF
 - Increased tracer retention on amyloid PET
 - Mini-Mental State Examination (MMSE) Score \geq 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 \geq 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 $<$ $40 \times 10^9/L$)
- 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

- 1
2
3
4
5
6
7
8
9
- 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

10
11
12

Related to magnetic resonance imaging:

- 13
14
15
16
17
18
19
20
- 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

21 198

199 **Treatment and examinations**

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

203

204 **Treatment**

205 For this study, ExPlas (plasma from fit donors) and Octaplasma are defined as Investigational
206 Medicinal Products. ExPlas is plasma drawn by plasmapheresis once a month for 4 months, from a
207 total of 30 donors (aged 18-40, BMI ≤ 27 kg/m² and VO_{2max} >55 mL/kg/min). All units will be virus
208 inactivated by the Intercept method (CERUS corporation, US), in accordance with the instructions from
209 the manufacturer and the procedures at the Blood Bank at St. Olavs Hospital.

210

211 Octaplasma is human pooled plasma produced by Octapharma (Lachen, Switzerland). The rationale
212 for including Octaplasma is to separate the effect of ExPlas from the “general untrained” plasma
213 pooled from thousands of donors (relatively young men). Placebo for this study is isotonic saline (0.9%
214 sodium chloride) (Fresenius Kabi, Halden, Norway). Comparison with isotonic saline allows
215 differentiation from a non-blood product. ExPlas and Octaplasma are stored at $\leq -18^{\circ}\text{C}$ until the time
216 of transfusion. The transfusion volume will be 200 mL at every time point for all three treatments.

217

218 **Cognitive test battery**

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

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

223

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

228

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

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

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

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

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

1
2
3 245 clinical trials because it is continuous and provides a greater variation in values (47). Both the CDR-
4 246 Global score and Sum of Boxes will be calculated.

5 247
6 248 **The Lawton Instrumental Activities of Daily Living Scale (IADL).** This IADL scale evaluate eight items,
7 249 related to complex everyday activities, and each can be scored 0 that equals “dependent” and 1 that
8 250 reflect “completely independent”. Change from 0 to 1 in any of the eight items is considered a
9 251 “clinically relevant change” (57).

10 252
11 253 **Unified Parkinson’s Disease Rating Scale (UPDRS).** The motor examination part of UPDRS will be used
12 254 to detect development of parkinsonian motor signs, such as tremor, rigidity, bradykinesia, and
13 255 postural-gait abnormalities (58).

14 256
15 257 **The 6-minute walk test**
16 258 Fitness level will be measured using the 6 minute walk-test which is a good alternative to direct
17 259 measurement of PeakVO₂ (59). The 6-minute walk test is considered safer for the current patient group
18 260 than a treadmill test.

19 261
20 262 **Structural- and functional MRI**
21 263 For increased sensitivity we will use multiparameric MRI at 7T MRI to assess brain structure and
22 264 function to uncover both neurodegenerative and cerebrovascular changes from baseline to 1, 2 and 5
23 265 years. The 7T protocol consists of the following scans: whole brain 3D MP2RAGE, gradient echo
24 266 SWI/Quantitative susceptibility mapping (QSM), and FLAIR, high resolution T2 weighted spin echo
25 267 sequence of the medial temporal lobe, multishell DTI, asl FLAIR, and rs-fMRI. The primary outcomes
26 268 are hippocampal and entorhinal cortex structure ((subfield)-volume/thickness, diffusion
27 269 characteristics, T2*-mapping) and function (blood flow, connectivity and BOLD signal). Other
28 270 quantitative measures include brain morphometry (e.g., parenchymal fraction, at-risk AD pattern
29 271 volume loss), and diffusion metrics in white and grey matter. Cerebrovascular pathology (perfusion,
30 272 white matter hyperintensities, perivascular spaces (quantitative from multishell DTI),
31 273 microhaemorrhages, micro- and macro- infarction) will be evaluated. For participants where 7T is
32 274 contraindicated, but not 3T, a similar examination will take place using 3T.

33 275
34 276 A secondary aim is to identify any effect of treatment group on MRI markers of both
35 277 neurodegenerative and cerebrovascular disease. MRI at 7T allows for a stronger signal and better
36 278 anatomical localization in less time, but with the stronger magnetic field there are also more
37 279 contraindications.

38 280
39 281 **Quality of Life**
40 282 Quality of life will be evaluated by the questionnaire SF-36, which contains questions about physical
41 283 and mental health, pain, vitality and general health perceptions (60, 61).

42 284
43 285 **Echocardiography**
44 286 All patients will undergo echocardiography examination at screening and four times during the first
45 287 year, and potentially at 2 and 5-years follow up. Screening echo is performed to ensure safety of
46 288 transfusions for patients included in the study. Patients with reduced cardiac function will be excluded
47 289 due to risk of developing heart failure triggered by fluid overload. Screening echocardiography serve
48 290 as baseline for included patients. Following echocardiography will be performed at week 5 (one week
49 291 after 4 weeks of transfusion treatment), week 20 (before treatment period two), week 48 (before
50 292 treatment period three), week 52 (one week after 4 weeks of transfusion treatment), and assumedly
51 293 at 2 and 5-years follow up. The echocardiography examination will be a complete examination of
52 294 cardiac structure and function, including ultrahigh framerate recordings at each time point.

295

296 Biomarkers in blood and spinal fluid

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

324

325 Blood sampling procedures

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

333

334 Spinal puncture procedures

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

344

345 Sample size and statistics

346 We do not expect that ExPlas will cause more adverse effects than Octaplasma, but have made the
347 following considerations about power calculations related to safety. The most common reaction to
348 transfusion of Octaplasma is allergy and occurs in up to 1% of the elderly. If we consider that ExPlas
349 would cause a dramatic increase in allergic reactions, of e.g. 35% vs. 1% after Octaplasma treatment,
350 19 participants will be needed in each group to show significant differences (with alpha 0.05 and power
351 of 0.8). There are substantial uncertainties in the assumptions for this power calculation. But
352 considering that another study found that transfusing plasma from young donors to patients (n=18)
353 with mild cognitive impairment or early AD was safe with no adverse events (39) we find it likely that
354 20 patients in each group will be enough to test safety in ExPlas.

355 The magnitude of a possible treatment effect of ExPlas is currently not established. The following
356 information has been established: i) a difference of 2 points on the Mini-Mental State Examination
357 Score (MMSE) primarily between those receiving ExPlas vs. Octaplasma will be clinically relevant after
358 1 and 2 years; ii) Based on several clinical studies in this population, we expect an average MMSE-NR-
359 3 of about 24 in our population, iii) Based on previous studies, we expect a standard deviation of
360 MMSE-NR-3 score of approximately 3. These assumptions suggest that 17 participants are needed in
361 each group (with alpha 0.05, and power 0.8) to demonstrate a clinically relevant effect of ExPlas. Given
362 potential dropouts, it is reasonable to include 20 in each group. The plan is therefore to include 60
363 patients in the study.

364
365 For the primary endpoints, counts will be reported and compared using recommended methods for
366 analysis of contingency tables (58). Secondary and other endpoints will be analyzed using mixed
367 models with the outcome variable as dependent variable, treatment group, time and their interaction
368 as categorical covariates, and patient as random effect. In these analyses, we will adjust for the
369 baseline value of the outcome variable, as recommended (66, 67).

371 Ethics

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

384 Organization

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

387
388 A safety committee consisting of two clinicians (one neurologist and one specialist in Transfusion
389 Medicine) has been appointed to ensure the safety of study participants. In case of adverse events,
390 the safety committee will evaluate whether treatments can continue or must be stopped. A study
391 nurse will observe the patients during and for one hour after infusion and a physician will evaluate the
392 patients in case of adverse effects. Neither the safety committee nor the attending physician
393 responsible for each infusion are involved in other parts of the study and they will not be blinded for
394 the treatment given.

395

396 Study monitors

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

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

411
412 The monitor will review the relevant CRFs for accuracy and completeness and will ask the site staff to
413 adjust any discrepancies as required. Sponsor's representatives (e.g., monitors, auditors) and/or
414 competent authorities will be allowed access to source data for source data verification in which case
415 a review of those parts of the hospital records relevant to the study will be required.

416

417 Data management

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

428

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

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

1
2
3 445 • Additional information according to local regulations and practice.
4 446 Data management will be performed by the Unit for Applied Clinical Research at NTNU (Berit
5 447 Bjelkåsen). The Data management procedures will be performed in accordance with the department's
6 448 SOPs and ICH guidelines. The data management process will be described in the study specific data
7 449 handling plan and the study specific data handling report after database closure. Data entered into the
8 450 eCRF will be validated as defined in the data validation plan. Validation includes, but is not limited to,
9 451 validity checks (e.g. range checks), consistency checks and customised checks (logical checks between
10 452 variables to ensure that study data are accurately reported) for eCRF data and external data (e.g.
11 453 laboratory data). A majority of edit checks will be triggered during data entry and will therefore
12 454 facilitate efficient 'point of entry' data cleaning. Data management personnel will perform both
13 455 manual eCRF review and review of additional electronic edit checks to ensure that the data are
14 456 complete, consistent and reasonable. The electronic edit checks will run continually throughout the
15 457 course of the study and the issues will be reviewed manually online to determine what action needs
16 458 to be taken. Manual queries may be added to the system by clinical data management or study
17 459 monitor. Clinical data managers and study monitors are able to remotely and proactively monitor the
18 460 patient eCRFs to improve data quality. All updates to queried data will be made by authorised study
19 461 centre personnel only and all modifications to the database will be recorded in an audit trail. Once the
20 462 queries have been resolved, eCRFs will be signed by electronic signature Any changes to signed eCRFs
21 463 will be approved and resigned by the Investigator. Once the full set of eCRFs have been completed and
22 464 locked, the Sponsor will authorise database lock and all electronic data will be sent to the designated
23 465 statistician for analysis. Subsequent changes to the database will then be made only by written
24 466 agreement. The data will be stored in a dedicated and secured area at NTNU. Data will be stored in a
25 467 de-identified manner, where each study participant is recognisable by his/her unique trial subject
26 468 number. The data will be stored until 15 years after database lock.
27
28
29
30

31 470 Patients and public involvement

32 471 To ensure a high study quality and relevance a user board consisting of three next kin of present and
33 472 past AD patients has been established. As we conduct research on a patient group that is considered
34 473 vulnerable, this board is particularly important. We have met the user group on several occasions while
35 474 working on the study protocol (first meeting in Feb 2017) and have received input on several matters,
36 475 such as how to ensure a tolerable load of participation for the individual patient/relatives. The board
37 476 will continue to consult the study team twice annually throughout the study period, on
38 477 implementation, results, and future developments. They are encouraged to give their opinions
39 478 regarding the project as a whole and particularly on the patients' well-being. The study group has
40 479 already made changes and adjustments in the planned protocols based on feedback from the user
41 480 group. The study has a user representative who participates in meetings and presentations of the study
42 481 to the general public. On initiative from the ExPlas user group, we are currently making three
43 482 information videos about "AD and participation in research studies", for AD patients and their families,
44 483 where patients and their next of kin tell their story to help new patients and their next of kin in the
45 484 coming process. These videos will also be used in the recruitment phase of the study to inform and
46 485 motivate to take part in ExPlas.
47
48
49

50 487 Dissemination

51 488 Direct communication with users and patient organisations: ExPlas Study group regularly present at
52 489 various meetings of patient organizations (such as the National Association for Public Health) and for
53 490 senior citizens' societies. This type of contact with the public, patients and relatives has proven
54 491 mutually useful. We plan to intensify participation in such meetings to inform about current knowledge
55 492 about prevention and treatment of AD, particularly via the established user groups. Communication
56 493 via Internet: One of the most important media for spreading the news and awareness will be the
57 494 Internet. The results and information (including videos) about the studies will be presented on CERG's
58 495 webpage (ntnu.edu/cerg), one of the most visited sites at Scandinavian universities; and ExPlas's own
59
60

1
2
3 496 Norwegian webpage (ntnu.no/cerg/explas). Scientific and non-scientific communication: General
4 497 communication activities include publication in open access peer reviewed journals, non-scientific
5 498 journals and at national- and international meetings, to reach the general public, patients, scientists,
6 499 and policy makers. Importantly, our group is closely linked and active partners in the Norwegian
7 500 Research School in Neuroscience, Physical Activity and Health (master program) and medical education
8 501 where we actively will present our research to the next generation of health care personnel and
9 502 scientists. We also have a journalist at CERG (Anders Revdal), available for ExPlas, who will be
10 503 responsible for communication.
11 504

13 505 DISCUSSION

14 506
15 507 To our knowledge, ExPlas will be the first study with the aim of studying safety and efficacy of
16 508 transfusion of plasma from well endurance trained donors to patients in the early symptomatic phase
17 509 of AD. Even if prevention probably will be the most effective way to reduce numbers of patients with
18 510 AD worldwide, we need a cure for this devastating disease which impacts the lives for both patients
19 511 and their families substantially. There is also a need to understand the mechanisms behind the
20 512 beneficial effect of physical exercise on the brain, and it seems logically to try to exploit this effect in
21 513 treatment of the early phase of symptomatic AD.
22 514

23 515 On June 7th, 2021, the U.S. Food and Drug Administration, approved aducanumab (marketed as
24 516 Aduhelm) for use in treatment of AD (68), due to its ability to reduce amyloid plaques in the brain,
25 517 under an accelerated approval pathway (69). Confirmation of the clinical benefit is still required to be
26 518 confirmed for continued approval (68). Independent of the usefulness of aducanumab in AD
27 519 therapeutics, other interventions capable of delaying the clinical onset of AD dementia should
28 520 continue to be studied. The findings from preclinical AD models (36-38, 70), and a small clinical trial
29 521 (39) clearly indicate that there is communication between the systemic environment and the
30 522 hippocampus. Systemic factors are capable of inducing changes, and even therapeutic effects, in the
31 523 brain without hindrance by the blood-brain barrier; potentially an effective strategy to reverse
32 524 neurodegeneration in the AD-brain (30). There are myriads of factors and processes that are set in
33 525 motion during and after exercise training, and much of this is reflected in the composition of the blood
34 526 (71). Thus, it is not likely that it is a single factor is orchestrating the beneficial effects of exercise, but
35 527 rather an interplay between several molecular factors that need to be discovered and understood to
36 528 develop the first-generation of exercise-mimicking drugs. This is a promising idea as a large population
37 529 of patients are simply unable to exercise, such as patients in the intensive care unit, the critically ill,
38 530 patients recovering from accidents, the morbidly obese and paralyzed patients. For these patients,
39 531 innovative exercise-mimicking therapies could be of benefit.
40 532

41 533 However, development of exercise-mimicking therapies is a very complex and time-consuming
42 534 undertaking, that should not delay the testing of a potential benefit of exercise trained plasma, with
43 535 most of its natural components, on safety and therapeutic effect in patients with AD. In the context of
44 536 lack of disease-modifying treatments, ExPlas is innovative and potentially groundbreaking if it is found
45 537 to be safe with few side effects and with similar promising results as seen in preclinical AD models (35-
46 538 38).
47 539

48 540 Another key question is at what stage of AD interventions such as ExPlas treatment can be expected
49 541 to have an effect. Today we know that AD-related changes in the brain are present 10-30 years before
50 542 symptoms develop. The optimal time window for treatment is probably as early as possible during this
51 543 period, to decelerate or stop the progressive loss of neurons before functions are lost. As in all
52 544 diseases, prevention will always be the optimal path. Depending upon outcomes in the ExPlas Study,
53 545 a natural next step may be treatment with ExPlas of patients in the preclinical phase of the disease
54 546 (62).

1
2
3 547 A small clinical trial found that plasma from young donors (young blood) transfused to patients with
4 548 mild to moderate AD dementia (MMSE score ranging from 12-24) was safe with no adverse events and
5 549 possibly beneficial with improvement in functional activity. In this study, 9 patients were randomized
6 550 to a cross-over cohort, receiving 4 once-weekly infusions of either 250 mL of plasma from male donors
7 551 (aged 18-30 years) or 250 mL of saline, followed by a 6-week washout and crossover to 4 once-weekly
8 552 infusions of the alternate treatment. In addition, 9 patients were included in an open-label design in
9 553 which patients received 4 once-weekly infusions of only young plasma. Considering the low number of
10 554 patients, short follow-up period and promising findings in the study by Sha *et al.* (39) there is reason
11 555 to believe that transfusion of exercise-trained plasma also is safe. With increased treatment periods
12 556 and extended follow-up, we believe the ExPlas Study is well designed also to evaluate the potential
13 557 therapeutic effect of exercise trained plasma. The relatively large number of patients will also likely
14 558 enable us to assess whether endpoints become differentially affected by *APOE 4* status. As the ExPlas
15 559 Study is the first of its kind it is not straightforward to undertake power calculations, and the results of
16 560 our study may be useful for planning of an appropriately sample sized study in the future.
17 561

20 562 We expect the ExPlas study to give new knowledge about whether transfusion of plasma from
21 563 exercise-trained donors is safe and indications on whether it has therapeutic effects. ExPlas will also
22 564 contribute to pioneering the discovery of molecular targets to potentially treat AD and lay the
23 565 foundation for first-generation exercise-mimicking drugs, by capturing the molecular signature of high-
24 566 fitness and molecular mechanisms provided by exercise.
25 567

26 568 **Contributor statement**

- 28 569 **Atefe R. Tari** Conception and design of the study, obtained funding, drafting the
29 570 manuscript and applications to ethical committee and Norwegian Medicine Agency for study
30 571 approval
- 32 572 **Helene H. Berg** Design of the study, drafting the manuscript, critical review of manuscript
- 34 573 **Vibeke Videm** Conception and design of the study, critical review of the manuscript,
35 574 supervision, obtained funding, applications to ethical committee and Norwegian Medicine Agency for
36 575 study approval
- 38 576 **Geir Bråthen** Conception and design of the study, critical review of the manuscript,
39 577 supervision, obtained funding, applications to ethical committee and Norwegian Medicine Agency for
40 578 study approval
- 41 579 **Linda R. White** Conception and design of the study, critical review of the manuscript,
42 580 supervision, obtained funding, applications to ethical committee and Norwegian Medicine Agency for
43 581 study approval
- 45 582 **Ragnhild Røsbjorgen** Critical review of the manuscript, supervision, established methods and
46 583 operating procedures for the study
- 48 584 **Katja Scheffler** Design of the study, critical review of the manuscript, established procedures
49 585 for AD biomarkers in blood and CSF for ExPlas
- 51 586 **Håvard Dalen** Critical review of the manuscript, established echocardiographic procedures
52 587 for AD patients in ExPlas
- 53 588 **Espen Holte** Critical review of the manuscript, established echocardiographic procedures
54 589 for AD patients in ExPlas
- 56 590 **Asta Håberg** Design of the study, critical review of the manuscript, obtained funding,
57 591 established structural and functional-MRI procedures for AD patients in ExPlas, applications to ethical
58 592 committee for study approval
- 60 593 **Geir Selbæk** Design of the study, critical review of the manuscript, obtained funding

- 1
2
3 594 **Stian Lydersen** Design of the study, critical review of the manuscript, making the statistical
4 595 plan and analyses in ExPlas
5
6 596 **Emrah Duzel** Critical review of the manuscript, established methods and operating
7 597 procedures for the study
8
9 598 **Sverre Bergh** Critical review of the manuscript, obtained funding
10
11 599 **Kjell Rune Halvorsrud** Design of the study, critical review of the manuscript, supervision,
12 600 established methods and operating procedures for the blood-donor part of ExPlas, supervision,
13 601 obtained funding
14 602 **Sigrid B. Sando** Conception and design of the study, critical review of the manuscript,
15 603 supervision, obtained funding, applications to ethical committee and Norwegian Medicine Agency for
16 604 study approval, Co-PI of ExPlas
17
18 605 **Ulrik Wisløff** Conception and design of the study, critical review of the manuscript,
19 606 supervision, obtained funding, applications to ethical committee and Norwegian Medicine Agency for
20 607 study approval, Co-PI of ExPlas
21
22 608
23 609 **Acknowledgement**
24
25 610 ExPlas study group acknowledge Randi Granbo, Tone Stav and Geir Suul for their active role in the
26 611 ExPlas user group.
27
28 612
29 613 **Competing interests**
30
31 614 None of the authors reports any competing interests or had financial relationships with any
32 615 organisations that might have an interest in the submitted work in the previous three years; no other
33 616 relationships or activities that could appear to have influenced the submitted work. All authors will
34 617 complete the ICMJE uniform disclosure form in due time.
35
36 618
37 619 **Funding**
38
39 620
 - Research Council of Norway;
40
41 621
 - The K.G. Jebsen Foundation for Medical Research, Norway;
42
43 622
 - Norwegian University of Science and Technology (NTNU);
44
45 623
 - Central Norway Regional Health Authority;
46
47 624
 - St Olavs hospital, Trondheim, Norway;
48
49 625
 - The National Association for Public Health, Norway
50
51 626
 - The Liaison Committee for Central Norway Regional Health Authority
52
53 627 Authors reports no financial relationships with any organisations that might have an interest in the
54 628 submitted work in the previous three years, or any other relationships or activities that could appear
55 629 to have influenced the submitted work.
56
57 630
58 631 **Country of Recruitment**
59
60 632 Norway

1
2
3 633 **Trial sponsor**

4 634 Øystein Risa, Head of Department

5 635 Department of Circulation and Medical Imaging, NTNU - Norwegian University of Science and

6 636 Technology, Box 8905 MTF, 7491 Trondheim, Norway. Tel: (+47) 92613734

7 637 E-mail: oystein.risa@ntnu.no

8 638

9
10
11
12 639 **Data sharing**

13 640 We are not permitted to share individual data from the current trial, but we are open to
14 641 collaborative research with researchers worldwide, who can have access to analysed data from our
15 642 university. We have also established a biobank of blood and genetic material that we plan to share
16 643 with researchers worldwide, but individual data must be analysed within our university only.

17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For peer review only

644 References

- 645
- 646 1. World Health Organization. Aging and Life Course 2015 [Available from:
647 <http://www.who.int/ageing/en/>.
- 648 2. alz.org. Alzheimer's Disease Facts and Figures [Report]. 2018 [updated 2018. 5,8].
649 Available from: <http://www.alz.org>.
- 650 3. Andrieu S, Coley N, Lovestone S, Aisen PS, Vellas B. Prevention of sporadic
651 Alzheimer's disease: lessons learned from clinical trials and future directions. *Lancet Neurol.*
652 2015;14(9):926-44.
- 653 4. Helsedirektoratet d. Demensplan 2025.
- 654 5. Gjøra L, Strand BH, Bergh S, Borza T, Brækhus A, Engedal K, et al. Current and Future
655 Prevalence Estimates of Mild Cognitive Impairment, Dementia, and Its Subtypes in a
656 Population-Based Sample of People 70 Years and Older in Norway: The HUNT Study. *J*
657 *Alzheimers Dis.* 2021;79(3):1213-26.
- 658 6. Kvello-Alme M, Bråthen G, White LR, Sando SB. The Prevalence and Subtypes of
659 Young Onset Dementia in Central Norway: A Population-Based Study. *J Alzheimers Dis.*
660 2019;69(2):479-87.
- 661 7. Association As. 2021 Alzheimer's Disease Facts and Figures. 2021.
- 662 8. Potter PE. Investigational medications for treatment of patients with Alzheimer
663 disease. *J Am Osteopath Assoc.* 2010;110(9 Suppl 8):S27-36.
- 664 9. Mullane K, Williams M. Alzheimer's therapeutics: continued clinical failures question
665 the validity of the amyloid hypothesis-but what lies beyond? *Biochem Pharmacol.*
666 2013;85(3):289-305.
- 667 10. Cummings JL, Morstorf T, Zhong K. Alzheimer's disease drug-development pipeline:
668 few candidates, frequent failures. *Alzheimers Res Ther.* 2014;6(4):37.
- 669 11. Livingston G, Huntley J, Sommerlad A, Ames D, Ballard C, Banerjee S, et al. Dementia
670 prevention, intervention, and care: 2020 report of the Lancet Commission. *Lancet.*
671 2020;396(10248):413-46.
- 672 12. Barnes DE, Yaffe K. The projected effect of risk factor reduction on Alzheimer's
673 disease prevalence. *Lancet Neurol.* 2011;10(9):819-28.
- 674 13. Colcombe SJ, Erickson KI, Raz N, Webb AG, Cohen NJ, McAuley E, et al. Aerobic fitness
675 reduces brain tissue loss in aging humans. *The journals of gerontology.* 2003;58(2):176-80.
- 676 14. de Bruijn RF, Ikram MA. Cardiovascular risk factors and future risk of Alzheimer's
677 disease. *BMC Med.* 2014;12:130.
- 678 15. Levine DA, Langa KM. Vascular cognitive impairment: disease mechanisms and
679 therapeutic implications. *Neurotherapeutics.* 2011;8(3):361-73.
- 680 16. Rovio S, Kareholt I, Helkala EL, Viitanen M, Winblad B, Tuomilehto J, et al. Leisure-
681 time physical activity at midlife and the risk of dementia and Alzheimer's disease. *The Lancet*
682 *Neurology.* 2005;4(11):705-11.
- 683 17. Qiu C, Xu W, Fratiglioni L. Vascular and psychosocial factors in Alzheimer's disease:
684 epidemiological evidence toward intervention. *J Alzheimers Dis.* 2010;20(3):689-97.
- 685 18. Qiu C. Preventing Alzheimer's disease by targeting vascular risk factors: hope and
686 gap. *J Alzheimers Dis.* 2012;32(3):721-31.
- 687 19. Exalto LG, Quesenberry CP, Barnes D, Kivipelto M, Biessels GJ, Whitmer RA. Midlife
688 risk score for the prediction of dementia four decades later. *Alzheimer's & dementia : the*
689 *journal of the Alzheimer's Association.* 2014;10(5):562-70.

- 1
2
3 690 20. Tolppanen AM, Solomon A, Soininen H, Kivipelto M. Midlife vascular risk factors and
4 691 Alzheimer's disease: evidence from epidemiological studies. *J Alzheimers Dis*.
5 692 2012;32(3):531-40.
- 6 693 21. Norton S, Matthews FE, Barnes DE, Yaffe K, Brayne C. Potential for primary
7 694 prevention of Alzheimer's disease: an analysis of population-based data. *Lancet Neurol*.
8 695 2014;13(8):788-94.
- 9 696 22. Kaminsky LA, Arena R, Beckie TM, Brubaker PH, Church TS, Forman DE, et al. The
10 697 importance of cardiorespiratory fitness in the United States: the need for a national registry:
11 698 a policy statement from the American Heart Association. *Circulation*. 2013;127(5):652-62.
- 12 699 23. Larson EB, Wang L, Bowen JD, McCormick WC, Teri L, Crane P, et al. Exercise is
13 700 associated with reduced risk for incident dementia among persons 65 years of age and older.
14 701 *Ann Intern Med*. 2006;144(2):73-81.
- 15 702 24. Tari AR, Nauman J, Zisko N, Skjellegrind HK, Bosnes I, Bergh S, et al. Temporal
16 703 changes in cardiorespiratory fitness and risk of dementia incidence and mortality: a
17 704 population-based prospective cohort study. *The Lancet Public Health*. 2019;4(11):e565-e74.
- 18 705 25. Hoffmann K, Frederiksen KS, Sobol NA, Beyer N, Vogel A, Simonsen AH, et al.
19 706 Preserving cognition, quality of life, physical health and functional ability in Alzheimer's
20 707 disease: the effect of physical exercise (ADEX trial): rationale and design.
21 708 *Neuroepidemiology*. 2013;41(3-4):198-207.
- 22 709 26. Lautenschlager NT, Cox KL, Flicker L, Foster JK, van Bockxmeer FM, Xiao J, et al. Effect
23 710 of physical activity on cognitive function in older adults at risk for Alzheimer disease: a
24 711 randomized trial. *Jama*. 2008;300(9):1027-37.
- 25 712 27. van Praag H, Shubert T, Zhao C, Gage FH. Exercise enhances learning and
26 713 hippocampal neurogenesis in aged mice. *J Neurosci*. 2005;25(38):8680-5.
- 27 714 28. van Praag H, Christie BR, Sejnowski TJ, Gage FH. Running enhances neurogenesis,
28 715 learning, and long-term potentiation in mice. *Proc Natl Acad Sci U S A*. 1999;96(23):13427-
29 716 31.
- 30 717 29. Maass A, Duzel S, Goerke M, Becke A, Sobieray U, Neumann K, et al. Vascular
31 718 hippocampal plasticity after aerobic exercise in older adults. *Molecular psychiatry*.
32 719 2015;20(5):585-93.
- 33 720 30. Bouchard J, Villeda SA. Aging and brain rejuvenation as systemic events. *J*
34 721 *Neurochem*. 2015;132(1):5-19.
- 35 722 31. Pereira AC, Huddleston DE, Brickman AM, Sosunov AA, Hen R, McKhann GM, et al. An
36 723 in vivo correlate of exercise-induced neurogenesis in the adult dentate gyrus. *Proc Natl Acad*
37 724 *Sci U S A*. 2007;104(13):5638-43.
- 38 725 32. Fabel K, Fabel K, Tam B, Kaufer D, Baiker A, Simmons N, et al. VEGF is necessary for
39 726 exercise-induced adult hippocampal neurogenesis. *The European journal of neuroscience*.
40 727 2003;18(10):2803-12.
- 41 728 33. Castellano JM, Mosher KI, Abbey RJ, McBride AA, James ML, Berdnik D, et al. Human
42 729 umbilical cord plasma proteins revitalize hippocampal function in aged mice. *Nature*.
43 730 2017;544(7651):488-92.
- 44 731 34. Tari AR, Norevik CS, Scrimgeour NR, Kobro-Flatmoen A, Storm-Mathisen J, Bergersen
45 732 LH, et al. Are the neuroprotective effects of exercise training systemically mediated?
46 733 *Progress in cardiovascular diseases*. 2019.
- 47 734 35. Villeda SA, Plambeck KE, Middeldorp J, Castellano JM, Mosher KI, Luo J, et al. Young
48 735 blood reverses age-related impairments in cognitive function and synaptic plasticity in mice.
49 736 *Nat Med*. 2014;20(6):659-63.

- 1
2
3 737 36. Katsimpardi L, Litterman NK, Schein PA, Miller CM, Loffredo FS, Wojtkiewicz GR, et al.
4 738 Vascular and neurogenic rejuvenation of the aging mouse brain by young systemic factors.
5 739 Science. 2014;344(6184):630-4.
- 7 740 37. Xia E, Xu F, Hu C, Kumal JPP, Tang X, Mao D, et al. Young Blood Rescues the Cognition
8 741 of Alzheimer's Model Mice by Restoring the Hippocampal Cholinergic Circuit. Neuroscience.
9 742 2019;417:57-69.
- 10 743 38. Zhao Y, Qian R, Zhang J, Liu F, Iqbal K, Dai CL, et al. Young blood plasma reduces
11 744 Alzheimer's disease-like brain pathologies and ameliorates cognitive impairment in 3xTg-AD
12 745 mice. *Alzheimers Res Ther.* 2020;12(1):70.
- 14 746 39. Sha SJ, Deutsch GK, Tian L, Richardson K, Coburn M, Gaudioso JL, et al. Safety,
15 747 Tolerability, and Feasibility of Young Plasma Infusion in the Plasma for Alzheimer Symptom
16 748 Amelioration Study: A Randomized Clinical Trial. *JAMA Neurol.* 2018.
- 18 749 40. Horowitz AM, Fan X, Bieri G, Smith LK, Sanchez-Diaz CI, Schroer AB, et al. Blood
19 750 factors transfer beneficial effects of exercise on neurogenesis and cognition to the aged
20 751 brain. *Science.* 2020;369(6500):167-73.
- 21 752 41. Aspenes ST, Nilsen TI, Skaug EA, Bertheussen GF, Ellingsen Ø, Vatten L, et al. Peak
22 753 oxygen uptake and cardiovascular risk factors in 4631 healthy women and men. *Med Sci*
23 754 *Sports Exerc.* 2011;43(8):1465-73.
- 25 755 42. Nauman J, Nes BM, Zisko N, Revdal A, Myers J, Kaminsky LA, et al. Personal Activity
26 756 Intelligence (PAI): A new standard in activity tracking for obtaining a healthy
27 757 cardiorespiratory fitness level and low cardiovascular risk. *Progress in cardiovascular*
28 758 *diseases.* 2019;62(2):179-85.
- 30 759 43. Aspenes ST, Nilsen TI, Skaug EA, Bertheussen GF, Ellingsen O, Vatten L, et al. Peak
31 760 oxygen uptake and cardiovascular risk factors in 4631 healthy women and men. *Med Sci*
32 761 *Sports Exerc.* 2011;43(8):1465-73.
- 34 762 44. Dubois B, Feldman HH, Jacova C, Hampel H, Molinuevo JL, Blennow K, et al.
35 763 Advancing research diagnostic criteria for Alzheimer's disease: the IWG-2 criteria. *Lancet*
36 764 *Neurol.* 2014;13(6):614-29.
- 37 765 45. Morris JC, Heyman A, Mohs RC, Hughes JP, van Belle G, Fillenbaum G, et al. The
38 766 Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part I. Clinical and
39 767 neuropsychological assessment of Alzheimer's disease. *Neurology.* 1989;39(9):1159-65.
- 41 768 46. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for
42 769 grading the cognitive state of patients for the clinician. *J Psychiatr Res.* 1975;12(3):189-98.
- 43 770 47. McDougall F, Edgar C, Mertes M, Delmar P, Fontoura P, Abi-Saab D, et al.
44 771 Psychometric Properties of the Clinical Dementia Rating - Sum of Boxes and Other Cognitive
45 772 and Functional Outcomes in a Prodromal Alzheimer's Disease Population. *J Prev Alzheimers*
46 773 *Dis.* 2021;8(2):151-60.
- 48 774 48. Reitan RM. Validity of the Trail Making Test as an indication of organic brain damage.
49 775 Perceptual and motor skills. 1958;8(3):271-6.
- 51 776 49. Espenes J, Hessen E, Eliassen IV, Waterloo K, Eckerström M, Sando SB, et al.
52 777 Demographically adjusted trail making test norms in a Scandinavian sample from 41 to 84
53 778 years. *Clin Neuropsychol.* 2020;34(sup1):110-26.
- 54 779 50. Brodaty H, Moore CM. The Clock Drawing Test for dementia of the Alzheimer's type:
55 780 A comparison of three scoring methods in a memory disorders clinic. *Int J Geriatr Psychiatry.*
56 781 1997;12(6):619-27.
- 58
59
60

- 1
2
3 782 51. Nutter-Upham KE, Saykin AJ, Rabin LA, Roth RM, Wishart HA, Pare N, et al. Verbal
4 783 fluency performance in amnesic MCI and older adults with cognitive complaints. *Arch Clin*
5 784 *Neuropsychol.* 2008;23(3):229-41.
- 7 785 52. Patterson C. World Alzheimer report 2018. Alzheimer's Disease International; 2018.
- 8 786 53. Elizabeth K Warrington MJ. Visual Object and Space Perception Battery (VOSP)1991.
- 9 787 54. Binetti T. Disorders of visual and spatial perception in the early stage of Alzheimers
10 788 disease1996.
- 11 789 55. Hughes CP, Berg L, Danziger WL, Coben LA, Martin RL. A new clinical scale for the
12 790 staging of dementia. *Br J Psychiatry.* 1982;140:566-72.
- 13 791 56. O'Bryant SE, Humphreys JD, Smith GE, Ivnik RJ, Graff-Radford NR, Petersen RC, et al.
14 792 Detecting dementia with the mini-mental state examination in highly educated individuals.
15 793 *Arch Neurol.* 2008;65(7):963-7.
- 16 794 57. Lawton MP, Brody EM. Assessment of older people: self-maintaining and
17 795 instrumental activities of daily living. *Gerontologist.* 1969;9(3):179-86.
- 18 796 58. Fagerland M, Lydersen, S., & Laake, P. *Statistical Analysis of Contingency Tables.*
19 797 Chapman and Hall/CRC2017.
- 20 798 59. Ries JD, Echternach JL, Nof L, Gagnon Blodgett M. Test-retest reliability and minimal
21 799 detectable change scores for the timed "up & go" test, the six-minute walk test, and gait
22 800 speed in people with Alzheimer disease. *Phys Ther.* 2009;89(6):569-79.
- 23 801 60. Geschke K, Fellgiebel A, Laux N, Schermuly I, Scheurich A. Quality of life in dementia:
24 802 impact of cognition and insight on applicability of the SF-36. *Am J Geriatr Psychiatry.*
25 803 2013;21(7):646-54.
- 26 804 61. Ware JE, Jr., Sherbourne CD. The MOS 36-item short-form health survey (SF-36). I.
27 805 Conceptual framework and item selection. *Med Care.* 1992;30(6):473-83.
- 28 806 62. Dubois B, Hampel H, Feldman HH, Scheltens P, Aisen P, Andrieu S, et al. Preclinical
29 807 Alzheimer's disease: Definition, natural history, and diagnostic criteria. *Alzheimers Dement.*
30 808 2016;12(3):292-323.
- 31 809 63. Bateman RJ, Xiong C, Benzinger TL, Fagan AM, Goate A, Fox NC, et al. Clinical and
32 810 biomarker changes in dominantly inherited Alzheimer's disease. *N Engl J Med.*
33 811 2012;367(9):795-804.
- 34 812 64. Dubois B, Hampel H, Feldman HH, Scheltens P, Aisen P, Andrieu S, et al. Preclinical
35 813 Alzheimer's disease: Definition, natural history, and diagnostic criteria. *Alzheimers Dement.*
36 814 2016;12(3):292-323.
- 37 815 65. Bye A, Røsjø H, Aspenes ST, Condorelli G, Omland T, Wisløff U. Circulating microRNAs
38 816 and aerobic fitness--the HUNT-Study. *PLoS One.* 2013;8(2):e57496.
- 39 817 66. J T, L B, T H, J R, M W, M H. Different ways to estimate treatment effects in
40 818 randomised controlled trials. *Contemp Clin Trials Commun.* 2018;10:80-5.
- 41 819 67. Coffman CJ, Edelman D, Woolson RF. To condition or not condition? Analysing
42 820 'change' in longitudinal randomised controlled trials. *BMJ Open.* 2016;6(12):e013096.
- 43 821 68. Cavazzoni P. FDA's Decision to Approve New Treatment for Alzheimer's Disease:
44 822 [fda.gov; 06/07/2021](https://www.fda.gov/drugs/news-events-human-drugs/fdas-decision-approve-new-treatment-alzheimers-disease) [Available from: [https://www.fda.gov/drugs/news-events-human-](https://www.fda.gov/drugs/news-events-human-drugs/fdas-decision-approve-new-treatment-alzheimers-disease)
45 823 [drugs/fdas-decision-approve-new-treatment-alzheimers-disease](https://www.fda.gov/drugs/news-events-human-drugs/fdas-decision-approve-new-treatment-alzheimers-disease).
46 824 69. Sevigny J, Chiao P, Bussièrè T, Weinreb PH, Williams L, Maier M, et al. The antibody
47 825 aducanumab reduces A β plaques in Alzheimer's disease. *Nature.* 2016;537(7618):50-6.
- 48 826 70. Villeda SA, Plambeck KE, Middeldorp J, Castellano JM, Mosher KI, Luo J, et al. Young
49 827 blood reverses age-related impairments in cognitive function and synaptic plasticity in mice.
50 828 *Nature Medicine.* 2014;20:659.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

829 71. Tari AR, Norevik CS, Scrimgeour NR, Kobro-Flatmoen A, Storm-Mathisen J, Bergersen
830 LH, et al. Are the neuroprotective effects of exercise training systemically mediated?
831 Progress in cardiovascular diseases. 2019;62(2):94-101.
832
833

For peer review only

1
2
3 834 **Figure text**

4 835

5 836

6 837 **Figure 1.** Flow chart of the ExPlas Study. Infusion solution, Sodium Chloride 0.9% (Saline); US,

7 838 ultrasound of the heart; fMRI, functional magnetic resonance imaging.

8 839

9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For peer review only

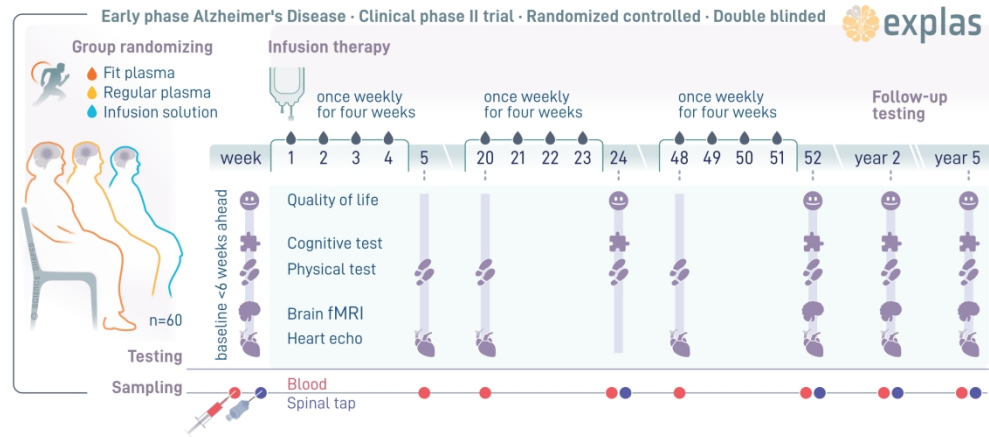


Figure 1. Flow chart of the ExPlas Study. Infusion solution, Sodium Chloride 0.9% (Saline); US, ultrasound of the heart; fMRI, functional magnetic resonance imaging.

533x239mm (118 x 118 DPI)



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

1				
2	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
3				
4				
5				
6				
7				
8	Methods: Participants, interventions, and outcomes			
9				
10	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
11				
12				
13				
14	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
15				
16				
17				
18				
19	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
20				
21				
22		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
23				
24				
25				
26				
27		11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	
28				
29				
30				
31		11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	p. 5/ 187 -189
32				
33				
34	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
35				
36				
37				
38				
39				
40				
41				
42	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
43				
44				
45				
46				
47	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
48				
49				
50				
51	Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	p. 13/ 460 -475
52				
53				
54				
55				
56				
57				
58				
59				
60				

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

1				
2		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
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				
21				
22				
23				
24				
25				
26				
27				
28				
29				
30				
31				
32				
33				
34				
35				
36				
37				
38				
39				
40				
41				
42				
43				
44				
45				
46				
47				
48				
49				
50				
51				
52				
53				
54				
55				
56				
57				
58				
59				
60				

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

1				
2	Ancillary and	30	Provisions, if any, for ancillary and post-trial care, and for	
3	post-trial care		compensation to those who suffer harm from trial participation	
4				
5	Dissemination	31a	Plans for investigators and sponsor to communicate trial results to	p. 13-14/
6	policy		participants, healthcare professionals, the public, and other relevant	477-493
7			groups (eg, via publication, reporting in results databases, or other	
8			data sharing arrangements), including any publication restrictions	
9				
10				
11		31b	Authorship eligibility guidelines and any intended use of professional	p. 15/ 559
12			writers	-598
13				
14		31c	Plans, if any, for granting public access to the full protocol, participant-	p. 17/ 631
15			level dataset, and statistical code	-635
16				
17	Appendices			
18				
19	Informed consent	32	Model consent form and other related documentation given to	Attachment
20	materials		participants and authorised surrogates	
21				
22				
23	Biological	33	Plans for collection, laboratory evaluation, and storage of biological	p. 17/ 631
24	specimens		specimens for genetic or molecular analysis in the current trial and for	-635
25			future use in ancillary studies, if applicable	
26				

*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](https://creativecommons.org/licenses/by-nc-nd/3.0/)" license.