Official Title: A Multi-center, Randomized, Double-blind, Placebo-controlled, 24 months Study in Patients with amnestic Mild Cognitive Impairment or Very Mild Alzheimer's Disease to Investigate the Safety, Tolerability and Immune Response of Repeated Subcutaneous Injections of ABvac40.

NCT Number: NCT03461276

Document Date: Protocol Version 5.0; 22 May 2020

Clinical Study Protocol

Protocol Title:	A Multi-center, Randomized, Double-blind, Placebo-controlled, 24 months Study in Patients with amnestic Mild Cognitive Impairment or Very Mild Alzheimer's Disease to Investigate the Safety, Tolerability and Immune Response of Repeated Subcutaneous Injections of ABvac40.
Investigational Product:	ABvac40
Sponsor's Name and Address:	ARACLON BIOTECH, S.L. a GRIFOLS company, Vía de la Hispanidad, 21 50009 Zaragoza, Spain
Sponsor's Telephone Number:	[redacted]
Study Number/Protocol Version Number/Date:	AB1601 Version V5.0 SPA including Amendment 3 22 of May 2020
Previous version	Version V4.0 SPA including Amendment 2 23 of July 2018
Additional Identifier	
EUDRACT Number:	2016-004352-30
IND/CTA Number	NA
Development Phase:	Phase 2

Protocol signature sheet

Study code: AB1601
EudraCT No: 2016-004352-30

All those who sign below agree with the study protocol "*AB1601 Clinical Trial Protocol V5.0 SPA including Amendment 3*". The information contained in the protocol is consistent with the current assessment of the risk/benefit ratio, as well as moral, ethical and scientific principles governing clinical research as set out in the latest version of the Declaration of Helsinki and Good Clinical Practice guidelines.

[redacted]		
Name	Date	Signature
[redacted]	13-Jul-2020 | 03:45 PDT	DocuSigned by: [redacted] Signer Name: [redacted] Signing Reason: I approve this document Signing Time: 13-Jul-2020 | 03:45 PDT [redacted]

Sponsor		
Name	Date	Signature
[redacted] Araclon	10-jul.-2020 | 08:33 PDT	DocuSigned by: [redacted] Nombre del firmante: [redacted] Motivo de la firma: Apruebo este documento Hora de firma: 10-jul.-2020 | 08:33 PDT [redacted]

Sponsor		
Name	Date	Signature
[redacted] Araclon	10-jul.-2020 | 06:16 PDT	DocuSigned by: [redacted] Nombre del firmante: [redacted] Motivo de la firma: He revisado este documento Hora de firma: 10-jul.-2020 | 06:16 PDT [redacted]

[redacted]		
Name	Date	Signature
[redacted]		

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PROTOCOL SYNOPSIS

Title of Study:

A Multicenter, Randomized, Double-blind, Placebo-controlled, 24 months Study in Patients with amnestic Mild Cognitive Impairment or Very Mild Alzheimer´s Disease to Investigate the Safety, Tolerability and Immune Response of Repeated Subcutaneous Injections of ABvac40.

Study Number:

AB1601

Phase:

Confirmatory Phase 2 clinical trial.

Study Objectives:

Part A (Randomized, double-blind, placebo-controlled – 18 to 24 months follow-up):

Primary Safety Objective

- To evaluate the safety and tolerability of repeated doses of ABvac40 in a population of patients with amnestic Mild Cognitive Impairment (a-MCI) or very mild Alzheimer's disease (vm-AD).

Primary Efficacy Objective (immunogenicity)

- To assess the immune response produced during the study by repeated doses of ABvac40 in a population of a-MCI or vm-AD.

Secondary (Exploratory) Efficacy Objectives

- To characterize the immune response elicited by repeated doses of ABvac40 in a population of a-MCI or vm-AD.
- To assess the changes in the disease biomarkers elicited by ABvac40 in the overall study population.
- To assess the changes in cognition and quality of life elicited by ABvac40 in the overall study population.

Part B (ABvac40 delayed start, open label [Part A assignment will remain blinded for investigators and patients] – 18 months follow-up):

Exploratory Safety Objective

- To evaluate the safety and tolerability of repeated doses of ABvac40 after delayed start in patients receiving placebo during Part A, and the long-term safety and tolerability of ABvac40 in patients receiving a booster after their Part A vaccination scheme.

Exploratory Efficacy Objectives

Immunogenicity

- To assess the immune response produced by repeated doses of ABvac40 after delayed start in patients receiving placebo during Part A.
- To assess the immune response triggered by a second ABvac40 booster in patients receiving ABvac40 during Part A.

Other

- To assess the changes in the disease biomarkers elicited by ABvac40 in the overall study population.
- To assess the changes in cognition and quality of life elicited by ABvac40 in the overall study population and according to the treatment scheme during Part A and B.
- To assess the immunological memory, characterizing the immune response ex vivo elicited by the booster in patients who have received ABvac40 during Part A.
- To assess the potential relationship between antibody titration, biomarkers and cognitive state.
- To define the optimal ABvac40 treatment scheme.

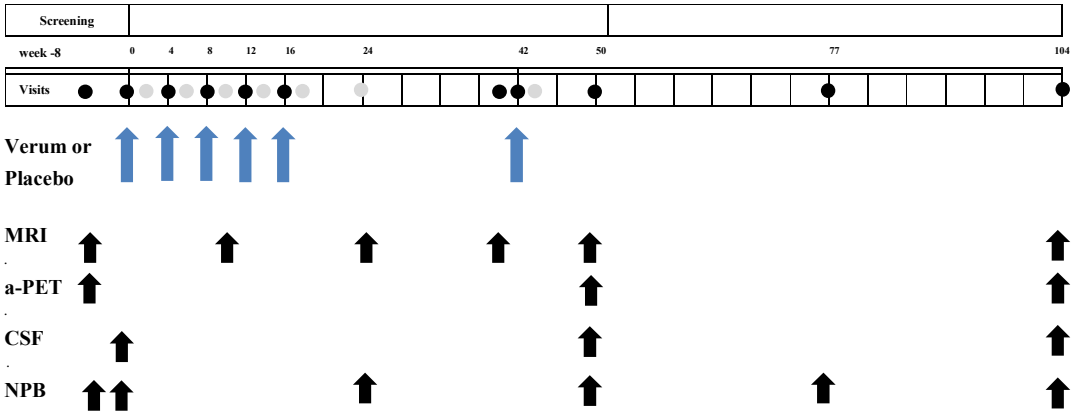
Overall Study Design and Description:

A multi-center, prospective, longitudinal, randomized, double-blind, placebo-controlled, two parallel treatment groups (verum and placebo), confirmatory phase 2 clinical trial has been designed (Part A).

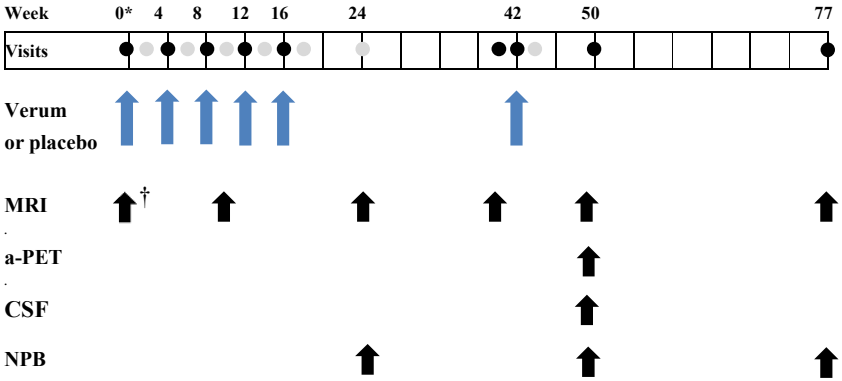
Additionally, a delayed-start vaccination, open label (Part A assignment will remain blinded for investigators and patients) extension will start after at least 18 months of Part A initiation (Part B).

OVERALL STUDY DESIGN

Part A:



Part B:



***At least 18 months after initiating Part A**

†A MRI will be performed before first vaccination of Part B only if the previous MRI was performed more than 6 months (±15 days) before V1B

STUDY SCHEME

Part A:

	Screening																																			
Month	-2	0			1			2			3			4			5	6	7	8	9		10			11	12	..	15	..	18	..	21	..	24
Week	-8	0	0	2	4	4	6	8	8	10	12	12	14	16	16	18		24			40	42	42	44		50		65		77		90		104
Day	-60	-3 /0*	3 ±3	14 ±3	28 ±3	31 ±3	42 ±3	56 ±3	59 ±3	70 ±3	84 ±3	87 ±3	98 ±3	112 ±3	115 ±3	126 ±3		168 ±7			280 ±7	294 ±7	297 ±7	308 ±7		350 ±15		455 ±15		539 ±15		630 ±15		728 ±15
Visit Nº (T=by phone)	0†	1* Vc	2 (T)	3 Sc	4 Vc	5 (T)	6 Sc	7 Vc	8 (T)	9 Sc	10 Vc	11 (T)	12 Sc	13 Vc	14 (T)	15 Sc		16 Int 6m			17 Sc	18 Vc	19 (T)	20 Sc		21 Sc		22 (T)		23 Sc		24 (T)		25 FV
Informed Consent	⊙[1]																																			
Vaccination ABvac40/placebo		⊙*			⊙			⊙			⊙			⊙								⊙§													
Medical History, Height (only at screening)	⊙[1]	⊙	⊙	⊙	⊙	⊙	⊙	⊙	⊙	⊙	⊙	⊙	⊙	⊙	⊙	⊙		⊙			⊙	⊙	⊙	⊙		⊙		⊙		⊙		⊙		⊙
Weight, BP, HR, RR, Tº	⊙[1]	⊙		⊙	⊙		⊙	⊙		⊙	⊙		⊙	⊙		⊙		⊙			⊙	⊙		⊙		⊙				⊙				⊙
Physical and neurological exam	⊙[1]	⊙		⊙	⊙		⊙	⊙		⊙	⊙		⊙	⊙		⊙		⊙			⊙	⊙		⊙		⊙				⊙				⊙
Neuropsych. Battery and IGE (exc. V0/V23)	⊙[1]	⊙																⊙								⊙				⊙				⊙
Quality of Life EQ-5D5L		⊙																								⊙								⊙
Eletrocardiogram	⊙[1]																	⊙#								⊙#				⊙#				⊙#
Urine Analysis (test strip)	⊙[2]	⊙		⊙			⊙			⊙			⊙			⊙		⊙			⊙			⊙		⊙				⊙				⊙
ApoE Genotyping		⊙																																	
Hemogram	⊙[2]	⊙		⊙			⊙			⊙			⊙			⊙		⊙			⊙			⊙		⊙				⊙				⊙
Biochemistry	⊙[2]	⊙		⊙			⊙			⊙			⊙			⊙		⊙			⊙			⊙		⊙				⊙				⊙
Biological Activity	⊙[2]	⊙		⊙			⊙			⊙			⊙			⊙		⊙			⊙			⊙		⊙				⊙				⊙
MRI	⊙[3]									⊙								⊙			⊙					⊙								⊙
a-PET	⊙[4]																									⊙								⊙
CSF		⊙																								⊙								⊙
Adverse events	⊙	⊙	⊙	⊙	⊙	⊙	⊙	⊙	⊙	⊙	⊙	⊙	⊙	⊙	⊙	⊙		⊙			⊙	⊙	⊙	⊙		⊙		⊙		⊙		⊙		⊙
Concomitant Medication	⊙[1]	⊙	⊙	⊙	⊙	⊙	⊙	⊙	⊙	⊙	⊙	⊙	⊙	⊙	⊙	⊙		⊙			⊙	⊙	⊙	⊙		⊙		⊙		⊙		⊙		⊙

MRI: Magnetic Resonance Imaging; a-PET: Amyloid PET; CSF: Cerebrospinal fluid; EKG: Electrocardiogram; exc.: except; IGE: Investigator Global Evaluation; NPB: Neuropsychological battery; FV: final visit; Vc: Vaccination; Sc: Safety control; Int: Interim analysis; T: Telephone call; RR: respiratory rate: Tº: body temperature

†The Screening Visit (V0) should be performed in 4 separate days:

[1]Day 1 of V0 (V0a):

- Signature of informed consent and verification of inclusion and exclusion criteria; medical history, vital signs, physical and neurological exam, neuropsychological battery, EKG, concomitant medication for AD and other diseases and delivery of Patient Card and Patient Diary; the scanned EKG must be sent to the Medical Advisor and no further assessments can be made to the subject until the approval is received at the center.
- If the patient meets all neuropsychological criteria, once the EKG has been approved by the Medical Advisor, the Day 2 of V0 (V0b) can be scheduled

[2]Day 2 of V0 (V0b):

- Urine analysis and blood sample extraction (for hemogram, biochemistry and biological activity)
- If no abnormal findings are found in the blood and urine tests, the Day 3 of V0 (V0c) can be scheduled.

[3]Day 3 of V0 (V0c):

- Magnetic Resonance Imaging
- Once the MRI has been approved by the MRI Central Reader, the Day 4 of V0 (V0d) can be scheduled

[4]Day 4 of V0 (V0d):

- a-PET
- Once the a-PET has been approved by the a-PET Central Reader, the patient can be randomized and the first day of V1 can be scheduled (a minimum of 4 days later, so that the appropriate material for CSF extraction and the investigational medicinal product (IMP) can be received at the center).

* The Inclusion Visit (V1) should be performed in 2 separate days; the vaccination should take place **at least 3 days** after the CSF extraction, **or the time needed until resolution of any AE occurring after the CSF extraction, whichever is longer** (to avoid interference with the assessment of AEs related to the vaccination)**, with a maximum of 10 days after the urine and blood sample extraction**:

- Day 1 of V1 (V1a):
  - Urine analysis, blood sample extraction (for ApoE genotyping, hemogram, biochemistry and biological activity) and CSF collection
- Day 2 of V1 (V1b):
  - Physical and neurological exam, neuropsychological battery and IGE, quality of life, vital signs (before vaccination), vaccination, vital signs (after vaccination), concomitant medication and AEs.

✓ A telephone control interview will take place within the first week (3± 3 days) after each vaccination visit and at V22 and V24.

✓ A safety visit will be scheduled two weeks after each vaccination and at V17 and V23.

✓ Blood samples will be collected at every safety control visit for safety control and characterization of immune response.

✓ Study visits should take place at the Days indicated above, within the following windows:

- During first 6 months: ± 3 days
- Between month 6 and month 12: ± 7 days
- Between month 12 and month 24: ± 15 days

✓ If a visit takes place out of window, the patient should be visited as soon as possible; For the five firsts immunizations the **minimum interval between vaccinations must be 25 days** and the **maximum interval must be 31 days**; if one vaccination is delayed for any reason, the next dose must be re-scheduled to fit this therapeutic window.

In this visit the scanned EKG must be sent to the Medical Advisor for review.

§ All patients who will attend their V18 after May 2020 must be tested for COVID-19 by PCR (Polymerase chain reaction test) method before receiving the study medication. A specific informed consent form (ICF) must be signed by the applicable patients authorizing this test (see Section 6.1.1.1 for further details)

Part B:

	Part B																										
Month	**0†**			**1**			**2**			**3**			**4**			**5**	**6**	**7**	**8**	**9**	**10**			**11**	**12**	**…**	**18**
Week	**0**	**0**	**2**	**4**	**4**	**6**	**8**	**8**	**10**	**12**	**12**	**14**	**16**	**16**	**18**		**24**			**40**	**42**	**42**	**44**		**50**		**77**
Day	**-3 /0***	**3 ±3**	**14 ±3**	**28 ±3**	**31 ±3**	**42 ±3**	**56 ±3**	**59 ±3**	**70 ±3**	**84 ±3**	**87 ±3**	**98 ±3**	**112 ±3**	**115 ±3**	**126 ±3**		**168 ±7**			**280 ±7**	**294 ±7**	**297 ±7**	**308 ±7**		**350 ±15**		**539 ±15**
Visit Nº (T=by phone)	**1B* Vc**	**2B (T)**	**3B Sc**	**4B Vc**	**5B (T)**	**6B Sc**	**7B Vc**	**8B (T)**	**9B Sc**	**10B Vc**	**11B (T)**	**12B Sc**	**13B Vc**	**14B (T)**	**15B Sc**		**16B Sc**			**17B Sc**	**18B Vc**	**19B (T)**	**20B Sc**		**21B Sc**		**22B EOS**
Informed Consent	o																										
Vaccination ABvac40/placebo§	o*			o			o			o			o								o						
Medical History	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o		o			o	o	o	o		o		o
Weight, BP, HR, RR, Tº	o		o	o		o	o		o	o		o	o		o		o			o	o		o		o		o
Physical and neurological exam			o	o		o	o		o	o		o	o		o		o			o	o		o		o		o
Neuropsych. Battery and IGE																	o								o		o
Quality of Life EQ-5D5L																									o		
Eletrocardiogram																	o#								o#		o
Urine Analysis (test strip)			o			o			o			o			o		o			o			o		o		o
Hemogram			o			o			o			o			o		o			o			o		o		o
Biochemistry			o			o			o			o			o		o			o			o		o		o
Biological Activity	o		o			o			o			o			o		o			o			o		o		o
MRI	o‡								o								o			o					o		o
a-PET																									o		
CSF																									o		
Adverse events	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o		o			o	o	o	o		o		o
Concomitant Medication	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o		o			o	o	o	o		o		o

MRI: Magnetic Resonance Imaging; a-PET: Amyloid PET; CSF: Cerebrospinal fluid; EKG: Electrocardiogram; exc.: except; IGE: Investigator Global Evaluation; NPB: Neuropsychological battery; EOS: end of study; Vc: Vaccination; Sc: Safety control; T: Telephone call; RR: respiratory rate: Tº: body temperature

† **At least 18 months after initiating Part A**

* The First Visit of Part B (V1B) will take place only when the results of the last MRI test performed (either at V21 or V25 or before first vaccination of Part B‡) are available and support the vaccination.

‡ A MRI will be performed before first vaccination of Part B only if the previous MRI was performed more than 6 months (±15 days) before V1B.

- ✓ A telephone control interview will take place within the first week (3 ±3 days) after each vaccination visit.
- ✓ A safety visit will be scheduled two weeks after each vaccination and at V17B.
- ✓ Blood samples will be collected at every safety control visit for safety control and characterization of immune response.
- ✓ Study visits should take place at the Days indicated above, within the following windows:
  - During first 6 months: ± 3 days
  - Between month 6 and month 12: ± 7 days
  - Between month 12 and month 18: ± 15 days
- ✓ If a visit takes place out of window, the patient should be visited as soon as possible; For the five first immunizations the **minimum interval between vaccinations must be 25 days** and the **maximum interval must be 31 days**; if one vaccination is delayed for any reason, the next dose must be re-scheduled to fit this therapeutic window.

In this visit the scanned EKG must be sent to the Medical Advisor for review

§ All patients must be tested for COVID-19 during the Part B vaccination visits before receiving the study medication (see Section 6.1.1.2 for further details)

Phases in the study: visits, assessments and dates:

Part A:

The Part A of the study will last for up to 104 weeks (from V0 to V25) and at least 18 months (up to V23) during which the patients will receive five monthly (every four weeks) immunizations plus a booster shot. During the study, two interim analyses (Int) will be performed to assess safety, tolerability and biological activity of the treatment (including data from the collected disease biomarkers at each of them). These analyses will be reviewed by an independent Data Safety Monitoring Board (DSMB). The first Int will be carried out once the first 30 patients have completed the 24-week visit (Int-6m) and the second one once all patients have completed the 24-week visit (Int-6m, eight weeks after the fifth monthly immunization) or in October 2020, whichever occurs first.. The double blinding of the study will be maintained throughout these two Int. Additional DSMB reviews may be made *ad hoc* in the event of safety issues.

Additionally, after all patients complete their 18-months visit (V23), a statistical analysis will be performed to determine whether the Part B of the study should be discontinued.

Within the first week (3± 3 days) after each vaccination visit, a telephone control interview will take place.
Two weeks after each vaccination, and at 40, 50, 77 and 104 weeks there will be a safety visit consisting on:

- Physical and neurological examination (assessment of vital signs; electrocardiogram in five visits: V0, V16, V21, V23 and the final visit (V25); body biometrics; collection of adverse events and concomitant medication) at all face to face visits (V0, V1, V3, V4, V6, V7, V9, V10, V12, V13, V15, V16, V17, V18, V20, V21, V23 and V25; and PDV, if applicable).
- Blood collection for safety control and measurement of biological activity at visits: V0, V1, V3, V6, V9, V12, V15, V16, V17, V20, V21, V23 and V25
- A MRI at visits V0, V9, V16, V17, V21 and V25.

At the screening visit (V0), the participants will receive an amyloid-PET scan. Subjects will undergo additional amyloid-PET scan at V21 and final visit (V25) for the assessment of amyloid burden. The subject will receive lumbar puncture for CSF analysis at baseline (V1), V21 and at the final visit (V25) for the assessment of changes in CSF biomarkers.

Part B:

The Part B of the study will last for 18 months (from V1B to V22B). The scheme of visits will mirror the Part A scheme.

The first visit of Part B (V1B) will take place at least 18 months after the patient started Part A (i.e. he/she should have completed V23 of Part A to enter Part B).

The patients randomized to the placebo group during Part A will receive five monthly (every four weeks) immunizations with ABvac40 plus a booster shot after 6 months (Delayed start group), whereas the patients randomized to the Verum group during Part A will receive placebo following this schedule, except in V13B, where they will receive an ABvac40 booster shot

(Booster group). Given the extended inclusion period of Part A, the V13B booster will take place at different timepoints relative to the patients' last immunization. For the analysis, they will be grouped in 2 periods: 12± 3 months and 18± 3 months after their last Part A immunization.

Within the first week (3± 3 days) after each vaccination visit, a telephone control interview will take place.
Two weeks after each vaccination, and at 40 and 50 weeks there will be a safety visit consisting on:

- Physical and neurological examination (assessment of vital signs, body biometrics and collection of adverse events and concomitant medication at all face to face visits (V1B, V3B, V4B, V6B, V7B, V9B, V10B, V12B, V13B, V15B, V16B, V17B, V18B, V20B, V21B and V22B, if applicable; electrocardiogram only in three visits: V16B, V21B and V22B).
- Blood collection for safety control and measurement of biological activity at visits: V1B, V3B, V6B, V9B, V12B, V15B, V16B, V17B, V20B, V21B and V22B.
- A MRI at visits V9B, V16B, V17B, V21B and V22B.
    - A MRI will be performed before first vaccination of Part B only if the previous MRI was performed more than 6 months (±15 days) before V1B

Patients will undergo an amyloid-PET scan at V21B for the assessment of amyloid burden. The patients will receive lumbar puncture for CSF analysis at V21B for the assessment of changes in CSF biomarkers.

Study Population:

Patients with amnestic Mild Cognitive Impairment (a-MCI) or very mild Alzheimer's Disease (vm-AD).

Number of Subjects Planned:

To ensure the primary safety and efficacy endpoints of the study a minimum of 60 subjects with a-MCI or vm-AD per treatment group (verum and placebo) are required. The amyloid-PET positive and amyloid-PET negative patients will be independently randomized following a ratio 1:1 between verum and placebo (Part A). Thus, the study contains two treatment groups.

To ensure the primary efficacy endpoint with a potency of 85%, at least 15 subjects per treatment group (verum and placebo; 30 in total) are required to complete the study. Since a total of 120 subjects will be initially included in the study and we estimate ~40% dropouts along the clinical trial, 70 are expected to complete the Part A of the protocol.

The patients' recruitment will be competitive until the required number of patients is completed. Approximately 22 international centres will be involved: Spain (17 sites), France (3 sites), Sweden (1 site) and Italy (1 site).

Diagnosis and Main Criteria for Inclusion:

The study population will consist of patients of both sexes with a-MCI or VM-AD, who meet all the selection criteria and give their informed consent to collaborate in the study, after being informed. The inclusion of each participant will be controlled by the principal investigator of each center.

Inclusion Criteria:

A subject must meet all the following inclusion criteria:

1. Male or female between 55 and 80 years of age, both inclusive, at the time of signing informed consent.
2. The patient (or legal representative, if applicable) and a close relative/caregiver must read the subject information sheet, agree to participate in the clinical trial and sign the informed consent form (the patient and a close relative/caregiver).
3. Presence of a stable caregiver to attend the patient study visits.
4. Mini-Mental Status Examination (MMSE) score between 24 and 30 points (inclusive), according to age and education level.
5. Clinical Dementia Rating (CDR) scale scoring 0.5.
6. Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) Score on the Delayed Memory Index (DMI) of 85 or lower.
7. The results of the patient's MRI brain scan must be concordant with the diagnosis of clinical a-MCI or vm-AD according to the following criteria: Scheltens scale, and measurement of white matter and past haemorrhages.
8. If the patient is receiving treatment for AD, must have been stable during the two months before the selection visit.
9. Treatment for concomitant diseases must be stable during the previous month before the treatment of the study.
10. Positive assessment of the candidate by the investigator for complying with the requirements and procedures of the study.

Exclusion Criteria:

A subject meeting any of the following exclusion criteria is NOT eligible for participation in the study.

1. Known allergy to components of the vaccine or prior history of anaphylaxis, a severe allergic reaction or a history of hypersensitivity to any component of the formulation. Allergy to fish or shellfish.
2. Active infectious disease (i.e. hepatitis B, C). Positive syphilis serology.
3. History or presence of autoimmune disease, except mild eczema, rhinitis or psoriasis.
4. Presence or history of immunodeficiency (i.e. HIV).
5. Significant kidney and/or liver disease, as defined by plasma creatinine ≥ 2.5 mg/dL (221 μmol/l) and/or total bilirubin > 3 mg/dL (51.3 μmol/l) measured at the local site laboratory.
6. History of asthma or reactive airway disease with bronchospasm in the last 6 months, or currently on regular treatment.
7. Major uncontrolled systemic condition (e.g. diabetes, congestive heart failure, hypertension).

8. History of cancer (≤5 years since the last specific treatment). Exceptions: basocellular carcinoma.
9. Significant alterations in hematological, biochemical or urine analytical parameters, particularly those relating to levels of vitamin B12, folic acid or thyroid tests.
10. History of any other central nervous system disorder, degenerative or non-degenerative neurological or psychiatric condition that, in the investigator's opinion could be the cause of the dementia, or could explain the cognitive impairment, or that might interfere with cognitive function directly or by its treatment.
11. Geriatric Depression Scale (GDS; abbreviated version), score >5
12. Has a "yes" answer to C-SSRS suicidal ideation items 4 or 5, or any suicidal behavior within 6 months before Screening, or has been hospitalized or treated for suicidal behavior in the past 5 years before Screening.
13. History or signs of cerebrovascular disease (ischemic or haemorrhagic stroke, transient ischemic attack), or diagnosis of possible, probable or clear vascular dementia according to NINDS-AIREN criteria.
14. Presence on MRI of a relevant pattern of microvascular disease (Leukoaraiosis, Fazekas score ≥2 in the deep white matter scale or ≥4 in the global score) or more than one lacunar or territorial infarcts. Any other MRI finding that, in the opinion of the investigator, might be a relevant contributing cause of subject´s cognitive impairment. Presence of up to 3 microhemorrhages will be acceptable.
15. History of bleeding disorder or predisposing conditions, blood clotting or clinically significant abnormal results on coagulation profile at Screening, as determined by the Investigator.
16. Patients being treated with anticoagulants or antiaggregant therapy (aspirin at a prophylactic dose ≤ 325 mg daily or clopidogrel at a dose ≤75 mg daily are allowed) should not be recruited in the study.
17. Modified Hachinski Ischemic Scale, score higher than 4.
18. Surgery (with general anaesthetic) within the previous three months to be included in the trial, or programed during the study period.
19. Treatment within 30 days prior to visit 0 with systemic corticosteroids or other immunosuppressant's.
20. Vaccination against influenza or any other vaccination within 2 months before first IMP dose.
21. Patients, who have previously been randomized in this trial.
22. Participation in another clinical trial within the previous 1 month to screening visit, or within the previous 12 months after the last dose to the screening visit in the case of subjects who participated in trials with a study drug whose intention was to modify the progression AD unless documentation of receipt of placebo is available. The patient cannot be included in the study if the experimental drug was an immunotherapeutic drug, including IVIG or a vaccine against Alzheimer's disease unless documentation of receipt of placebo is available.
23. Patients with alcohol or drug abuse or dependence.
24. Absolute (having a pacemaker or implantable defibrillator) or relative (bare metal stent or stent implanted in the last six months) contraindications to MRI examination. Feeling of claustrophobic do not let perform MRI or PET scan.

25. Patients unlikely to comply with the protocol (e.g., unable to return for follow-up visits).
26. Women of childbearing potential, pregnant or nursing.
    Note: All women will be considered to be of childbearing potential unless they are postmenopausal (amenorrheic for at least 12 consecutive months, in the appropriate age group, and without other known or suspected cause) or have been sterilized surgically (i.e. bilateral tubal ligation, total hysterectomy, or bilateral oophorectomy, all with surgery at least 1 month before dosing)
27. Significant alterations in the EKG that are associated with an added risk for the patient.

Investigational Product, Dose and Mode of Administration

Study Medication

Name: ABvac40

Active ingredient: Conjugated Aβx-40 to KLH

Transporter: Phosphate buffer containing 0.35% aluminium hydroxide (adjuvant)

Pharmaceutical Form: Injection ready vials

Posology: Part A: Verum group: Six administrations: the first five administered once every 4 weeks (28±3 days) and the sixth at week 42 (V18), 26 weeks after the fifth immunization. Each administration consists of 1mL injection of ABvac40 (containing 0.2 mg of Abetax-40).

Part B: Delayed start group: Six administrations: the first five administered once every 4 weeks (28±3 days) and the sixth at week 42 (V18B), 26 weeks after the fifth immunization.
Booster group: One administration at week 16 (V13B).
Each administration consists of 1mL injection of ABvac40 (containing 0.2 mg of Abetax-40).

Method of administration: Subcutaneous injection.

Duration of Treatment:

The participation in the study for each patient will last between 36 and 42 months (Part A + Part B). They will be exposed to the study's drug for an initial period of 16 weeks (V1 to V13 [Part A – Verum group] or V1B to V13B [Part B – Delayed start group]). After 6 months, they will receive the 6th vaccination (V18/V18B). Only the patients assigned to the Verum group in Part A will receive a 7th vaccination at V13B.

Reference Therapy, Dose and Mode of Administration
Comparator

Name: Placebo

Active ingredient: None

Transporter: Phosphate buffer containing 0.35% aluminium hydroxide (adjuvant)

Pharmaceutical Form: Injection ready vials

Posology: Part A: Placebo group: Six administrations: the first five administered once every 4 weeks (28±3 days) and the sixth at week 42 (V18), 26 weeks after the fifth. Each administration consists of 1mL injection of ABvac40 vehicle.

Part B: Booster group: Five administrations: the first four administered once every 4 weeks (28±3 days) and the fifth at week 42 (V18B), 30 weeks after the forth. Each administration consists of 1mL injection of ABvac40 vehicle.
Method of administration: Subcutaneous injection.

Key Study Variables:

Part A:

Primary Safety Variable:

The rate (%) of Adverse Events (AEs).

Primary Efficacy Variable:

Maximal increment of specific anti-Aβ40 antibody signal (antibody titers estimated by ELISA (signal optical density (OD) values) in each subject with regard to the pre-treatment visit (either V0 or V1)

Secondary Safety Variables:

Other secondary safety endpoints will be evaluated, including:

- withdrawal criteria (continuation decision)
- number of withdrawn patients due to adverse events (AEs); cause of withdrawal
- serious adverse events (SAEs)
- physical and neurological examination
- concomitant medication
- vital signs (blood pressure, heart rate, respiratory rate, body temperature), body mass (weight, height).
- brain MRI
- electrocardiogram (EKG)
- analytical haematology, toxicology (reactive protein), biochemistry, coagulation, serology and urine test strip results.

Secondary Efficacy Variables:

- Characterization of the immune response:
  - Levels of anti-KLH and anti-Aβ42 antibodies in plasma
  - Level of anti-Aβ40 antibodies in CSF
  - Levels of cytokines in plasma including IL-2, IL-4, IL-5, IL-6, IL-10, IL-13, IL-15, TNF-α and IFN-γ.
  - Analysis of the peripheral blood cell subsets by immunophenotyping.
  - Levels of antibody secreting cells and cytokine-secreting cells (including IFN-γ, TNF-α and IL-13)
- Assessment of disease biomarkers:
  - Levels of Aβ peptides in plasma
  - Cortical fibrillary amyloid deposition assessed by PET scans.
  - Levels of CSF biomarkers (Aβ42, Tau, P-tau, neurofilament light and neurogranin) and other Aβ peptide species
  - Brain volumetric and atrophy of the hippocampus using magnetic resonance imaging
- Assessment of cognition and quality of life:
  - Mini Mental State Examination (MMSE)
  - Clinical Dementia Rating scale (CDR)
  - Repeatable Battery for the Assessment of Neuropsychological Status (RBANS)
  - Alzheimer's Disease Cooperative Study – Activities of Daily Living, Mild Cognitive Impairment (ADCS-ADL MCI)

- Columbia Suicide Severity Rating Scale (C-SSRS)
- Trail Making Test (TMT)
- Investigator Global Evaluation (IGE)
- Euroqol 5 Dimensions (EQ-5D-5L)

Part B:

Exploratory Safety Variables:

- Rate (%) of AEs
- Withdrawal criteria (continuation decision)
- Number of withdrawn patients due to AEs; cause of withdrawal
- SAEs
- Physical and neurological examination
- Concomitant medication
- Vital signs (blood pressure, heart rate, respiratory rate, body temperature), body mass (weight, height).
- Brain MRI
- EKG
- Analytical haematology, toxicology (reactive protein), biochemistry, coagulation, serology and urine test strip results

Exploratory Efficacy Variables:

- Maximal increment of specific anti-Aβ40 antibody signal (antibody titers estimated by ELISA (signal OD values) in each patient with regard to the pre-treatment visit (either V0/V1 [Booster group] or V1B [Delayed start group]).
- Characterization of the immune response:
  - Level of anti-Aβ40 antibodies in CSF
  - Levels of antibody secreting cells
- Assessment of disease biomarkers:
  - Levels of Aβ peptides in plasma
  - Cortical fibrillary amyloid deposition assessed by PET scans.
  - Levels of CSF biomarkers (Aβ42, Tau, P-tau, neurofilament light and neurogranin) and other Aβ peptide species
  - Brain volumetric and atrophy of the hippocampus using magnetic resonance imaging
- Assessment of cognition and quality of life:
  - MMSE
  - CDR scale
  - RBANS
  - ADCS-ADL MCI
  - C-SSRS
  - TMT
  - IGE
  - EQ-5D-5L

Study Assessments and Procedures:

Medical Evaluation:

To be performed at all visits, except telephone interviews. Consists on the following tests:

- Physical and neurological examination
- Vital Signs
- Electrocardiogram (EKG) (at visits V0, V16, V21, V23 and V25 [Part A], and at V16B, V21B and V22B [Part B]) (at all visits, except V22B, assessed centrally by the Medical Advisor)
- Body biometrics

Neuropsychological tests:

Throughout the study, a series of neuropsychological tests will be performed.

Part A:

At the pre-selection (V0) MMSE, CDR and RBANS will be performed to decide if the participants meet inclusion criteria. In the baseline (V1), Int-6m (V16), V21, V23 and final visit (V25) the complete Neuropsychological battery and the test for quality of life will be performed as per the following table:

Neuropsychological tests						
Neuropsychological Battery	***V0 Screening Week -8***	***V1 Baseline Week 0***	***V16 Int-6m Week 24***	***V21 Int-12m Week 52***	***V23 Week 77***	***V25 Final visit Week 104***
MMSE (24-30 at inclusion)	x	-	x	x	x	x
CDR (global score of 0.5 at inclusion)	x	-	x	x	x	x
RBANS (score ≤ 85 at inclusion)	x	-	x	x	x	x
COLUMBIA-SSRS (no answer "YES" in item 4 or 5 at inclusion)	x	-	x	x	x	x
ADCS-ADL MCI	-	x	x	x	x	x
TMT-B	-	x	x	x	x	x
GDS abbreviated (score≤ 85 at inclusion)	x	-	-	-	-	-
IGE	-	x	x	x	-	x
Quality of Life Tests						
EQ-5D-5L	-	x	-	x	-	x

Part B:

At V16B, V21B and V22B visits, the complete Neuropsychological battery will be performed. In addition, the test for quality of life will be performed in V21B.

Blood tests:

- Complete blood analysis for safety and characterization of immune response:
  Part A: V0, V1, V3, V6, V9, V12, V15, V16, V17, V20, V21, V23 and V25. Part B: V1B, V3B, V6B, V9B, V12B, V15B, V16B, V17B, V20B, V21B and V22B.
- Genotype ApoE (to be carried out in V1 [Part A])

Assessment of autoimmunity markers (to be held in V0, V21 and V25 [Part A] and V21B [Part B]): Antinuclear antibodies

- Native anti-DNA antibodies
- Anti-thyroglobulin antibodies
- Anti-TPO antibodies

Urine Analysis:

- A urine analysis will be done at visits V0, V1, V3, V6, V9, V12, V15, V16, V17, V20, V21, V23 and V25 (Part A) and V3B, V6B, V9B, V12B, V15B, V16B, V17B, V20B, V21B and V22B (Part B). Urine samples will be analysed locally with a test strip. If no relevant abnormalities are found, local results will be collected in the eCRF (and corresponding source document). If any abnormality is found, the urine sample will be sent to central laboratory for reanalysis.

The Sponsor Medical Advisor:

- The Medical Advisor will review all EKGs centrally, and will inform the sites about any abnormal finding.
- Medical Monitor 24/7

Neuroimaging:

- Brain MRI at visits:
  Part A: V0, V9, V16, V17, V21 and V25
  Part B: V1B (only in the absence of a MRI performed within 6 months (±15 days)), V9B, V16B, V17B, V21B and V22B.
- MRI Central Reader will issue his assessment after every MRI (except V22B). This assessment will be taken into account for subjects' eligibility and /or continuity. In V22B will be performed a MRI to identify any long-term safety issue.
- Amyloid-PET evaluation at visits V0, V21 and V25 (Part A) and V21B (Part B).

CSF collection for the following analysis at
Part A: V1, V21 and at visit V25. Part B: V21B

- Total proteins
- Glucose
- Albumin
- Assessment of biomarkers (Aβ42, tau, P-tau, neurogranin, neurofilament light and other Aβ peptide species)
- Assessment of anti-Aβ antibodies

Treatment:

- Vaccination (at visits V1, V4, V7, V10, V13 and V18 [Part A] and V1B, V4B, V7B, V10B, V13B and V18B [Part B])

Medical History (at all visits):

- Relevant past and concomitant diseases
- Adverse events
- Concomitant medication

Statistical Methods:

Part A:

Primary Safety Analysis:

The primary safety variable is the frequency (%) of Adverse Events (AEs) over the course of the study. The primary safety endpoint will be analyzed descriptively in the Safety population.

The hypothesis for the primary endpoint for safety is that ABvac40 will be safe for human administration, according to the observed pattern of AEs.

The trial will be considered satisfactory if the pattern of AEs (overall or grouped as neurological, psychiatric or cardiovascular) is consistent with a good safety and tolerability profile of ABvac40 for human administration at the final of the study.

For this primary safety analysis, the amyloid-PET negative and the amyloid-PET positive subjects will be pooled together.

Primary Efficacy Analysis (immunogenicity):

The primary efficacy variable is the maximal increment of specific anti-Aβ40 antibody signal (antibody titers estimated by ELISA [signal OD values]) in each subject with regard to the pre-treatment visit (either V0 or V1). For this primary efficacy analysis, the amyloid-PET negative and the amyloid-PET positive subjects will be pooled together.

The trial will be considered successfully confirmatory regarding efficacy (immunogenicity) of ABvac40 if the average maximal increment (MΔ) of anti-Aβ40 antibody signal (OD in ELISA) in the verum group is >0 OD than the average MΔ in the placebo group.

Secondary Safety Analysis:

The safety and tolerability analysis will be based on the safety population, comprising all subjects who took at least one dose of study medication. For this secondary safety analysis, the amyloid-PET negative and the amyloid-PET positive subjects will be pooled together.

Safety and tolerability assessments will be based on the following parameters:

- withdrawal criteria.
- number of withdrawn patients due to adverse events (AEs); cause of withdrawal
- serious adverse events (SAEs)
- physical and neurological examination
- concomitant medication
- vital signs (blood pressure, heart rate, respiratory rate, body temperature), body mass (weight, height).
- brain MRI
- electrocardiogram (ECG or EKG)
- analytical haematology, immunology, toxicology (reactive protein, cytokines, T cell activation), biochemistry, coagulation, serology and urine test strip results.

The number (%) of withdrawals due to AEs and the reason(s) for these withdrawals will be summarized using appropriate descriptive statistics.

All adverse events, results from physical and neurological examinations, results of new onset and/or worsening of signs found from physical examination, safety analytics, vital signs and EKG recorded during the study will be presented in tables by treatment group, and frequencies

by system-organ class will be calculated. These results will be analyzed descriptively and incidences summarized.

Blood pressure, heart rate, respiratory rate, body temperature, body mass and analytic parameters as well as changes from baseline will be analyzed using descriptive statistics for each measured time point and for the end point (defined as the last observation available).

Patient demographics, baseline characteristics, general treatment for AD and concomitant medication will be analyzed using descriptive statistics.

As an exploratory analysis, the incidence of AEs (overall or grouped as neurological, psychiatric or cardiovascular) will be compared between the group receiving the active treatment (ABvac40) and the control group receiving placebo.

Secondary Efficacy Analysis:

The trial will be considered satisfactory if the difference of the average maximal increment (MΔ) of anti-Aβ40 antibody signal (OD in ELISA) in the treated group is > 1.778 OD than the average MΔ in the placebo group; the difference that was reached between both groups in the phase I clinical trial of ABvac40.

Secondary efficacy variables will be analyzed in an exploratory manner, using descriptive statistics. The secondary efficacy variables regarding assessment of disease biomarkers and cognition will be analyzed in the overall group and separately in the amyloid-PET negative (verum and placebo) and the amyloid-PET positive (verum and placebo) subjects.

Statistical comparison between groups of treatment (placebo/treatment) will be done for each time point and for the end point (defined as the last observation available).

The change from baseline in absolute value will be analyzed for each time point and for the end point (defined as the last observation available).

Mixed-model repeated-measures (MMRM) analyses will be performed for all the primary and secondary efficacy variables (section 7.1) to assess between-group differences in change scores from baseline (V0/V1) to 12-months visit (V21), and separately to the final visit (V25). Patient demographics, baseline characteristics, general treatment for AD and concomitant medication will be analyzed using descriptive statistics.

Part B:

The analyses for Part B will mirror the analyses of Part A, without testing any hypothesis. The detailed statistical analyses will be described in the Statistical Analysis Plan.

Determination of Sample Size:

This trial is intended as a confirmatory assay for the primary safety and primary efficacy variables.

For the safety primary endpoint, the sample size may be calculated to ensure ≥95% probability of detecting an adverse event that occurs with a rate of at least 5% within the ABvac40 treated dose cohort. Under these assumptions, a minimum of 60 patients in the active dose group will be needed (and 60 in the placebo group).

This sample size (60 patients in the active group and 60 in the placebo group), considering ~40% dropouts along the clinical trial, will imply that approximately 70 patients will complete the study. These 70 patients will have greater than 85% power to detect a treatment increment of 1.778 (the one found in ABvac40 Phase I trial) in maximum change from baseline in anti-Aβ40 antibody signal (OD in ELISA without preadsorption) between the active group and placebo group, using a one-sided T test at a significance level of 0.025 (and considering standard deviations of 2.0 and 1.0 in the active and placebo groups, respectively). The study will not be powered to demonstrate efficacy in clinical or other biomarker outcomes.

Anticipated study duration:

First Patient In: February 2018

Last Patient In: September 2019

Last Patient Out (Part B): May 2022

Thus, the expected duration of the trial is 51 months, from February 2018 to May 2022.

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GLOSSARY AND ABBREVIATIONS

AChEI	Acetil Cholinesterasa
AD	Alzheimer´s Disease
ADAS-cog	Alzheimer's Disease Assessment Scale- Cognitive Subscale
ADCS-ADL	Alzheimer's Disease Cooperative Study – Activities of Daily Living
ADCS-iADL	Alzheimer's Disease Cooperative Study Activities of Daily Living inventory instrumental items
ADR	Adverse drug reaction
AE	Adverse event
AEMPS	Agencia Española de Medicamentos y Productos Sanitarios
a-MCI	Amnestic Mild Cognitive Impairment
ANCOVA	Analysis of Covariance
a-PET	Amyloid PET
AR	Adverse reaction
BL	Baseline visit
CDR	Clinical Dementia Rating scale
CDR-SB	Clinical Dementia Rating-Sum of Boxes
CHMP	Committee for Medicinal Products for Human Use
CI	Confidence interval
eCRF	Case report form/electronic case report form
CRO	Contract research organization
CSF	Cerebrospinal Fluid
C-SSRS	Columbia Suicide Severity Rating Scale
DBP	Diastolic Blood Pressure
DSMB	Data Safety Monitoring Board
EDC	Electronic data capture
EKG	Electrocardiogram
EMA	European Medicines Agency
EQ-5D	Euroqol 5 dimensions
FDA	Food and Drug Administration
FV	Final visit

HAV	Hepatitis A virus
HBV	Hepatitis B virus
IB	Investigator's Brochure
ICF	Informed consent form
ICH GCP	International Conference on Harmonization Good Clinical Practice
IgA	Immunoglobulin A
IGE	Investigator Global Evaluation
IgG	Immunoglobulin G
IgM	Immunoglobulin M
Int	Interim analysis
IMP	Investigational medicinal product
IRB/EC	Institutional Review Board/Ethics Committee
ITT	Intent-to-treat
IV	Intravenous
IVIG	Intravenous Immune Globulin (generic terminology)
IWG	International Working Group
kg	Kilogram
KLH	Keyhole limpet hemocyanin
LC-MS	Liquid Chromatography-Mass Spectrometry
MedDRA	Medical Dictionary for Regulatory Activities
M-AD	Mild Alzheimer´s Disease
MCI	Mild Cognitive Impairment
MCI-DAD	Mild Cognitive Impairment due to Alzheimer´s Disease
MMSE	Mini Mental State Examination
MMRM	Mixed-Model Repeated-Measures
MRI	Magnetic Resonance Imaging
m	Month
NIA-AA	National Institute on Aging - Alzheimer´s Association
NPB	Neuropsychological battery
OD	Optical density
P-AD	Prodromal Alzheimer´s Disease

PET	Positron emission tomography
p-TAU	Phosphorilated Tau
RBANS	Repeatable Battery for the Assessment of Neuropsychological Status
SAE	Serious adverse event
SBP	Systolic blood pressure
Sc	Safety control
SCR	Screening visit
SD	Standard deviation
SDCC	Safety and Data Control Committee
T	Temperature / Telephone call
TEAE	Treatment-emergent adverse event
TMT	Trail Making Test
US	United States
Vc	Vaccination
VM-AD	Very Mild Alzheimer's Disease
V	Visit
WBC	White blood cell

1 GENERAL INFORMATION

Protocol Title
A Multi-center, Randomized, Double-blind, Placebo-controlled, 24 months Study in Patients with amnestic Mild Cognitive Impairment or Very Mild Alzheimer's Disease to Investigate the Safety, Tolerability and Immune Response of Repeated Subcutaneous Injections of ABvac40.

Sponsor/monitor/technical coordination: Investigators and study administrative structure.

Sponsor
ARACLON BIOTECH, S.L. a GRIFOLS company,
Vía de la Hispanidad, 21
50009 Zaragoza, Spain

Study Coordinator Name: [redacted]

Sponsor´s Scientific Personell
[redacted]r Araclon Biotech. Zaragoza
[redacted] Araclon Biotech. Zaragoza

Data Safety Monitoring Board
[redacted]

Information regarding the Data Safety Monitoring Board (DSMB) is detailed in Section 9.1.5.1.

2 BACKGROUND INFORMATION

The first trial in-humans of ABvac40 (Phase I; registered under EUDRACT nº: 2012-004933-18) including 24 individuals (16 in verum and 8 placebo) diagnosed with mild to moderate Alzheimer's Disease (AD) showed a good safety and tolerability profile of the investigational medicine product (IMP). Additionally, the phase I study explored the immunogenicity of ABvac40 which resulted positive in the majority of the individuals belonging to the verum group, particularly in those receiving the third immunization included after amending the initial two-injection protocol

Recently, the European Medical Agency (EMA) Committee for Medicinal Products for Human Use (CHMP) released a "Draft guideline on the clinical investigation of medicines for the treatment of Alzheimer's disease and other dementias" (EMA/CHMP/539931/2014) which reviewed the last conceptual advancements on the natural history of AD. This document recognizes the new sets of research diagnostic criteria for AD published by the International Working Group (IWG) and the National Institute on Aging - Alzheimer´s Association (NIA-AA) and admit that from a regulatory perspective both are accepted for diagnosis of AD for research purposes and for trial enrichment. However, it is important to note that MCI due to AD (MCI-DAD) according to the NIA-AA criteria and those for Prodromal AD (P-AD) as published by IWG differ in several aspects, particularly with regard to the mandatory presence or not of positive AD biomarkers.

Additionally the EMA advises to perform phase II trials in the same population expected to be targeted at phase III; assumes the usefulness of biomarkers to better characterize the earlier stages of the disease; and admits that subjects with MCI-DAD/P-AD and mild AD (M-AD) may be studied together.

Thus, in line with this CHMP document, the present ABvac40 phase II clinical trial will be carried out on MCI-DAD/P-AD and/or very mild AD (vm-AD) patients, characterized by their amyloid-positron emission tomography (a-PET) neuroimaging profile.

The present study is aimed to confirm the Phase I safety and immunogenicity results in an adequately powered Phase II trial, including a larger population sample and a new batch of the IMP. Taken into account the results of Phase I, we will further explore the therapeutic range of ABvac40 by increasing the number of immunizations up to 5 administered at monthly intervals plus a delayed booster shot. This approach will allow exploring the development of the immunological memory to gain an insight on the possible future vaccination schedule. In addition, we will address a deeper characterization of the immune response, including the exploration of its effects on the biomarkers of β-amyloid (Aβ) cortical pathology and cognitive impairment. Taken together these data will be crucial for a founded "go no-go" decision into Phase III and an appropriated confirmatory designing.

In this clinical trial we will follow-up the EMA recommendations about study population and the biomarkers selected. The EMA includes the category "prodromal AD/MCI due to AD" in its Discussion paper EMA / CHMP / 539931/2014 which will lead to the revision of the current legislation on medicinal products for the treatment of AD (EMA / CHMP / 617734/2013).

The EMA has published favorable qualification opinions for the use of levels of biomarkers as tools for the enrichment of clinical trials with anti-amyloid therapies (EMA / CHMP / SAWP / 893622/2011; and EMA / CHMP / SAWP / 102001/2011).

In the same line the EMA has issued favorable qualification opinions to the use of PET amyloid imaging as a biomarker for enrichment for use in pre-dementia AD clinical trials (EMA / CHMP / SAWP / 892998/2011) and the determination of hippocampal atrophy by magnetic resonance imaging (MRI) for use in clinical trials for regulatory purpose in pre-dementia stage of Alzheimer's disease (EMA / CHMP / SAWP / 809208 / PET-amyloid 2011).

2.1 Name and Description of the Investigational Product(s)

See Section 4.4 Study Treatments for detail.

Study Medication

Name: ABvac40

Active ingredient: Aβx-40 peptide conjugated to keyhole limpet hemocyanin (KLH)

Transporter: Phosphate buffer containing 0.35% aluminium hydroxide (adjuvant)

Pharmaceutical Form: vials ready to inject

Posology: Part A: Verum group: Six administrations: the first five administered once every 4 weeks (28±3 days) and the sixth at week 42 (V18), 26 weeks after the fifth. Each administration consists of 1mL injection of ABvac40 (containing 0.2 mg of Abetax-40).

Part B: Delayed start group: Six administrations: the first five administered once every 4 weeks (28±3 days) and the sixth at week 42 (V18B), 26 weeks after the fifth immunization. Booster group: One administration at week 16 (V13B).

Each administration consists of 1mL injection of ABvac40 (containing 0.2 mg of Abetax-40).

Method of administration: Subcutaneous injection.

Comparator

Name: Placebo

Active ingredient: None

Transporter: Phosphate buffer containing 0.35% aluminium hydroxide (adjuvant)

Pharmaceutical Form: Injection ready vials

Posology: Part A: Placebo group: Six administrations: the first five administered once every 4 weeks (28±3 days) and the sixth at week 42 (V18), 26 weeks after the fifth.

Each administration consists of 1mL injection of ABvac40 vehicle.

Part B: Booster group: Five administrations: the first four administered once every 4 weeks (28±3 days) and the fifth at week 42 (V18B), 30 weeks after the forth.

Each administration consists of 1mL injection of ABvac40 vehicle.

Method of administration: Subcutaneous injection.

See section 4.4 Study Treatment for details.

2.2 Relevant Findings from Nonclinical and Clinical Trials

For a detailed description of pre-clinical studies in the development of ABvac40 (see investigator´s brochure (IB) document).

ABvac40 has undergone a clinical development program, a phase I clinical trial in humans (AB1203) approved by the Agencia Española de Medicamentos y Productos Sanitarios (AEMPS) on 24 July 2013, entitled "A randomized, placebo-controlled, parallel group, double-blinded, single-center phase-I, pilot study to assess tolerability and safety of repeated subcutaneous administration of two single-doses of ABvac40 applied to patients with mild to moderate Alzheimer's Disease"

The primary objective of the AB1203 study was to evaluate the safety and tolerability of two immunizations with ABvac40, compared to placebo, in 24 patients with mild or moderate Alzheimer's disease, randomly distributed into two distinct groups with a verum: placebo ratio of 2:1. Each immunization consisted of injecting subcutaneously one millilitre (1) of ABvac40 or placebo. The secondary objective was to evaluate in an exploratory manner ABvac40 biological activity by determining plasma levels of anti-Aß40 antibodies and Aβ peptides.

An Interim Analysis was carried out with the first 8 randomized patients without disclosing the blind of the study. According to the safety and biological activity data obtained in these first eight participants who had completed the initial study protocol, it was decided to amend the protocol to include an additional injection of ABvac40 (or placebo as corresponded) in the remaining sixteen patients.

This protocol amendment was approved by the Ethics Committee on 2 December 2014 and by the AEMPS by administrative silence. This additional injection of ABvac40 (or placebo) was administered 29 days after the second administration in the remaining sixteen patients included in the trial.

On July 30th 2015, the final visit to the last participant in the trial was performed. On October 27th 2015, after the database was closed, the final meeting of the Safety and Data Control Committee (SDCC) was held, the trial randomization was unveiled and the AEMPS was notified of the end of the clinical trial.

For a complete description of the Phase I results see the Clinical Study Report. In brief, the analysis of the Phase I primary endpoint – to evaluate the safety and tolerability of ABvac40 immunizations – carried out in the Safety and Intent-to-treat (ITT) populations (24 patients) –

showed no statistical significant differences between the control and the treatment group regarding the frequency of overall adverse events (AEs) and grouped as neurological, psychiatric and cardiovascular (see table 1). There were no AEs leading to discontinuation of study treatment. All AEs recorded in the database were of mild severity and no action was needed. Of particular relevance, no incident vasogenic edema or sulcal effusion (ARIA-Edema) was detected throughout the study period and only one cortical micro-hemorrhage (ARIA-Hemorrhage) was detected by MRI after the 2º injection in a patient (S002) who at the end turned out to belong to the placebo group.

Table 1: Safety and Tolerability of ABvac40 at Phase I Clinical Trial

Safety and tolerability of ABvac40 at Phase I							
Safety / ITT population (N=24)	*Total (N=24)*		*ABvac40 (N=16)*		*Placebo (N=8)*		*P-value**
%AEs / Patients	**# AEs**	**# Patients**	**# AEs**	**# Patients**	**# AEs**	**# Patients**	**P-value***
Total of AEs	71	18 (75.00%)	42	11 (68.75%)	29	7 (87.50%)	**0.6214**
Neurological AEs	15	10 (41.67%)	9	5 (31.25%)	6	5 (62.50%)	**0.2038**
Psychiatric AEs	3	3 (12.50%)	2	2 (12.50%)	1	1 (12.50%)	**1.000**
Cardiovascular AEs	2	2 (8.33%)	1	1 (6.25%)	1	1 (12.50%)	**1.000**

* Fisher's exact test p-value

The analysis of ABvac40 biological activity – carried out in the ITT population (24 patients) – showed a significant difference between groups of treatment at the final visit only for the mean optical density (OD) without pre-adsorption (representing level of anti-Aβ40 antibodies): median of 2.67 (range, 0.7-4.1) in the ABvac40 group and median of 0.60 (range, 0.6-1.3) in the placebo group (p=0.0129). Although the level of response had a considerable individual component as could be expected, 87.5 % of patients in the verum group were considered responders (those which, at any visit, showed a 3 standard deviations (SD) increase in OD with regard to the pre-immune sample) a figure which reached 91 % of the population that received the 3 immunization shots in the amended protocol. The Mean OD with pre-adsorption indicates that over 95% of the signal registered in the plasma samples from the patients treated with verum was due to the presence of specific anti-Aβ40 antibodies.

The other parameters of biological activity analyzed did not were significant different between the two groups of treatment at the final visit.

In summary, the clinical trial was considered satisfactory because the average frequency of AEs (overall or grouped as neurological, psychiatric or cardiovascular) was not significantly different between the group that received the active treatment (ABvac40) and the placebo group. In fact, the incidence of AEs tended to be lower in the active treatment group,

particularly with regard to the Neurological and Cardiovascular events. Therefore, the active treatment (ABvac40) shows a good safety and tolerability profile.

Additionally, the first interim analysis of the present ongoing Phase II clinical trial was conducted in July 2019, after the first 36 patients have completed their 24-week visit. The results showed that ABvac40 has a favorable profile concerning futility criteria, safety and immunogenicity data, encouraging the amendment of the study protocol to include the delayed-start extension (Part B).

Encouraged by these results and taking into account that ABvac40 is an active vaccine directed specifically against the Aβ-40 peptide, a cross-over for the subjects receiving placebo will be offered after they complete their 18-months visit (V23), aiming to obtain additional safety data (patient-years of ABvac40 exposure) and to be able to assess its efficacy after a delayed-start. Furthermore, the long-term safety and tolerability of ABvac40 in patients receiving a booster after their Part A vaccination scheme will be assessed.

2.3 **Known and Potential Risks and Benefits to Human Subjects**

The ABvac40 formula is designed to avoid the known potential risks of immunization against AD (meningoencephalitis, microhemorrhages and angiogenic cerebral edema) while maintaining an efficient immune response (see IB document).

In this regard, the immunogen peptide of ABvac40 (Aβx-40) does not contain the epitope recognized by T cells whose activation was considered the primary cause of aseptic meningoencephalitis in the AN1792 vaccine study. The occurrence of microhemorrhages and cerebral edema has been associated with passive immunization but especially antibodies (passive immunization) or induced antibodies (active immunization) against the N-terminal of Aβ. However, ABvac40 is designed to recognize the C-terminal segment of Aβ. These segments are not accessible to antibodies as long as the Aβ molecule is not secreted in body fluids.

Moreover, the ABvac40 formulation contains a Th2 type adjuvant, widely used in vaccines for human use, which directs the immune response towards the production of circulating antibodies minimizing the pro-inflammatory cell response.

The safety assumptions on which the design of ABvac40 is based have been upheld by the results of the Phase I clinical trial (see Clinical Study Report document) and all experiments carried out to date including three formal toxicology tests.

The potential benefit for participants in the Phase II trial, that we believe to be sufficiently substantiated, rests in the fact that the vaccination can elicit an immune response against Aß40 peptide potentially capable of modifying the course of the disease. We maintain that this potential benefit could occur in both the amyloid-PET positive and amyloid-PET negative amnestic-MCI and vm-AD patients intended to be recruited for this clinical trial.

This idea is mainly supported, firstly, by studies showing that a considerable proportion of MCI patients are experiencing accelerated rates of Aβ deposition and cognitive/functional decline before reaching current threshold for amyloid positivity [26] and secondly, in reports that ~70% of a-MCI that meet the core clinical criteria defined by the eligibility criteria in the present clinical trial (independent of their amyloid-PET or CSF Aβ42 status) progress to dementia in a 26.6 month follow-up (range 6-68 months) [27]. In those a-MCI and vm-AD patients deemed as Aβ-negative, the clinical symptomatology may be driven by soluble Aβ isoforms and/or perivascular deposits of Aβ40, not detectable by current Aβ neuroimaging, and susceptible to being modified by our anti-Aβ40 treatment. In addition, there is no alternative pharmacological treatment approved for these MCI patients who have such a gloomy prognosis and the treatments currently available for AD are merely symptomatic; they do not alter the course of the disease, and are effective only in a limited percentage of patients and for a limited time. Although this situation in no way justifies any excessive risks, assuming that ABvac40 has showed a good safety and tolerability profile and anti-Aβ40 specific immunogenicity in previous clinical trial (Phase I), participants in the trial and in the future, other patients affected by Alzheimer's disease could benefit from this treatment, especially if treatment could be offered in the early stages of the disease.

Furthermore, on the basis of current knowledge, the foreseeable risks of clinical use of the ABvac40 vaccine are considered scarce and reasonably controllable.

In summary from our point of view this ABvac40 clinical trial presents a risk-benefit balance that seems positive for both study participants and for society.

The results of the first interim analysis of the present ongoing Phase II clinical trial bolsters this conclusion.

2.4 Description of and Justification for the Route of Administration, Dosage Regimen, and Treatment Period(s)

2.4.1 Administration of Investigational Products

ABvac40 is administered by subcutaneous injection which proved to be well tolerated in previous pre-clinical and clinical trials. For this Phase II study, five monthly (every four weeks) single-dose injections plus a 6 months (26 weeks) delayed booster of verum (or placebo, as corresponds) are planned for the Part A.

For the Part B, patients receiving placebo in Part A will cross-over to receive verum following the aforementioned scheme (Delayed start group), whereas patients receiving verum in Part A will receive placebo following the same scheme (except in V13B when they will receive a booster of verum) aiming to maintain the blinding for both patients and investigators (Booster group).

2.4.2 Justification for Selection of Doses/Timing of Investigational Products

Pre-clinical trials on rabbits allowed Non-Observed Adverse Effect Level (NOAEL) doses to be set for ABvac40 to about 0.1 mg of Abetax-40 / kg of live weight. That NOAEL meant that the Human-Equivalent Dose (HED) could be taken to about 0.0322 mg / kg (following indications given by the FDA in its "Guidance for Industry Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers"), i.e. up to 2 mg of the antigenic peptide for a person weighing about 60 kg.

However, taking account of the nature of ABvac40 (an active vaccine) and the recommendations contained in the EMEA / CHMP "Guidelines on Strategies to Identify and Mitigate Risks for First-in-Human Clinical Trials" for this first use in humans, it was decided to use a dose of 0.2 mg of Abetax-40, i.e. a dose ten times smaller than the HED calculated using the NOAEL.

Consideration must be given to the fact that those guidelines were put in place for healthy volunteers, so they may lead to insufficient calculated doses for administering to aged individuals who generally suffer from immuno-senescence and could need repeated injections to mount a significant immune response.

Based on the safety and tolerability results of Phase I and pre-clinical investigations (see IB document), we think that there is a safe margin for adding in this Phase II trial two further monthly inoculations plus the delayed booster shot to the trial, which will allow to expand the therapeutic range of ABvac40 and better explore its ability to generate immunological memory.

Additionally, based on the conclusions of the DSMB after the first interim analysis (July 2019), an additional booster shot will be administered to the patients receiving verum during the Part A of the present trial, aiming to better explore ABvac40 ability to generate immunological memory.

2.5 **Compliance Statement**

This study will be conducted under the conditions described in this protocol and in compliance with International Conference on Harmonization Good Clinical Practice (ICH GCP) and all applicable regulatory requirements.

2.6 **Study Population**

Patients (between 55-80 years of age) diagnosed either with a-MCI or vm-AD will be included in the trial. To ensure the primary safety and efficacy endpoints of the study a minimum of 60 subjects with a-MCI or vm-AD per treatment group (verum and placebo) are required. The amyloid-PET positive and amyloid-PET negative patients will be independently randomized following a ratio 1:1 between verum and placebo (Part A).

To ensure the primary efficacy endpoint with a potency of 85% at least 15 subjects per treatment group (verum and placebo; 30 in total) are required to complete the study. Since a total of 120 subjects will be initially included in the study and we estimate ~40% dropouts along the clinical trial, 70 are expected to complete the Part A of the protocol.

Participants in Part A will have to complete their 18-month visit (V23) in order to be eligible for the Part B of the study.

All patients who have participated in Part A, have not presented any drug reaction and have no MRI safety issues before the first immunization in Part B, could participate in Part B.

2.7 **Relevant Data and Literature Review**

Anti-Aß immunotherapies were considered very likely to be effective in the prevention of AD, especially if patients are treated before the onset of the disease or in it's very early stage (for a review see reference [1] in section 16).

However, in the last ten years most phase III clinical trials on anti-amyloid targeting drugs have failed to meet their primary endpoints (for recent reviews, see [2, 3]). In general, these disappointing results have been attributed to inadequate selection of either the subjects to be recruited, the stage of disease to be treated or the trial's endpoints to be assessed [3, 4]. Thus, the amyloid-cascade hypothesis is still leading the field supported by lines of evidence from laboratories and clinics worldwide [5]. In line with this, different anti-amyloid monoclonal antibodies (crenezumab, ganterezumab, solanezumab) are still being tested in ongoing phase III clinical trials, either in prodromal or pre-clinical AD, as disease modifying therapies (see, www.clinicaltrials.gov). Of note, it has been recently reported that a human anti-Aβ monoclonal antibody (aducanumab) tested in a phase I clinical trial in patients with prodromal or mild AD, reduces brain Aβ in a dose- and time-dependent manner. This is accompanied by a slowing of clinical decline measured by Clinical Dementia Rating—Sum of Boxes and Mini Mental State Examination scores [6]. On the other hand, active immunizations with short Aβ fragments have proved to be safer than the fibrillary Aβ1-42 used as immunogen in Wyeth's AN1792 trial. Thus, three active vaccines of this new kind (CAD-106, ACC-10 and AD02) have recently completed phase II showing a good safety profile [7-11].

Araclon-Grifols has developed an active vaccine directed specifically against the Aβ1-40 peptide. The reasons supporting this strategy directed against the Aβ1-40 peptide are initially based in several papers published in the 90s pointing to the relevance of Abeta 40 for Alzheimer's disease.

Among the first, is a paper published by Näslund & col [12] who found that Aβ1-40 was the predominant variant in sporadic AD whereas the principal variant in non-demented brains was Aβ1-42. Furthermore, the ratio Aβ1-40/Aβ1-42 was 10-fold greater on average in brains from sporadic AD than in non-demented controls.

A little later, Wang & col [13] reported that a progressive shift of brain Aβ1-40 and Aβ1-42 from soluble to insoluble pools and a profound increase in the levels of insoluble Aβ1-40 plays mechanistic roles in the onset and/or progression of AD. In line with this, Shin & col [14] found that Aβ1-40 but not Aβ1-42 contributes to the experimental formation of Alzheimer disease amyloid fibrils in rat brain.

Although Aβ1-42 is more hydrophobic and prone to aggregate, Aβ1-40 can indeed produce diffusible aggregates which are highly toxic to cells [15].

Furthermore, we saw in our laboratory that specific antibodies against Aβ40 were able to label intra-cellular neurofibrillary tangle-like structures in the entorhinal and hippocampal cortex of AD brains [16]. These Aβx-40 neurofibrillary tangles like structures did not co-localize in the same neurons with the tau neurofibrillary tangles. Thus it appears that in AD brains there are two different neurodegenerating populations one filled with tau neurofibrillary tangles and other filled with C-terminal fragments of Aβx-40 [16].

Moreover, we found an outstanding difference between the brains of two patients with Down syndrome suggesting that the onset of dementia may be accompanied by the deposition of Aβ40 in the parenchyma [17].

Other recent studies have reported higher CSF or plasma levels of Aβ40 in AD patients with Down syndrome compared to non-dements controls [18-21].

Currently, soluble Aβ species (dimers and/or oligomers) are considered to interact with synapsis causing alterations in their functional activity leading to cognitive decline and eventually dementia. Increased concentration of these soluble isoforms is supposed to precede deposition of fibrillary Aβ in senile plaques and would not be detectable by amyloid-PET scans [22, 23].

Recently, two phase 3 trials (Expedition-1 and -2 sponsored by Lilly), randomized 2,052 people with mild to moderate AD to receive infusions of Solanezumab or placebo once a month for 80 weeks. Although Expedition-1 and -2 overall showed no improvement on the primary outcome measures of Alzheimer's Disease Assessment Scale-Cognitive Subscale (ADAS-Cog)-11 and Alzheimer's Disease Cooperative Study – Activities of Daily Living (ADCS-ADL), statistically significant benefits were seen in a pooled analysis of the subgroup of patients with mild AD from both trials [24].

Supported by these results, Lilly started Expedition-3, in patients with mild AD and demonstrated brain amyloid burden (Florbetapir PET scans). Cognition (ADAS-cog 14) was set as the single primary outcome and function (Alzheimer's Disease Cooperative Study Activities of Daily Living inventory instrumental items (ADCS-iADL)) as a secondary outcome. On November 23, 2016, and then at the CTAD 2016 (San Diego, December 2016) Lilly announced, that primary and secondary outcome results were trending in the direction of a treatment benefit, but effects were small and fell short of statistical significance.

It is possible that the observed effect favoring Solanezumab in the pooled subpopulation of mild AD from Expedition-1 and -2 were lost precisely because of the selection of people with demonstrated brain amyloid burden who might have been in a too advanced disease stage as to positively respond to the Solanezumab; an antibody specifically targeting soluble Aβ isoforms while current radiotracers for PET are uptaken by insoluble fibrillary Aβ aggregates deposited in senile plaques.

Polyclonal antibodies induced after active immunization with ABvac40 binds both soluble and insoluble Aβ40 isoforms. Thus, a priori, it cannot be ruled out an effect in patients with MCI or vm-AD and a negative amyloid-PET scans (around 50% prevalence [25]). In line with this, it has been recently reported that rates of multiple modes of neurodegeneration, cognitive decline, and functional decline in MCI patients are observed to accelerate prior to the current

threshold for amyloid positivity [26]. Thus, a considerable proportion of patients with MCI would not meet inclusion criteria for a clinical trial based on the current threshold for amyloid positivity, though on average, they are experiencing cognitive and functional decline associated with pre-threshold levels of CSF Aβ42. In fact, these patients would never meet criteria for preclinical or prodromal AD during the course of their progression because they demonstrated clinically significant cognitive and functional impairment prior to becoming amyloid-positive [26]. Furthermore, it has been found that ~70% of a-MCI meeting the NIH-AA core clinical criteria (required for eligibility in the present clinical trial), progress to dementia in a 26.6 month follow-up (range 6-68 months; independently of their amyloid-PET or CSF Aβ42 status [27].

Taken together, these results suggest that the Aβ burden required to elicit an MCI profile could vary across patients. Some may be susceptible to minimal levels of Aβ deposition, showing clinical symptoms at earlier stages of Aβ cerebral pathology, while others would only show clinical symptoms after prolonged Aβ deposition.
Furthermore, we hypothesized that in those a-MCI and vm-AD patients deemed as Aβ-negative, the clinical symptomatology may be primarily driven by soluble Aβ isoforms and/or perivascular deposits of Aβ40, not detectable by current Aβ neuroimaging, and nevertheless susceptible to being modified by our anti-Aβ40 treatment. We assume that by making amyloid eligibility more liberal, there may be some loss in specificity; however, this loss may be worth the gains made by treating a less progressed population. In conclusion, we think that treatment with ABvac40 may benefit a-MCI and vm-AD patients, regardless of their amyloid-PET status as determined by the currently validated thresholds.

Nevertheless, it is well know that on average the progression to dementia in amyloid-PET negative MCI or vm-AD patients is less probable and significantly slower than in their counterparts with demonstrated amyloid burden, and this is why we decided to use this biomarker to stratify (not to select or to reject) the subjects for the assessment of the secondary exploratory efficacy objectives [28-30].

Thus, in line with the EMA/CHMP/539931/2014 document (Draft guideline on the clinical investigation of medicines for the treatment of Alzheimer's disease and other dementias) , the present ABvac40 phase II clinical trial will be carried out on a-MCI and/or vm-AD patients, characterized by their amyloid-PET neuroimaging profile as either Aβ-positives or Aβ-negatives. Eligible subjects must meet clinical criteria for either a-MCI as defined by the National Institute on Aging–Alzheimer's Association [31] ; or vm-AD as defined by National Institute of Neurological and Communicative Diseases and Stroke/Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) (see section 2, pag 29).

Patients will be recruited regardless their amyloid-PET state (positive or negative), which will be assessed as per the currently validated visual reading for flutemetamol. The amyloid-PET positive and amyloid-PET negative patients will be independently randomized following the same verum:placebo ratio of 1:1 [Part A].

The amyloid-PET positive and amyloid-PET negative subjects within each treatment group will be pooled together for the analysis of safety, tolerability and primary efficacy (immunogenicity) variables. However, the secondary efficacy variables regarding assessment of disease biomarkers and cognition (section 7.1.2) will be compared between verum and

placebo groups both in the overall group and separately in the amyloid-PET positive and amyloid-PET negative subjects.

3 STUDY OBJECTIVES AND PURPOSE

The purpose of this Phase II study is to confirm in patients with a-MCI or vm-AD the level of safety and tolerability obtained in the ABvac40 Phase I clinical trial in patients with mild to moderate AD. In addition, the study is aimed to better characterize the immune response elicited by ABvac40 and to explore its effects on AD biomarkers.

3.1 Part A (Randomized, Double-Bblind, placebo-controlled – 18 to 24 months follow-up)

3.1.1 Safety Objectives

3.1.1.1 Primary Safety Objective

- To evaluate the safety and tolerability of repeated doses of ABvac40 in a population of patients with a-MCI or vm-AD.

3.1.2 Efficacy Objectives

3.1.2.1 Primary Efficacy (Immunogenicity) Objective

- To assess the immune response produced during the study by repeated doses of ABvac40 in a population of a-MCI or vm-AD.

3.1.2.2 Secondary (Exploratory) Efficacy Objectives

- To characterize the immune response elicited by repeated doses of ABvac40 in a population of a-MCI or vm-AD.
- To assess the changes in the disease biomarkers elicited by ABvac40 in the overall study population.
- To assess the changes in cognition and quality of life elicited by ABvac40 in the overall study population.

3.2 Part B (ABvac40 delayed start, open label [Part A assignment will remain blinded for investigators and patients] – 18 months follow-up)

3.2.1 Exploratory Safety Objective

- To evaluate the safety and tolerability of repeated doses of ABvac40 after delayed start in patients receiving placebo during Part A, and the long-term safety and tolerability of ABvac40 in patients receiving a booster after their Part A vaccination scheme.

3.2.2 Exploratory Efficacy Objectives

3.2.2.1 Immunogenicity

- To assess the immune response produced by repeated doses of ABvac40 after delayed start in patients receiving placebo during Part A.
- To assess the immune response triggered by a second ABvac40 booster in patients receiving ABvac40 during Part A.

3.2.2.2 Other

- To assess the changes in the disease biomarkers elicited by ABvac40 in the overall study population.
- To assess the changes in cognition and quality of life elicited by ABvac40 in the overall study population and according to the treatment scheme during Part A and B.
- To assess the immunological memory, characterizing the immune response ex vivo elicited by the booster in patients who have received ABvac40 during Part A
- To assess the potential relationship between antibody titration, biomarkers and cognitive state.
- To define the optimal ABvac40 treatment scheme.

4 STUDY DESIGN

A multi-center, prospective, longitudinal, randomized, double-blind, placebo-controlled, two parallel treatment groups (verum and placebo), confirmatory phase 2 clinical trial has been designed (Part A).

Additionally, a delayed-start vaccination, open label (Part A assignment will remain blinded for investigators and patients) exploratory extension will start after at least 18 months of Part A initiation (Part B).

4.1 Endpoints

4.1.1 Part A

The primary endpoint for safety is the incidence of AEs (overall or grouped as neurological, psychiatric or cardiovascular).

The hypothesis for the primary endpoint for safety is that ABvac40 will be safe for human administration, according to the observed pattern of AEs. The trial will be considered satisfactory if the pattern of AEs (overall or grouped as neurological, psychiatric or cardiovascular) is consistent with a good safety and tolerability profile of ABvac40 for human administration at the final visit.

Additionally, the following secondary safety endpoints will be evaluated including:

- number of withdrawn patients due to adverse events (AEs)
- number of withdrawn patients due to other causes
- number of serious adverse events (SAEs)
- number of clinically significant changes in physical and neurological examination
- number and type of concomitant medications
- number of clinically significant changes in vital signs (blood pressure, heart rate, respiratory rate, body temperature), body mass (weight, height).
- number of clinically significant changes in brain MRI
- number of clinically significant changes in electrocardiogram (EKG)
- number of clinically significant changes in analytical haematology, biochemistry, coagulation, serology and urine test strip results.

The primary efficacy endpoint is the average maximal increment (MΔ) of anti-Aβ40 antibody signal (OD in ELISA) in each subject with regard to the pre-treatment visit (either V0 or V1).

For the primary efficacy analysis, the trial will be considered successfully confirmatory regarding efficacy (immunogenicity) of ABvac40 if the average maximal increment (MΔ) of anti-Aβ40 antibody signal (OD in ELISA) in the verum group is >0 OD than the average MΔ in the placebo group. For the secondary efficacy analysis, the trial will be considered satisfactory if the difference of the average maximal increment (MΔ) of anti-Aβ40 antibody signal (OD in ELISA) in the treated group is > 1.778 OD than the average MΔ in the placebo

group; the difference that was reached between both groups in the phase I clinical trial of ABvac40.

Additionally, the following secondary efficacy endpoints will be evaluated including:

- Characterization of the immune response:
  - Levels of anti-KLH and anti-Aβ42 antibodies in plasma
  - Level of anti-Aβ40 antibodies in CSF
  - Levels of cytokines in plasma including IL-2, IL-4, IL-5, IL-6, IL-10, IL-13, IL-15, TNF-α and IFN-γ.
  - Analysis of the peripheral blood cell subsets by immunophenotyping.
  - Levels of antibody secreting cells and cytokine-secreting cells (including IFN-γ, TNF-α and IL-13)
- Assessment of disease biomarkers:
  - Levels of Aβ peptides in plasma
  - Cortical fibrillary amyloid deposition assessed by PET scans.
  - Levels of CSF biomarkers (Aβ42, Tau, P-tau, neurofilament light and neurogranin) and other Aβ peptide species
  - Brain volumetric and atrophy of the hippocampus using magnetic resonance imaging
- Assessment of cognition and quality of life:
  - Mini Mental State Examination (MMSE)
  - Clinical Dementia Rating scale (CDR)
  - Repeatable Battery for the Assessment of Neuropsychological Status (RBANS)
  - Alzheimer's Disease Cooperative Study – Activities of Daily Living, Mild Cognitive Impairment (ADCS-ADL MCI)
  - Columbia Suicide Severity Rating Scale (C-SSRS)
  - Trail Making Test (TMT)
  - Investigator Global Evaluation (IGE)
  - Euroqol 5 Dimensions (EQ-5D)

4.1.2 Part B

All the endpoints of Part B will be explorative. No hypothesis will be tested.

Both the primary and secondary safety endpoints mentioned in Section 4.1.1 will also be evaluated in Part B (only as exploratory safety endpoints).

Regarding efficacy, the following exploratory endpoints will be evaluated:

- Presence of specific antibodies against Aβ40 in plasma
- Characterization of the immune response:
  - Level of anti-Aβ40 antibodies in CSF
  - Levels of antibody secreting cells
- Assessment of disease biomarkers:
  - Levels of Aβ peptides in plasma
  - Cortical fibrillary amyloid deposition assessed by PET scans.

  - Brain volumetric and atrophy of the hippocampus using magnetic resonance imaging
- Assessment of cognition and quality of life:
  - MMSE
  - CDR scale
  - RBANS
  - ADCS-ADL MCI
  - C-SSRS
  - TMT
  - IGE
  - EQ-5D-5L

4.2 Study Design and Plan

The expected duration of the trial will be of 51 months, from February 2018 to May 2022.

First Patient In: February 2018

Last Patient In: September 2019

Last Patient Out (Part B): May 2022

4.2.1 Part A

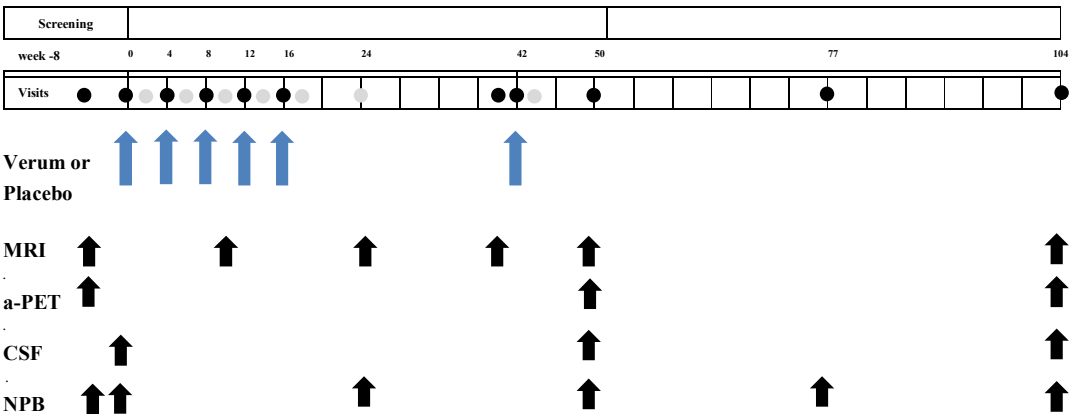
The Part A of the study will last for up to 24 months (from V0 to V25) and at least 18 months (up to V23) during which the patients will receive five monthly (every four weeks) immunizations plus a booster shot.

During the study, two interim analyses (Int) will be performed to assess safety, tolerability and biological activity of the treatment (including data from the collected disease biomarkers at each of them). These analyses will be reviewed by an independent Data Safety Monitoring Board (DSMB). Information regarding the DSMB is detailed in Section 9.1.5.1. The first Int will be carried out once the first 30 patients have completed the 24-week visit (Int-6m) and the second one once all patients have completed the 24-week visit (Int-6m, eight weeks after the fifth monthly immunization) or in October 2020, whichever occurs first. The double blinding of the study will be maintained throughout these two Int. Additional DSMB reviews may be made *ad hoc* in the event of safety issues.

Additionally, after all patients complete their 18-months visit (V23), a statistical analysis will be performed to determine whether the Part B of the study should be discontinued.

The study outline for Part A is summarized in the following figure:

Table 2: Overall Study Design (Part A)



MRI: Magnetic Resonance Imaging; a-PET: Amyloid PET; NPB: Neuropsychological battery.

Visit Description

Over the first 50 weeks of the Phase II clinical trial, 21 visits will take place. Additionally, a one year long-term follow-up period has been programed. This additional follow-up consists of four additional visits (V22-V25) at quarterly intervals.
The study visits can be grouped as follows:

- Pre-selection or Screening Visit (V0)
- Vaccination Visits (V1, V4, V7, V10, V13 and V18*)
- Safety control visits
  - Phone interview: V2, V5, V8, V11, V14 ,V19, V22 and V24
  - Face-to-face visits: V3, V6, V9, V12, V15, V16 (Int-6m), V17, V20, V21, V23 and V25 (Final visit)

* All patients who will attend their V18 after April 2020 must be tested for COVID-19 by PCR (Polymerase chain reaction test) method before receiving the study medication. A specific informed consent form (ICF) must be signed by the applicable patients authorizing this test (see Section 6.1.1.1 for further details)

The following table shows the schedule of study procedures and events.

Table 3: Schedule of Study Procedures and Events (Part A)

	Screening																																		
Month	-2	0			1			2			3			4			5	6	7	8	9		10			11	12	..	15	..	18	..	21	..	24
Week	-8	0	0	2	4	4	6	8	8	10	12	12	14	16	16	18		24			40	42	42	44		50		65		77		90		104
Day	-60	-3 /0*	3 ±3	14 ±3	28 ±3	31 ±3	42 ±3	56 ±3	59 ±3	70 ±3	84 ±3	87 ±3	98 ±3	112 ±3	115 ±3	126 ±3		168 ±7			280 ±7	294 ±7	297 ±7	308 ±7		350 ±15		455 ±15		539 ±15		630 ±15		728 ±15
Visit Nº (T=by phone)	0†	1* Vc	2 (T)	3 Sc	4 Vc	5 (T)	6 Sc	7 Vc	8 (T)	9 Sc	10 Vc	11 (T)	12 Sc	13 Vc	14 (T)	15 Sc		16 Int 6m			17 Sc	18 Vc	19 (T)	20 Sc		21 Sc		22 (T)		23 Sc		24 (T)		25-FV
Informed Consent	●[1]																																		
Vaccination ABvac40/placebo		●*			●			●			●			●								●§												
Medical History, Height (only at screening)	●[1]	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●		●			●	●	●	●		●		●		●		●		●
Weight, BP, HR, RR, Tº	●[1]	●		●	●		●	●		●	●		●	●		●		●			●	●		●		●				●				●
Physical and neurological exam	●[1]	●		●	●		●	●		●	●		●	●		●		●			●	●		●		●				●				●
Neuropsych. Battery and IGE (exc. V0/V23)	●[1]	●																●								●				●				●
Quality of Life EQ-5D5L		●																								●								●
Eletrocardiogram	●[1]																	●#								●#				●#				●#
Urine Analysis (test strip)	●[2]	●		●			●			●			●			●		●			●			●		●				●				●
ApoE Genotyping		●																																	
Hemogram	●[2]	●		●			●			●			●			●		●			●			●		●				●				●
Biochemistry	●[2]	●		●			●			●			●			●		●			●			●		●				●				●
Biological Activity	●[2]	●		●			●			●			●			●		●			●			●		●				●				●
MRI	●[3]									●								●			●					●								●
a-PET	●[4]																									●								●
CSF		●																								●								●
Adverse events	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●		●			●	●	●	●		●		●		●		●		●
Concomitant Medication	●[1]	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●		●			●	●	●	●		●		●		●		●		●

MRI: Magnetic Resonance Imaging; a-PET: Amyloid PET; CSF: Cerebrospinal fluid; EKG: Electrocardiogram; exc.: except; IGE: Investigator Global Evaluation; NPB: Neuropsychological battery; FV: final visit; Vc: Vaccination; Sc: Safety control; Int: Interim analysis; T: Telephone call; RR: respiratory rate: Tº: body temperature

†The Screening Visit (V0) should be performed in 4 separate days:

[1]Day 1 of V0 (V0a):
- Signature of informed consent and verification of inclusion and exclusion criteria; medical history, vital signs, physical and neurological exam, neuropsychological battery, EKG, concomitant medication for AD and other diseases and delivery of Patient Card and Patient Diary; the scanned EKG must be sent to the Medical Advisor and no further assessments can be made to the subject until the approval is received at the center.
- If the patient meets all neuropsychological criteria, once the EKG has been approved by the Medical Advisor, the Day 2 of V0 (V0b) can be scheduled

[2]Day 2 of V0 (V0b):
- Urine analysis and blood sample extraction (for hemogram, biochemistry and biological activity)
- If no abnormal findings are found in the blood and urine tests, the Day 3 of V0 (V0c) can be scheduled.

[3]Day 3 of V0 (V0c):
- Magnetic Resonance Imaging
- Once the MRI has been approved by the MRI Central Reader, the Day 4 of V0 (V0d) can be scheduled

[4]Day 4 of V0 (V0d):
- a-PET
- Once the a-PET has been approved by the a-PET Central Reader, the patient can be randomized and the first day of V1 can be scheduled (a minimum of 4 days later, so that the appropriate material for CSF extraction and the IMP can be received at the center).

* The Inclusion Visit (V1) should be performed in 2 separate days; the vaccination should take place **at least 3 days** after the CSF extraction, **or the time needed until resolution of any AE occurring after the CSF extraction, whichever is longer** (to avoid interference with the assessment of AEs related to the vaccination)**, with a maximum of 10 days after the urine and blood sample extraction**:

➢ Day 1 of V1 (V1a):
- Urine analysis, blood sample extraction (for ApoE genotyping, hemogram, biochemistry and biological activity) and CSF collection

➢ Day 2 of V1 (V1b):
- Physical and neurological exam, neuropsychological battery and IGE, quality of life, vital signs (before vaccination), vaccination, vital signs (after vaccination), concomitant medication and AEs.

✓ A telephone control interview will take place within the first week (3± 3 days) after each vaccination visit and at V22 and V24.
✓ A safety visit will be scheduled two weeks after each vaccination and at V17 and V23.
✓ Blood samples will be collected at every safety control visit for safety control and characterization of immune response.
✓ Study visits should take place at the Days indicated above, within the following windows:
- During first 6 months: ± 3 days
- Between month 6 and month 12: ± 7 days
- Between month 12 and month 24: ± 15 days

✓ If a visit takes place out of window, the patient should be visited as soon as possible; For the five first immunizations **the minimum interval between vaccinations must be 25 days and the maximum interval must be 31 days**; if one vaccination is delayed for any reason, the next dose must be re-scheduled to fit this therapeutic window.

In this visit the scanned EKG must be sent to the Medical Advisor for review.
§ All patients who will attend their V18 after April 2020 must be tested for COVID-19 by PCR method before receiving the study medication. A specific ICF must be signed by the applicable patients authorizing this test (see Section 6.1.1.1 for further details)

4.2.2 Part B

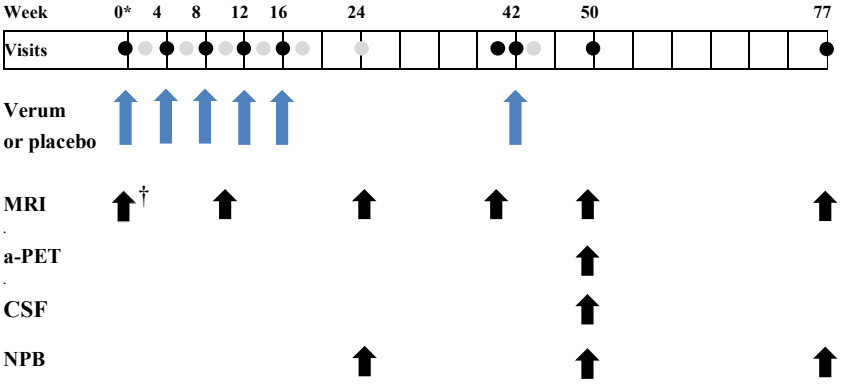
The Part B of the study will last for 18 months (from V1B to V22B) during which the patients will receive five monthly immunizations plus a booster shot. The first visit of Part B (V1B) will take place at least 18 months after the patient started Part A (i.e. he/she should have completed V23 of Part A to enter Part B).

The patients randomized to the placebo group during Part A will receive five monthly (every four weeks) immunizations with ABvac40 plus a booster shot after 6 months (Delayed start group), whereas the patients randomized to the Verum group during Part A will receive placebo following this schedule, except in V13B, where they will receive an ABvac40 booster shot (Booster group). Given the extended inclusion period of Part A, the V13B booster will take place at different timepoints relative to the patient's last immunization. For the analysis, they will be grouped in 2 periods: 12± 3 months and 18± 3 months after last immunization.

There will not be interim analyses for this Part B, but the DSMB reviews may be made *ad hoc* in the event of safety issues.

The study outline for Part B is summarized in the following figure:

Table 4: Overall Study Design (Part B)



*At least 18 months after initiating Part A

† A MRI will be performed before first vaccination of Part B only if the previous MRI was performed more than 6 months (±15 days) before V1B

Visit Description

Over the first 12 months of the Part B, 21 visits will take place. The first visit of Part B (V1B) will take place at least 18 months after the patient started Part A (i.e. he/she should have completed V23 of Part A to enter Part B).

The study visits can be grouped as follows:

- Vaccination Visits*: V1B, V4B, V7B, V10B, V13B and V18B
- Safety control visits

- Phone interview: V2B, V5B, V8B, V11B, V14B and V19B
- Face-to-face visits: V3B, V6B, V9B, V12B, V15B, V16B, V17B, V20B, V21B and V22B

* All patients must be tested for COVID-19 during the Part B vaccination visits before receiving the study medication (see Section 6.1.1.2 for further details).

The following table shows the schedule of study procedures and events.

Table 5: Schedule of Study Procedures and Events (Part B)

	Part B																										
Month	**0†**			**1**			**2**			**3**			**4**			**5**	**6**	**7**	**8**	**9**	**10**			**11**	**12**	**…**	**18**
Week	**0**	**0**	**2**	**4**	**4**	**6**	**8**	**8**	**10**	**12**	**12**	**14**	**16**	**16**	**18**		**24**			**40**	**42**	**42**	**44**		**50**		**77**
Day	**-3 /0***	**3 ±3**	**14 ±3**	**28 ±3**	**31 ±3**	**42 ±3**	**56 ±3**	**59 ±3**	**70 ±3**	**84 ±3**	**87 ±3**	**98 ±3**	**112 ±3**	**115 ±3**	**126 ±3**		**168 ±7**			**280 ±7**	**294 ±7**	**297 ±7**	**308 ±7**		**350 ±15**		**539 ±15**
Visit Nº (T=by phone)	**1B* Vc**	**2B (T)**	**3B Sc**	**4B Vc**	**5B (T)**	**6B Sc**	**7B Vc**	**8B (T)**	**9B Sc**	**10B Vc**	**11B (T)**	**12B Sc**	**13B Vc**	**14B (T)**	**15B Sc**		**16B Sc**			**17B Sc**	**18B Vc**	**19B (T)**	**20B Sc**		**21B Sc**		**22B EOS**
Informed Consent	o																										
Vaccination ABvac40/placebo§	o*			o			o			o			o								o						
Medical History	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o		o			o	o	o	o		o		o
Weight, BP, HR, RR, Tº	o		o	o		o	o		o	o		o	o		o		o			o	o		o		o		o
Physical and neurological exam			o	o		o	o		o	o		o	o		o		o			o	o		o		o		o
Neuropsych. Battery and IGE																	o								o		o
Quality of Life EQ-5D5L																									o		
Eletrocardiogram																	o#								o#		o
Urine Analysis (test strip)			o			o			o			o			o		o			o			o		o		o
Hemogram			o			o			o			o			o		o			o			o		o		o
Biochemistry			o			o			o			o			o		o			o			o		o		o
Biological Activity	o		o			o			o			o			o		o			o			o		o		o
MRI	o‡								o								o			o					o		o
a-PET																									o		
CSF																									o		
Adverse events	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o		o			o	o	o	o		o		o
Concomitant Medication	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o		o			o	o	o	o		o		o

MRI: Magnetic Resonance Imaging; a-PET: Amyloid PET; CSF: Cerebrospinal fluid; EKG: Electrocardiogram; exc.: except; IGE: Investigator Global Evaluation; NPB: Neuropsychological battery; EOS: end of study; Vc: Vaccination; Sc: Safety control; T: Telephone call; RR: respiratory rate: Tº: body temperature

† **At least 18 months after initiating Part A**

* The First Visit of Part B (V1B) will take place when the results of the last MRI test performed (either at V21 or V25 or before first vaccination of Part B‡) are available and support the vaccination.

‡ A MRI will be performed before first vaccination of Part B only if the previous MRI was performed more than 6 months (±15 days) before V1B.

- ✓ A telephone control interview will take place within the first week (3 ±3 days) after each vaccination visit.
- ✓ A safety visit will be scheduled two weeks after each vaccination and at V17B.
- ✓ Blood samples will be collected at every safety control visit for safety control and characterization of immune response.

- ✓ Study visits should take place at the Days indicated above, within the following windows:
  - During first 6 months: ± 3 days
  - Between month 6 and month 12: ± 7 days
  - Between month 12 and month 18: ± 15 days
- ✓ If a visit takes place out of window, the patient should be visited as soon as possible; For the five first immunizations the **minimum interval between vaccinations must be 25 days** and the **maximum interval must be 31 days**; if one vaccination is delayed for any reason, the next dose must be re-scheduled to fit this therapeutic window.

In this visit the scanned EKG must be sent to the Medical Advisor for review.

§ All patients must be tested for COVID-19 during the Part B vaccination visits before receiving the study medication (see Section 6.1.1.2 for further details)

4.3 Measures Taken to Minimize/Avoid Bias

4.3.1 Subject Numbering

Within each study site, subjects in the study will receive a consecutive subject number. Subject numbers are generated beginning with the study center number (2 digits assigned by the sponsor) preceded by “S” followed consecutively with a unique number for each subject (3 digits, including leading zeros). For example, if the investigator’s center number is 01, subject number will be S01-001, S01-002, S01-003, etc. Subject numbers, once assigned, will not be reused at any center.

4.3.2 Randomization

Subjects will be randomized into one of two arms, based on a computer-generated 1:1 ratio randomization schedule issued to a Randomization Manager not involved in the study. A randomization number will be assigned to each subject in addition to the subject number at Baseline visit (V1). A stratified randomization will be performed to obtain separate randomization schemes for the amyloid-PET positive and amyloid-PET negative subjects following a 1:1 verum:placebo ratio.

A Randomization Manager from the CRO (Clinical Research Organization) not involved in the study will prepare two separate randomization lists, one without treatment details (only with the treatment code) to be sent to CRO Study Project Manager to proceed with the patients randomization and one with treatment details to be sent to the Independent Representative of the Sponsor (IRS) for the packaging and labeling of the study medication, and it will be kept secret until the end of the study, or until the database is closed. . This will ensure that the statistician involved in the study analysis is blinded until the study is complete or until the database is closed. The randomization list will be generated using SAS software version 9.2 or equivalent.

For the DSMB, a randomization list will be sent to the Independent Statistician (IS) in order to prepare and confidentially distributing to each member of the DSMB interim reports that include data summaries, tables, and listings containing unblinded data.

The patients will be then numbered by two codes, the Screening number (S01-001 to S0n-00n) and the randomization number (R001 to R00n). Randomization numbers will be consecutive, with three digits preceded by the letter "R" (for example, R001, R002, R003, etc). The nurse or investigator responsible for the study at the center will distribute the IMP blisters following the randomization numeration.

4.3.3 Blinding

Only the IRS and the IS will work without blinding in order to label study medication and to prepare all the DSMB documentation, respectively. The IS will be a statistician who does not participate in any other activities related to the planning and execution of the study and who will be part of the DSMB team (as non-voting member). In this way, the blinding will be assure during the Int.

For the Part B of the study, both the sites' PI and the patients will remain blinded.

Three sets of opaque and blind emergency envelopes will be prepared. The sites' PI will be given a closed envelope bound to each treatment kit (blister) for each subject randomized containing information of the treatment that person receives; the PI can open this in a medical emergency (see section 8.3). The investigator or person, who opens the envelope, must record the date and reasons for opening the sealed envelope in the Case Report Form and in the randomization envelope.

The two remaining sets of emergency envelopes will be given to the global CRO [redacted] and to the pharmacovigilance responsible person.

Details printed on the emergency envelope:
- It should be opened only by the Principal Investigator (or assigned delegate in an unavoidable situation) in case of emergency.
- Partial/complete breakage of blinding should be adequately documented.
- An accidental opening of the envelope should be reported to the sponsor and must be documented.
  - Code of the study:
  - Patient No.: R-XXX

Details printed inside the emergency envelope:
- Patient No.: R-XXX
- Treatment: verum or placebo

4.4 Study Treatments

4.4.1 Treatments to Be Administered

The treatments to be administered are verum (ABvac40) and placebo (ABvac40 vehicle without its active ingredient).

The following table shows when the vaccination will occur:

Table 6: Treatment Time points

#	Treatment visits	Week #	Month #
Part A			
1	V1	0	0
2	V4	4	1
3	V7	8	2
4	V10	12	3
5	V13	16	4
6	V18*	42	10

Part B			
1	V1B†	0	0
2	V4B†	4	1
3	V7B†	8	2
4	V10B†	12	3
5	V13B†	16	4
6	V18B†	42	10

***** All patients who will attend their V18 after April 2020 must be tested for COVID-19 by PCR method before receiving the study medication. A specific ICF must be signed by the applicable patients authorizing this test (see Section 6.1.1.1 for further details)

† All patients must be tested for COVID-19 during the Part B vaccination visits before receiving the study medication (see Section 6.1.1.2 for further details)

Verum

Name: ABvac40

Active ingredient: Conjugated Aβx-40 to KLH

Vehicle: Phosphate buffer containing 0.35% aluminium hydroxide (adjuvant)

Pharmaceutical Form: Injection ready vials

Posology: Part A: Verum group: Six administrations: the first five administered once every 4 weeks (28±3 days) and the sixth at week 42 (V18), 26 weeks after the fifth. Each administration consists of 1 mL injection of ABvac40 (containing 0.2 mg of Abetax-40).

Part B: Delayed start group: Six administrations: the first five administered once every 4 weeks (28±3 days) and the sixth at week 42 (V18B), 26 weeks after the fifth immunization. Booster group: One administration at week 16 (V13B).

Each administration consists of 1mL injection of ABvac40 (containing 0.2 mg of Abetax-40).

Method of administration: Subcutaneous injection.

Placebo

Name: Placebo

Active ingredient: Nil

Vehicle: Phosphate buffer containing 0.35% aluminium hydroxide (adjuvant)

Pharmaceutical Form: Injection ready vials

Posology: Part A: Placebo group: Six administrations: the first five administered once every 4 weeks (28±3 days) and the sixth at week 42 (V18), 26 weeks after the fifth. Each administration consists of 1mL injection of ABvac40 vehicle.

Part B: Booster group: Five administrations: the first four administered once every 4 weeks (28±3 days) and the fifth at week 42 (V18B), 30 weeks after the forth. Each administration consists of 1mL injection of ABvac40 vehicle.

4.4.2 Labeling of Investigational Product(s)

Investigational products are labeled according to the requirements of local law and legislation. Label text (see Label Models document) will be approved according to agreed Araclon-Grifols procedures, and a copy of the labels will be made available to the study site.

4.4.3 Packaging of Investigational Product(s)

The final steps of IMP manufacturing (Fill & Finish) are carried out by [redacted]

Thes companies will also be responsible for preparing the vials of the comparator or placebo. It shall be carried out in accordance with GMP guidelines. The material will be always sent and stored refrigerated at 2-8°C. The investigator is responsible for the proper storage of IMP at the center.

Each patient will be assigned a treatment blister which consists of 8 vials (6 for treatment and two as backup) packaged in a labeled cardboard box [Part A].

Medication for Part B will consist of 6 vials packaged in a labeled cardboard box.

4.4.4 Storage of Investigational Product(s)

The study IMP will be sent to the Pharmacy of each participant center keeping the restrictions of temperature during the courier supply (2-8ºC).The investigator is responsible for the proper storage of IMP at the center.

The treatment blisters will be stored separately from the standard practice products, refrigerated (2-8°C) in a dark and closed place to which only authorized personnel have access.

The supplies of medication are exclusively for the purposes indicated in this protocol and cannot be used for any other purpose. The researcher will not destroy any label or partially used or unused product supply. At the end of the study and, as appropriate during the course of the study, the investigator will return all used and unused medication packaging, medication labels and a copy of the completed medication-dispensing notebook to the sponsor. All supplies will be recounted at the end of the study.

[redacted] will store IMP and placebo spare vials which can be used for replacement, if necessary.

4.5 Expected Duration of Subject Participation in the Study

Part A

The expected duration of a study subject's participation in Part A will be approximately of 18 (minimum) to 24 (maximum) months.

After a Screening Visit (V0), subject randomization will occur at the Baseline Visit (V1) to be held within the 60 days after the screening visit. Each participant will receive a maximum of six vaccinations, the first five immunizations will be administered at four-week intervals and the sixth, a booster shot, will take place 26 weeks after the fifth vaccination (at month 10).

Part B

The expected duration of the study subject's participation in Part B will be of 18 months. Each participant will receive a maximum of six vaccinations, the first five immunizations will be administered at four-week intervals and the sixth, a booster shot, will take place 26 weeks after the fifth vaccination (at month 10). 32 weeks after (at month 18), a long-term safety visit will be done.

4.6 Discontinuation Criteria for Individual Subjects and Study

4.6.1 Discontinuation Criteria for Individual Subjects

See Section 5.3 Subject Withdrawal Criteria

4.6.2 Premature Termination of Study/Closure of Center

The sponsor, Institutional Review Board/Ethics Committee (IRB/EC), and/or regulatory authorities have the right to close this study or a study center, and the investigator/sponsor has the right to close a center, at any time, although this should occur only after consultation between involved parties. The IRB/EC and the DSMB must be informed. Should the study/center be closed prematurely, all study materials (except documentation that has to remain stored at site) must be returned to the sponsor. The site principal investigator will retain all other documents until notification given by the sponsor for destruction.

Completion of the clinical trial is expected when all patients included in the study have finished the clinical trial, the database has been closed and the final report is submitted to the health authorities.

The sponsor, Araclon Biotech, S.L. a GRIFOLS company, reserves the right to stop the trial prematurely. If the clinical trial is terminated or suspended, the sponsor shall immediately inform the investigators and the regulatory authority. The IRB shall be informed immediately, noting the sponsor's reason(s) for terminating or suspending the study.

If the trial is prematurely terminated, the investigator will inform all patients involved in the trial, ensuring that adequate patient monitoring will be continued and all data up to the date of termination will be collected and stored.

Reasons for premature discontinuation of the trial can be:

- Occurrence of serious unexpected ADR that the Data and Statistics Center (DSC), the investigator and Araclon-Grifols, deem cause to prevent the continuation of the trial.
- Incidence of repeated SAEs probably related with the IMP, which constitute a potential health risk in the opinion of the Critical Event Committee (CEC).
- The emergence of new scientific data on the investigational product that does not justify the continuation of the trial.

A study center can be closed by the sponsor for the following reasons:

- Lack of enrollment
- Non-compliance with the requirements of the study protocol
- Non-compliance with ICH GCP
- Lack of personnel
- Serious discrepancies with regard to verification of the original documents
- Inadequate quality of eCRFs
- Serious and/or persistent failure to comply with the protocol, GCP of the ICH, and/or relevant requirements of the investigator/institution.

In addition, if after analyzing the study data from Part A (up to month 18 – V23) no clinical differences have been observed between the Placebo and the Verum group according to the following stopping rules, the sponsor could prematurely finalize the study. A least one of the following criteria must have been fulfilled in the Verum group:

1. For PET negative population: If the lower bound of the 95% exact CI for the proportion of negative PET patients treated with vaccine is greater than the proportion in Placebo group after 12 months (V21).
2. For PET positive population: If the lower bound of 95% exact CI for the proportion of Reduction of cerebral amyloid plaques of positive PET patients treated with vaccine is greater than the proportion in Placebo group after 12 month (V21).
3. For PET positive population: If the lower bound of the 95% exact CI for the mean of decline in Clinical Dementia Rating-Sum of Boxes (CDR-SB) in the positive PET patients treated with vaccine is greater than the mean in Placebo group after 18 months (V23).

4.7 Accountability Procedures for Investigational Product(s)

Investigational product is to be used only for the study in accordance with the directions given in this protocol. The Investigator, or designee such as the study pharmacist, is responsible for the distribution of the investigational product in accordance with directions given in the protocol and pharmacy manual.

The IMP and its comparator or placebo, packaged in individual blisters containing 8 vials, will be supplied by [redacted] to a central depot.

Upon requested by the study sites, once the baseline visit (V1 or V1B) for a new subject has been set up, central depot will send the treatment kits and their corresponding emergency envelopes with an acknowledgment form. The Pharmacist must sign the form and send it to the person responsible for managing the study at central depot to confirm receipt of the IMP kits.

The investigator is responsible for maintaining accurate records of the investigational product for his/her site. Investigational product inventory/dispensing documentation verifying the receipt, dispensing, destruction, or return must be maintained and kept current by the investigator, or designee. The inventory must be made available for inspection by the monitor. Investigational product supplies (including placebo) must be accounted for by the monitor and inventory/dispensing logs must be verified by the monitor prior to IMP return or destruction. Written documentation of any used and unused inventory is required. At the end of the study, a copy of the inventory/dispensing log(s) will be retrieved by the monitor and returned to Araclon-Grifols.

4.8 Maintenance of Treatment Randomization Codes

For a randomized and blinded study, access to the actual randomization schedules or codes must be strictly controlled during the course of the study. Unveiling the blinding of treatment assignment during the study should only occur if it is absolutely necessary to identify treatment assignment in an emergency situation (eg, due to a serious adverse event). The date and reason for the unblinding must be recorded in the subject's medical record and on the subject's eCRF. (See section 8.3).

4.9 Data to Be Recorded on the eCRFs

This section identifies the data to be recorded on the eCRF (and corresponding source documents) at the different visits unless elsewhere specified (laboratory manuals and/or data management guidelines). Other data which are initially recorded in written or electronic files from efficacy or safety assessments will be subsequently uploaded to the subjects' eCRF (see section 7.2 and 8.2, respectively)

4.9.1 Part A

4.9.1.1 Screening phase: visit V0 (weeks -8 to 0)

During this period, a pre-selection visit (V0) will be carried out up to a maximum of 60 days before the start of treatment (V1). In the pre-selection (V0) visit the investigator will assess the patient's suitability for inclusion in the clinical trial.

Before any assessment or intervention for the study is completed, the investigator will inform the patient and caregiver, about the trial. Both will receive a written detailed description of all

activities and requirements involved of the trial (Patient's Information and caregiver Leaflet), and the investigator will ensure that they completely understand the clinical trial.

Once understood and accepted, they will proceed to sign the informed consent.

The investigator will keep a selection record in which suitability of each patient as deduced at the screening visit will be reported, independent of whether or not the patient ends up participating in the clinical trial. This selection record will be saved along with the original documents of the clinical trial.

After signing the informed consent and to verify the suitability of patients, the following assessments will be carried out preferably in four separate days:

- Day 1 of V0 (V0a):
  - Signature of informed consent and verification of inclusion and exclusion criteria; medical history, vital signs, physical and neurological exam, neuropsychological battery, EKG, concomitant medication for AD and other diseases (all relevant treatments at the Investigator's opinion administered during the 6 months prior to the screening visit) and delivery of Patient Card and Patient Diary; the scanned EKG must be sent to the Medical Advisor and no further assessments can be made to the subject until the approval is received at the center.
  - If the patient meets all neuropsychological criteria, once the EKG has been approved by the Medical Advisor, the Day 2 of V0 (V0b) can be scheduled
- Day 2 of V0 (V0b):
  - Urine analysis and blood sample extraction (for hemogram, biochemistry and biological activity)
  - If no abnormal findings are found in the blood and urine tests, the Day 3 of V0 (V0c) can be scheduled.
- Day 3 of V0 (V0c):
  - Magnetic Resonance Imaging
  - Once the MRI has been approved by the MRI Central Reader, the Day 4 of V0 (V0d) can be scheduled
- Day 4 of V0 (V0d):
  - a-PET
  - Once the a-PET has been approved by the a-PET Central Reader, the patient can be randomized and the first day of V1 can be scheduled (a minimum of 4 days later, so that the appropriate material for CSF extraction and the IMP can be received at the center).

Data to be recorded in the eCRF (and corresponding source documents) at the Pre-selection visit (V0):

- Diagnosis of AD.
- Check inclusion and exclusion criteria.
- Demographic information (age, sex, smoking status, ethnicity, education level).
- Vital signs (blood pressure, heart rate, respiratory rate, body temperature).

- Body mass (height and weight).
- Medical and surgical history.
- History of illness.
- General treatment for AD.
- Physical examination.
- Neurological examination.
- Record concomitant medication.
- Mini-Mental Status Examination (MMSE).
- Clinical Dementia Rating Scale score (CDR).
- RBANS score.
- Sheikh & Yesavage Geriatric Depression Scale score.
- Electrocardiogram (EKG).
- MRI and verify: Hachinski Ischemic Scale, Scheltten Scale concordant with the diagnosis of a-MCI or vm-AD.
- Laboratory tests blood analysis.
- Urine test strip
- a-PET (only after MRI results)
- "Patient card" given to the patient and the caregiver.
- Decision on eligibility.
- Columbia Suicide Severity Rating Scale (C-SSRS)

In addition, the investigator will document, in the patient's medical record, the participation in this clinical trial.

4.9.1.2 Selection and Active Phase: visits V1-V21 (weeks 0-50)

Eligible patients will be randomized at baseline visit (V1).

The Inclusion Visit (V1) should be performed in 2 separate days; the vaccination should take place **at least 3 days** after the CSF extraction, **or the time needed until resolution of any AE occurring after the CSF extraction, whichever is longer** (to avoid interference with the assessment of AEs related to the vaccination)**, with a maximum of 10 days after the urine and blood sample extraction**:

- Day 1 of V1 (V1a):
  - Urine analysis, blood sample extraction (for ApoE genotyping, hemogram, biochemistry and biological activity) and CSF collection

- Day 2 of V1 (V1b):
  - Physical and neurological exam, neuropsychological battery and IGE, quality of life, vital signs (before vaccination), vaccination, vital signs (after vaccination), concomitant medication and AEs.

The Active Phase period consist in two different types of visits: vaccination visits (V1, V4, V7, V10, V13 and V18) and safety visits (phone interview: V2, V5, V8, V11, V14, V19 and face-to-face visits: V3, V6, V9, V12, V15, V16, V17, V20 and V21).

Data to be recorded in the eCRF (and corresponding source documents), at the Vaccination Visits (V1, V4, V7, V10, V13 and V18):

- Medical Evaluation
  - Physical and neurological examination
  - Vital signs (blood pressure, heart rate, respiratory rate, body temperature) before and after the Vaccination
  - Corporal biometry (weight)
- Medical History:
  - Record relevant past and concomitant diseases
  - Record concomitant medication.
  - Record any adverse events.
- Randomization at Visit 1.
- Lumbar puncture to obtain CSF, only at V1.
- Treatment:
  - Administer the study treatment
  - Assess vaccine reaction, local and systemic.
  - 2 hour after vaccination vital signs and a thorough examination of the patient to assess any reaction to the vaccine will be carried out and any adverse events (AEs) recorded.
- Neuropsychological Battery (V1; only ADS-ADL MCI and TMT)
- Quality of Life EQ-5D-5L (V1)
- Laboratory tests (hemogram, biochemistry and biological), and ApoE genotyping (only at V1).
- COVID-19 PCR test result (only at V16)

Data to be recorded in the eCRF (and corresponding source documents) at the safety phone interview (V2, V5, V8, V11, V14 and V19):

- Medical History:
  - Local or systemic reactions to the vaccine
  - Concomitant Medication
  - Adverse events

Data to be recorded in the eCRF (and corresponding source documents) at the safety face-to-face visits [V3, V6, V9, V12, V15, V16 (Int-6m), V17, V20 and V21]:

- Medical History:
  - Local or systemic reactions to the vaccine
  - Adverse Events, Assess vaccine reaction
  - Concomitant Medication information update
- Medical Evaluation
  - Physical and neurological examination
  - Vital signs (blood pressure, heart rate, respiratory rate, body temperature).
  - Corporal biometry (weight)

- Decision on subject's continuity
- Laboratory tests (hemogram, biochemistry and biological)
- Urine test strip
- MRI (V9)

In V16 (Int-6m), the following data will be additionally recorded in the eCRF (and corresponding source documents):

- Electrocardiogram
- Overall assessment carried out by the investigator
- Neuropsychological Battery and IGE
- MRI

In V21, it will be additionally recorded in the eCRF (and corresponding source documents):

- Electrocardiogram
- Overall assessment carried out by the investigator
- Neuropsychological Battery and IGE
- QoL EQ-5D-5L
- MRI
- a-PET (only after MRI results)
- Lumbar puncture to obtain CSF.

4.9.1.3 Follow-up Period: Visits V22 - V25 (weeks 51 to 104)

This period consist in two phone interviews (V22 and V24), two face-to-face visits [V23 and V25 (Final visit)]. Data to be recorded in the eCRF (and corresponding source documents) at these visits are the same than those of the phone interview visits and face-to-face visits, respectively described above, in addition to Neuropsychological Battery and EKG (V23 and V25) and IGE and QoL (V25 only). V25 also includes MRI, a-PET (only after MRI results) and lumbar puncture to obtain CSF.

4.9.2 Part B

Before any assessment or intervention for the study is completed, the investigator will inform the patient and caregiver, about the Part B of the trial. Both will receive a written detailed description of all activities and requirements involved of the Part B (Patient's Information and caregiver Leaflet), and the investigator will ensure that they completely understand the clinical trial.

Once understood and accepted, they will proceed to sign the informed consent. This will take place between Last Visit of Part A and First Visit of Part B (V1B).

The V1B should take place when the results of the last MRI test performed (either at V21 or V25 or within 6 months (±15 days) of V1B) are available and support the vaccination.

The Part B consist in two different types of visits: vaccination visits (V1B, V4B, V7B, V10B, V13B and V18B) and safety visits (phone interview: V2B, V5B, V8B, V11B, V14B, V19B and face-to-face visits: V3B, V6B, V9B, V12B, V15B, V16B, V17B, V20B, V21B and V22B).

Data to be recorded in the eCRF (and corresponding source documents), at the Vaccination Visits (V1B, V4B, V7B, V10B, V13B and V18B):

- Medical Evaluation
  - Physical and neurological examination (except at V1B)
  - Vital signs (blood pressure, heart rate, respiratory rate, body temperature) before and after the Vaccination
  - Corporal biometry (weight)
- Medical History:
  - Record relevant past and concomitant diseases
  - Record concomitant medication.
  - Record any adverse events.
- Treatment:
  - Administer the study treatment
  - Assess vaccine reaction, local and systemic.
  - 2 hour after vaccination vital signs and a thorough examination of the patient to assess any reaction to the vaccine will be carried out and any adverse events (AEs) recorded.
- Laboratory tests (hemogram, biochemistry and biological; at V1B: only biological)
- COVID-19 related information

Data to be recorded in the eCRF (and corresponding source documents) at the safety phone interview (V2B, V5B, V8B, V11B, V14B and V19B):

- Medical History:
  - Local or systemic reactions to the vaccine
  - Concomitant Medication
  - Adverse events

Data to be recorded in the eCRF (and corresponding source documents) at the safety face-to-face visits [V3B, V6B, V9B, V12B, V15B, V16B, V17B, V20B, V21B and V22B]:

- Medical History:
  - Local or systemic reactions to the vaccine
  - Adverse Events, Assess vaccine reaction
  - Concomitant Medication information update
- Medical Evaluation
  - Physical and neurological examination
  - Vital signs (blood pressure, heart rate, respiratory rate, body temperature).
  - Corporal biometry (weight)
  - Decision on subject's continuity (except in V22B)
- Laboratory tests (hemogram, biochemistry and biological)
- Urine test strip
- MRI (V9B, V16B, V17B, V21B and V22B only)

In V16B, the following data will be additionally recorded in the eCRF (and corresponding source documents):

- Electrocardiogram
- Overall assessment carried out by the investigator
- Neuropsychological Battery and IGE

In V21B, it will be additionally recorded in the eCRF (and corresponding source documents):

- Electrocardiogram
- Overall assessment carried out by the investigator
- Neuropsychological Battery and IGE
- QoL EQ-5D-5L
- a-PET
- Lumbar puncture to obtain CSF

Finally, in V22B, it will be additionally recorded in the eCRF (and corresponding source documents):

- Electrocardiogram
- Overall assessment carried out by the investigator
- Neuropsychological Battery and IGE

4.9.3 Premature discontinuation visit (PDV)

In the event that a patient prematurely discontinues completely the study for any reason, a final assessment of the clinical trial (premature discontinuation visit) must be carried out as soon as possible after discontinuing the study, indicating the reason for discontinuation.
All patient documents should be as complete as possible.

For patients who prematurely discontinue the clinical trial the same assessments as the 50-weeks visit (V21, see 4.9.1) should be performed. However, if a randomized patient withdraws the study before receiving the first IMP administration the following assessments will not be needed: electrocardiogram; urine analysis (test strip); hemogram, biochemistry, biological activity, MRI, a-PET, CSF.

If a patient discontinues early from the study treatment, he/she should continue with the remaining safety assessment visits (without any more treatment administration nor related phone interviews) (see 5.3.4 for more details).

4.9.4 Information collected on selection failures

Subjects who have signed informed consent but were not randomized for any reason shall be recorded in the selection record and complete V0 in the eCRF. No further information will be collected on these subjects.

5 SELECTION AND WITHDRAWAL OF SUBJECTS

Study Population:

The study population will consist of a representative group of patients of both sexes with a-MCI or vm-AD, who meet all the selection criteria and give their informed consent to collaborate in the study, after being informed. The a-MCI will meet the clinical criteria defined by the National Institute on Aging–Alzheimer's Association. The vm-AD will meet the criteria for probable AD defined by National Institute of Neurological and Communicative Diseases and Stroke/Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA).

The patients' recruitment will be competitive until the required number of patients is completed. Approximately 22 international centres will be involved: Spain (17 sites), France (3 sites), Sweden (1 site) and Italy (1 site).

5.1 Inclusion Criteria

A subject must meet all the following inclusion criteria:

1. Male or female between 55 and 80 years of age, both inclusive, at the time of signing informed consent.
2. The patient (or legal representative, if applicable) and a close relative/caregiver must read the subject information sheet, agree to participate in the clinical trial and sign the informed consent form (the patient and a close relative/caregiver).
3. Presence of a stable caregiver to attend the patient study visits.
4. Mini-Mental Status Examination (MMSE) score between 24 and 30 points (inclusive), according to age and education level.
5. Clinical Dementia Rating (CDR) scale scoring 0.5.
6. Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) Score on the Delayed Memory Index (DMI) of 85 or lower.
7. The results of the patient's MRI brain scan must be concordant with the diagnosis of clinical a-MCI or vm-AD according to the following criteria: Scheltens scale, and measurement of white matter and past haemorrhages.
8. If the patient is receiving treatment for AD, must have been stable during the two months before the selection visit.
9. Treatment for concomitant diseases must be stable during the previous month before the treatment of the study.
10. Positive assessment of the candidate by the investigator for complying with the requirements and procedures of the study.

5.2 Exclusion Criteria:

A subject meeting any of the following exclusion criteria is NOT eligible for participation in the study.

1. Known allergy to components of the vaccine or prior history of anaphylaxis, a severe allergic reaction or a history of hypersensitivity to any component of the formulation. Allergy to fish or shellfish.
2. Active infectious disease (i.e. hepatitis B, C). Positive syphilis serology.
3. History or presence of autoimmune disease, except mild eczema, rhinitis or psoriasis.
4. Presence or history of immunodeficiency (i.e. HIV).
5. Significant kidney and/or liver disease, as defined by plasma creatinine ≥ 2.5 mg/dL (221 μmol/l) and/or total bilirubin > 3 mg/dL (51.3 μmol/l) measured at the local site laboratory.
6. History of asthma or reactive airway disease with bronchospasm in the last 6 months, or currently on regular treatment.
7. Major uncontrolled systemic condition (e.g. diabetes, congestive heart failure, hypertension).
8. History of cancer (≤5 years since the last specific treatment). Exceptions: basocellular carcinoma.
9. Significant alterations in hematological, biochemical or urine analytical parameters, particularly those relating to levels of vitamin B12, folic acid or thyroid tests.
10. History of any other central nervous system disorder, degenerative or non-degenerative neurological or psychiatric condition that, in the investigator's opinion could be the cause of the dementia, or could explain the cognitive impairment, or that might interfere with cognitive function directly or by its treatment.
11. Geriatric Depression Scale (GDS; abbreviated version), score >5.
12. Has a “yes” answer to C-SSRS suicidal ideation items 4 or 5, or any suicidal behavior within 6 months before Screening, or has been hospitalized or treated for suicidal behavior in the past 5 years before Screening.
13. History or signs of cerebrovascular disease (ischemic or haemorrhagic stroke, transient ischemic attack), or diagnosis of possible, probable or clear vascular dementia according to NINDS-AIREN criteria.
14. Presence on MRI of a relevant pattern of microvascular disease (Leukoaraiosis, Fazekas score ≥2 in the deep white matter scale or ≥4 in the global score) or more than one lacunar or territorial infarcts. Any other MRI finding that, in the opinion of the investigator, might be a relevant contributing cause of subject´s cognitive impairment. Presence of up to 3 microhemorrhages will be acceptable.
15. History of bleeding disorder or predisposing conditions, blood clotting or clinically significant abnormal results on coagulation profile at Screening, as determined by the Investigator.
16. Patients being treated with anticoagulants or antiaggregant therapy (aspirin at a prophylactic dose ≤ 325 mg daily or clopidogrel at a dose ≤75 mg daily are allowed) should not be recruited in the study.
17. Modified Hachinski Ischemic Scale, score higher than 4.

18. Surgery (with general anaesthetic) within the previous three months to be included in the trial, or programed during the study period.

19. Treatment within 30 days prior to visit 0 with systemic corticosteroids or other immunosuppressant's.

20. Vaccination against influenza or any other vaccination within 2 months before first IMP dose.

21. Patients, who have previously been randomized in this trial.

22. Participation in another clinical trial within the previous 1 month to screening visit, or within the previous 12 months after the last dose to the screening visit in the case of subjects who participated in trials with a study drug whose intention was to modify the progression AD unless documentation of receipt of placebo is available. The patient cannot be included in the study if the experimental drug was an immunotherapeutic drug, including IVIG or a vaccine against Alzheimer's disease unless documentation of receipt of placebo is available.

23. Patients with alcohol or drug abuse or dependence.

24. Absolute (having a pacemaker or implantable defibrillator) or relative (bare metal stent or stent implanted in the last six months) contraindications to MRI examination. Feeling of claustrophobic do not let perform MRI or PET scan.

25. Patients unlikely to comply with the protocol (e.g., unable to return for follow-up visits)

26. Women of childbearing potential, pregnant or nursing.

Note: All women will be considered to be of childbearing potential unless they are postmenopausal (amenorrheic for at least 12 consecutive months, in the appropriate age group, and without other known or suspected cause) or have been sterilized surgically (i.e. bilateral tubal ligation, total hysterectomy, or bilateral oophorectomy, all with surgery at least 1 month before dosing)

27. Significant alterations in the EKG that are associated with an added risk for the patient

5.3 Subject Withdrawal Criteria

5.3.1 Screen Failures

Screening evaluations, those performed at pre-selection or screening visit (V0) will be used to determine the eligibility of each subject for enrollment. Subjects who fail to meet eligibility criteria during screening evaluations will be considered screen failures and will not participate in the study.

5.3.2 Removal of Subjects

Subjects may withdraw or be withdrawn from the study for the following reasons:

1. At their own request or at the request of their legally acceptable representative.
2. If, in the site's principal investigator's opinion, after consulting to the Medical Advisor, continuation in the study would be detrimental to the subject's well-being.
3. At the specific request of the sponsor if, in the Medical Advisor's opinion, continuation in the study could be detrimental to the subject´s well-being or in case that the subject have incurred in a major violation of the protocol.

4. In case of AEs related to the IMP, particularly allergic reactions or micro- or macro-hemorrhages detected by MRI in the safety control visits (see sections 8.2.6 and 8.3.10).

Subjects who withdraw after being randomized will remain in the study database and their data until the date of discontinuation will be analyzed.

In all cases, the reason for withdrawal must be recorded in the eCRF and in the subject's records.

5.3.3 Subject Replacement

It is expected that 120 patients (60 in verum and 60 in placebo) with a-MCI or vm-AD will be included in the clinical trial as per protocol, from whom ~ 40% will prematurely withdraw. However, since all enrolled patients who receive any amount of study drug will be valid for the primary safety analysis and the number of patients expected to complete the clinical trial taking into account a 40% of premature discontinuations (approximately 70) will have more than 85% power to evaluate the primary efficacy endpoint, there will be no need to replace withdrawn patients who have received at least one dose of the IMP or placebo, as correspond. Therefore, patients who have been randomized and did not received any dose of IMP or placebo will be replaced.

5.3.4 Follow-up of Subjects Withdrawn from Study Treatment

Subjects who receive any amount of investigational product and discontinue early from the study treatment will be requested to continue with the remaining safety assessment visits (without any more treatment administration nor related phone interviews). The following visits are those considered minimum “safety assessment visits” to be performed in subjects withdrawn from study treatment (as applicable according to the time of withdrawal):

- Safety control visits
  - Face-to-face visits: V3, V6, V9, V12, V15, V16 (Int-6m), V17, V20, V21, V23 and V25 (Final visit) [Part A]; V3B, V6B, V9B, V12B, V15B, V16B, V17B, V20B, V21B and V22B [Part B]

There is no need to perform any additional Vaccination Visit nor phone interviews.

Withdrawn subjects should receive at least a premature discontinuation visit within the 15 days after their last treatment’s administration (PDV; see section 4.9.3). If a randomized subject withdraws the study before receiving the first IMP administration, the following assessments of the PDV will not be needed: electrocardiogram; urine analysis (test strip); hemogram, biochemistry, biological activity, MRI, a-PET, CSF.

In accordance with good clinical practice (GCP), patients that discontinue the trial prematurely due to adverse events will be followed-up until resolution of the AE, properly documenting both the cause of premature withdrawal as well as management of data collected and the subsequent treatment received by the patients.

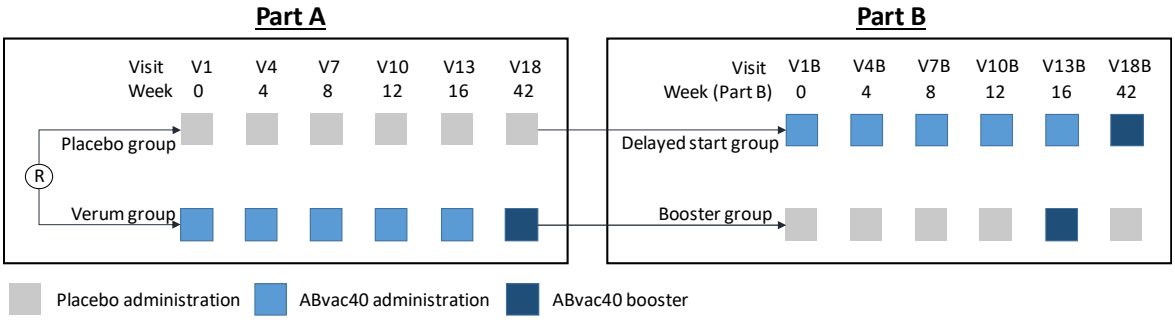
6 TREATMENT OF SUBJECTS

See Section 4.4 for the treatment(s) to be administered, including the name(s) of all the product(s), the dose(s), the dosing schedule(s), the route/mode(s) of administration.

6.1 Administration and Timing of Investigational Products for Each Subject

Treatment will consist of the administration of six 1 mL doses of verum (the IMP; ABvac40 vaccine) or placebo (comparator; the vaccine vehicle without the API) along a "treatment period" spanning for 42 weeks (10 months) for both Part A and Part B (see Figure 1). The first five doses will be administered at four-week intervals on visits V1, V4, V7, V10 and V13 [Part A] and V1B, V4B, V7B, V10B and V13B [Part B]. The sixth dose, a delayed booster shot, will be administered at V18 [Part A] or V18B [Part B], 26 weeks after the fifth dose.

Figure 1 Treatment administration schedule scheme



ABvac40 dose (or placebo) is dispensed in 1 mL vials, ready for direct injection. Each patient will be assigned a treatment kit (blister) containing 8 vials (verum or placebo as corresponds) including 2 spare vial. For Part B treatment kits will contain 6 vials of verum or placebo.

All treatments will take place at the trial center and will be administer by trained staff from each site.

Injections will be administered subcutaneously (sc) proceeding with special care to avoid intravenous administration, gently aspirating with the syringe before injecting. As a first option the lateral aspect of the arm will be used, about 8-10 cm above the elbow. As a prerequisite the regional lymph node group must be intact, if neither arm has intact lymphatic drainage, the injection will be administered to an anterior thigh, roughly half way between the groin and knee.

After each treatment the patient will remain under observation for 2 hours in the center. At the end of this time, vital signs will be assessed and a thorough examination carried out including inspection of the injection site to assess any reaction to the vaccine.

6.1.1 COVID-19 Testing

6.1.1.1 Part A

All patients who will attend their V18 after April 2020 must be tested for COVID-19 by PCR method before receiving the study medication (Urgent safety notification has been send to Competent Authorities).

The aforementioned patients must sign a specific ICF authorizing this test. In case they refuse, they will continue participating in the study but they will not receive the study treatment.

After authorization, the required samples will be taken by a trained study staff or delegate and sent to the central laboratory ([redacted]) where a standard PCR test for COVID-19 detection will be performed.

After 24-48h, the patient will be communicated with the results. In case the patient test negative for COVID-19, he or she will continue with the study procedures (including receiving the study treatment) normally.

In case the patient test positive for COVID-19, the PCR test will be repeated until the patient test negative and is asymptomatic. During this time, the patient will not receive the study treatment. Under any delay scenario, the subsequent visits will be re-scheduled accordingly.

6.1.1.2 Part B

All patients must be tested for COVID-19 during the Part B vaccination visits before receiving their study medication, unless the patient has already overcome the disease (it has to be documented) and remains asymptomatic.

At each vaccination visit, the patients will perform a rapid serological test at the study site to detect SARS-CoV-2 antibodies in serum.

In case the patient test positive and he or she is completely asymptomatic for COVID-19, he will continue with the study normally.

In case the patient test negative, a PCR test for COVID-19 detection will be performed. The required samples will be taken by a trained study staff or delegate and sent to the central laboratory ([redacted]) where a standard PCR test for COVID-19 detection will be performed.

After 24-48h, the patient will be communicated with the results. In case the patient test negative and remains completely asymptomatic for COVID-19, he or she will continue with the study procedures (including receiving the study treatment) normally.

In case the patient test positive for COVID-19, the PCR test will be repeated until the patient test negative and is asymptomatic. During this time, the patient will not receive the study treatment. The administration of the study treatment can be delayed for up to one month. In case it should be delayed beyond one month, the vaccination will be skipped. Under any delay scenario, the subsequent visits will be re-scheduled accordingly.

6.2 Prior and Concomitant Therapy

Patients are allowed to continue their usual treatment for Alzheimer's disease during the trial; it should remain stable throughout the study duration.

For subjects receiving at least one dose of the IMP, all relevant treatments (at the Investigator's opinion) administered during the 6 months prior to the screening visit for any indication and for Alzheimer's disease will be recorded in the eCRF, including the trade and generic names of the medication, the dose, the route of administration, and the duration of the medication (frequency). For subjects who do not receive at least one dose of the IMP (e.g. screening failure subjects), only the concomitant medication used to resolve SAEs reported during the screening period, will be collected in the eCRF.

6.2.1 Prohibited Medications Prior to Study Participation

As described in the Exclusion Criteria:

• Treatment within 30 days prior to visit 0 with systemic corticosteroids or other immunosuppressant's.

• Vaccination against influenza or any other vaccination, within 2 months before first IMP dose.

• Participation in another clinical trial within the previous 1 month to screening visit, or within the previous 12 months after the last dose to the screening visit in the case of subjects who participated in trials with a study drug whose intention was to modify the progression AD. The patient cannot be included in the study if the experimental drug was an immunotherapeutic drug, including IVIG or a vaccine against Alzheimer's disease.

• Patients with alcohol or drug abuse or dependence.

6.2.2 Prohibited Concomitant Medications during the Study

Administration of any of the following drugs interferes with the assessment and interpretation of clinical trial results and, therefore, any patient taking these drugs is not eligible for participation in the trial. These drugs are also prohibited during the whole study follow-up:

- Treatment with immunosuppressive drugs
- Treatment with experimental immunotherapeutics including IVIG or vaccines for Alzheimer's disease

6.2.3 Restricted Concomitant Medications during the Study

This section describes medications that are restricted but not prohibited during the study participation:

- Sedatives will not be permitted the night before the neuropsychological tests, particularly neuroleptics.

- Avoid administration of the influenza vaccine or any other vaccination during the 1 month prior and 15 days after each vaccination or until next Safety visit, whatever occurs earlier.

- If a patient with MCI needs to start a dementia treatment or a patient with AD needs modification of their dementia treatment before Int-6m visit (or V16B) or between Int-6m and V21visits (or between V16B and V21B), the investigator should discuss with him/her the benefit/risk balance in order to evaluate if the change can wait until after the Int-6m (or V16B) or V21 visit (or V21B), respectively. If the modification needs to be done before Int-6m (or V16B) visit or between Int-6m and V21 visit (or between V16B and V22B), respectively, the subject must be discontinued from the study treatment, but not from the study follow-up (see Section 5.3.4).

- Treatment with benzodiazepines and nootropics is authorized only at regular and stable doses in steady state (chronic use). Any dose change or treatment modification during the study will be reported both as an Adverse Event and as a change in the Concomitant medication section of the eCRF. If a patient needs to modify the dose before Int-6m (or V16B) visit or between Int-6m and V21 visits (or between V16B and V22B), the decision for the patient to continue with the study treatment will depend on clinical judgment and will be assessed on a case-by-case basis by the Medical Advisor.

6.3 Treatment Compliance

Treatment compliance is ensured because all treatments will take place at the trial center and will be administered by trained staff from each site.

Nevertheless, the reasons for any deviation from the administration of less than 100% of the investigational product dose as prepared must be recorded in the case report form (eCRF) and in the subject’s medical records.

7 ASSESSMENT OF EFFICACY

7.1 Efficacy Variables

7.1.1 Part A

7.1.1.1 Primary Efficacy Variable

- Maximal increment of specific anti-Aβ40 antibody signal (antibody titers estimated by ELISA [signal optical density (OD) values]) in each subject with regard to the pre-treatment visit (either V0 or V1)

7.1.1.2 Secondary Efficacy Variables

- Characterization of the immune response:
  - Levels of anti-KLH and anti-Aβ42 antibodies in plasma
  - Level of anti-Aβ40 antibodies in CSF
  - Levels of cytokines in plasma including IL-2, IL-4, IL-5, IL-6, IL-10, IL-13, IL-15, TNF-α and IFN-γ (Sandwich ELISA)
  - Analysis of the peripheral blood cell subsets by immunophenotyping
  - Levels of antibody-secreting cells and cytokine-secreting cells (including IFN-γ, TNF-α and IL-13)
- Assessment of disease biomarkers:
  - Levels of Aβ peptides in plasma
  - Levels of cortical fibrillary amyloid deposition; as standardized uptake value ratio (SUVR) in amyloid-PET scans.
  - Levels of CSF biomarkers (Aβ42, Tau, P-tau, neurofilament light and neurogranin) and other Aβ peptide species
  - Brain volumetric and atrophy of the hippocampus assessed by magnetic resonance imaging.
- Assessment of cognition, suicide risk and quality of life:
  - Mini Mental State Examination (MMSE)
  - Clinical Dementia Rating scale (CDR)
  - Repeatable Battery for the Assessment of Neuropsychological Status (RBANS)
  - Columbia Suicide Severity Rating Scale (C-SSRS).
  - ADCS-ADL MCI
  - Trail Making Test (TMT)
  - Investigator Global Evaluation (IGE)
  - Euroqol 5 Dimensions (EQ-5D)

7.1.2 Part B

7.1.2.1 Exploratory Efficacy Variables

- Maximal increment of specific anti-Aβ40 antibody signal (antibody titers estimated by ELISA (signal OD values) in each patient with regard to the pre-treatment visit (either V0/V1 [Booster group] or V1B [Delayed start group]).
- Characterization of the immune response:
  - Level of anti-Aβ40 antibodies in CSF
  - Levels of antibody secreting cells
- Assessment of disease biomarkers:
  - Levels of Aβ peptides in plasma
  - Cortical fibrillary amyloid deposition assessed by PET scans.
  - Levels of CSF biomarkers (Aβ42, Tau, P-tau, neurofilament light and neurogranin) and other Aβ peptide species
  - Brain volumetric and atrophy of the hippocampus using magnetic resonance imaging
- Assessment of cognition and quality of life:
  - MMSE
  - CDR scale
  - RBANS
  - ADCS-ADL MCI
  - C-SSRS
  - TMT
  - IGE
  - EQ-5D-5L

7.2 Methods and Timing for Assessing, Recording, and Analyzing Efficacy Parameters

7.2.1 Observations and Measurements

The following is a description of the procedures/assessments to take place at each study visit. See the Section 4.2 Study Design and Plan (Schedules of Study Procedures and Events in Table 3 and Table 5).

The primary efficacy variable is average maximal increment (MΔ) of specific anti-Aβ40 antibody signal (antibody titers estimated by ELISA [signal optical density (OD) values]) in each subject with regard to the pre-treatment visit (either V0 or V1). Additionally, the other efficacy assessments will be exploratory analyzed to enrich the ABvac40 proof of concept. In particular, the efficacy analysis will consist i) in the assessment and characterization of the immune response elicited by ABvac40, ii) assessment of the AD biomarkers and iii) assessment of the cognition and quality of life of the subjects along the study.

For a summary of the procedures/assessments to take place at each study visit see table 3 and table 5 in section 4.2.

7.2.1.1 Assessment and characterization of the immune response

For the assessment and characterization of the immune response blood samples will be collected at visits V0, V1, V3, V6, V9, V12, V15, V16, V17, V20, V21, V23 and V25 (Part A), and at visits V1B, V3B, V6B, V9B, V12B, V15B, V16B, V17B, V20B, V21B and V22B (Part B). All these analysis, together with the levels of Aβ peptides in plasma, will be centralized at the Araclon-Grifols.

The laboratory tests to be performed with these samples are listed below. (Detailed descriptions of the laboratory test procedures are located in the study Laboratory Manual document):

- Characterization of the antibodies generated by vaccination with ABvac40: it will proceed to the titration of antibodies generated against the immunogenic peptide (Aβx-40), other vaccine components (KLH) and Aβ-42 peptide. The specificity and isotype of these antibodies will also be characterized. These analyzes will be carried out in plasma samples by Enzyme-Linked ImmunoSorbent Assay (ELISA).
  For the Part A, patients in the verum group will be classified as "Positive Responders" when their maximal increment (MΔ) of anti-Aβ40 specific antibody signal (OD in ELISA) from the pre-treatment visit (either V0 or V1) is equal or greater than 3 times the standard deviation of the titration ELISA. The antibody titers (defined as the inverse of the maximal plasma sample dilution which showed an OD increase ≥ 3 SD with regard to the preimmune sample) of Positive Responder patients will be reported.
- Analysis of cytokines in plasma: quantitation of a panel of cytokines in order to characterize the type of immune response (Th1 or Th2) by sandwich ELISA [Part A].
- Analysis of antibody-secreting cells: quantitation of B-lymphocytes secreting antibodies against Aβ or other components of the vaccine. This analysis will be performed by Enzyme-Linked ImmunoSpot (ELISpot) assay.
- Analysis of cytokine-secreting cells: quantitation of cells secreting cytokines in order to characterize the potency and type of immune response (Th1 or Th2). This analysis will be performed by ELISpot assay [Part A].
- Immunophenotyping of circulating blood cells: Quantitation of the relative number of peripheral blood mononuclear cells (cluster of differentiations; CD) to monitor the effect of the vaccine at cellular level. This analysis will be performed by multicolor flow cytometry [Part A].

Analysis for antibody titration and determination of Aβ peptide and cytokine levels will be carried out at Int-6m (V16) and Final visit (V25/V23) [Part A] and at V22B (Part B), once the samples from all the subjects are available. Analysis of antibody-secreting cells [Part A and Part B], cytokine-secreting cells [Part A] and the immunophenotyping [Part A] will be performed subject by subject, immediately after each visit upon receiving the sample in the laboratory.

7.2.1.2 Assessment of the AD biomarkers

- Quantification of Aβ peptide species: levels of Aβ peptides in plasma, including Aβ40 Aβ42 and Aβ17 will be analyzed with ABtest sandwich ELISA and/or liquid chromatography-mass spectrometry (LC-MS).

- The level of cortical fibrillary amyloid deposition will be assessed at the pre-immune stage (V0), V21 and Final visit (V25) [Part A], and at V21B [Part B] by amyloid-PET scans, which will be assessed centrally by the a-PET Central Reader. The Aβ-PET standardized uptake value ratio (SUVR) is a surrogate marker of cortical Aβ pathology and will be assessed to explore the effect of ABvac40 on the cerebral Aβ load. An additional perfusion PET scan study will be performed within the first 5 min after tracer injection in order to adequately adjust the assessment of Aβ burden (SUVRs) for possible changes in patients brain perfusion caused by treatment or any other factor occurring along the study period.

  Detailed procedures for acquiring, recording and assessing Aβ-PET are described in the PET Site Operations Guide)

- The levels of CSF biomarkers (Aβ40, Aβ42, tau, P-tau, neurofilament light and total protein and albumin) together with levels of other Aβ peptide will be assessed at the pre-immune stage (V1), V21 and at the Final visit (V25) [Part A] and at V21B [Part B] by ELISA sandwich and/or LC-MS.

  Additionally, CSF levels of anti-Aβ40 antibodies will be determined by titration ELISA to deepen in the knowledge of ABVac40 mechanism of action.

  Detailed procedures for acquiring, recording and assessing CSF biomarkers are described in the Laboratory Manual document).

- Brain volumetric and atrophy will be assessed at visits V0, V16, V21 and V25 by MRI as an exploratory indication of ABvac40 efficacy. An additional MRI will be performed before first vaccination of Part B only if the previous MRI was performed more than 6 months (±15 days) before V1B to identify any safety findings. During Part B, they will be assessed at V16B, V21B and V22B by MRI. Additional MRI will be performed at V9 and V17 to control for safety (V9B and V17B in Part B) (see section 8.2.6).
  Detailed procedures for acquiring, recording and assessing MRI are described in the Neuroimaging Management document).

7.2.1.3 Assessment of cognition and quality of life

Assessment of cognition and quality of life will be carried out at visits V0, V1, V16, V21 during the active phase of the study and at V23 and V25 during the follow-up. The tests to be used are summarized in the following table.

Table 7 Neuropsychological tests:

Neuropsychological tests						
Neuropsychological Battery	***V0 Screening Week -8***	***V1 Baseline Week 0***	***V16 Int-6m Week 24***	***V21 Int-12m Week 52***	***V23 Week 77***	***V25 Final visit Week 104***
MMSE (24-30 at inclusion)	X	-	X	X	X	X
CDR (global score of 0.5 at inclusion)	X	-	X	X	X	X
RBANS (score ≤ 85 at inclusion)	X	-	X	X	X	X
COLUMBIA-SSRS (no answer "YES" in item 4 or 5 at inclusion)	X	-	X	X	X	X
ADCS-ADL MCI	-	X	X	X	X	X
TMT-B	-	X	X	X	X	X
GDS abbreviated (score≤ 85 at inclusion)	X	-	-	-	-	-
IGE	-	X	X	X	-	X
Quality of Life Tests						
EQ-5D-5L	-	X	-	X	-	X

Detailed procedures for acquiring, recording and assessing cognition and quality of life are described in the Assessment Management document.

The answers of the patients to these tests will be registered in paper/electronic form in the study records; In addition and only for CDR and RBANS and as long as the patient specifically consent to it, the answers will be also registered through audio recording to ensure that if necessary a diagnosis verification could be carried out posteriorly. These audio recording will be destroyed at the end of the study.

At V16B, V21B and V22B visits of Part B, the complete Neuropsychological battery will be performed. In addition, the test for quality of life will be performed in V21B.

7.2.2 Description of Laboratory Tests and Procedures

Detailed procedures for acquiring, recording and assessing laboratory tests are described in the Laboratory Manual document. The following table summarizes the name, description and location of Laboratory tests and procedures for blood, CSF and urine analysis (see Table 8).

Table 8. Name, description and location of Laboratory tests

Name, Description, and Location of Laboratory Tests and Procedures			
		Description	**Location**
Blood Analysis	Hematology	Hematocrit, hemoglobin, erythrocytes, VCM, MCHC, leukocytes, neutrophils, lymphocytes, monocytes. eosinophils, basophils, thrombocytes	[redacted]
	Biochemistry	**Sustrates:** Glucose, Total cholesterol, triglycerides, creatinine, Total bilirubin, Total proteins, Albumin, Uric acid, BUN, C-reactive protein, apolipoprotein E, Homoscysteine, Ferritin **Enzymes:** SGOT (AST), SGPT (ALT), Alkaline phosphatase, GGT, LDH, CPK **Electroliters:** Sodium, Potassium, Calcium, Chloride, inorganic phosphorus	[redacted]
	Coagulation	APTT, fibrinogen, INR (prothrombin time)	[redacted]
	Vitamin	B12 vitamin, Folic acid	[redacted]
	Hormones	Free T4, TSH	[redacted]
	Others	HbA1c	[redacted]
	Serology	Syphilis (VDRL, TPHA), HIV, Hepatitis B, Hepatitis C	[redacted]
	Reumatology Factors	RF, ANA	[redacted]
	ANTIBODIES : Visits V0 and V21	Anti-nuclear antibodies (ANA), native anti-DNA antibodies, anti-thyroglobulin antibodies, Anti-TPO antibodies	[redacted]
	Additional Special Tests, Visit V1	ApoE gen	Araclon Biotech
	Biological Activity: Antibody Titration, Measure of ABetas, Inflammation Factors	**Concentration of specific antibodies to Aβ40, KLH and Aβ42 peptides** in plasma **Levels of antibody-secreting cells and cytokine-secreting cells** (including IFN-γ, TNF-α and IL-13). **Levels of Aβ peptides in plasma.** **Levels of citoquines in plasma:** IL-2, IL-4, IL-5, IL-6, IL-10, IL-13, IL-15, TNF-α and IFN-γ. **Number of peripheral blood mononuclear cell**s (cluster of differentiations; CD) including CD3, CD4, CD8, CD19, CD25, CD27 and CD69.	Araclon Biotech
Urinalysis	Microscopic evaluation is done only with cause.	Analyzes will be performed with test strips at the trial center. If clinically relevant abnormalities are detected, a urine sample will be sent to the laboratory to analyze sediment.	[redacted]
CSF Analysis		Cells Total Proteins Glucose Levels of Aβ1-40, Aβ1-42; Total tau and Phosphorylated tau	Special Lab

If sample collected for laboratory analyses are non-analyzable or non-reportable due to various factors, they will need to be recollected by contacting the subject for unscheduled visit(s) so that additional samples (blood, urine) can be collected from subjects and assayed for critical analytes at any study time point. In this case, the data from the last sample analyzed at the corresponding study time point will be recorded in the eCRF.

Backup sample of blood will be collected at screening visit (V0). This sample will be used together with sample from baseline visit (V1) to assess pre-treatment levels of critical analytes.

Samples will be stored/retained in the Araclon-Grifols facilities for additional or repeated analysis until Araclon-Grifols deems that all analysis in support of the study have been completed. After his period, the samples will be destroyed. If an additional, not related with the initially programed analysis is intended subjects will be requested to extend their informed consent.

Biological samples will be managed according to local regulations of each participating country. Araclon-Grifols will comply with all pertinent legislation to maintain the confidentiality of all health-related information obtained in the study.

8 ASSESSMENT OF SAFETY

8.1 Safety Parameters

The primary safety variable is the rate (%) of Adverse Events (AEs). The trial will be considered satisfactory if the pattern of AEs (overall or grouped as neurological, psychiatric or cardiovascular) is consistent with a good safety and tolerability profile of ABvac40 for human administration at the final visit (V25/V22B).

Additionally the following secondary safety variables will be evaluated:

- withdrawal criteria (continuation decision)
- number of withdrawn patients due to adverse events (AEs); cause of withdrawal
- serious adverse events (SAEs)
- physical and neurological examination
- concomitant medication
- vital signs (blood pressure, heart rate, respiratory rate, body temperature), body mass index (weight, height).
- brain MRI
- electrocardiogram (EKG)
- analytical haematology, toxicology (reactive protein), biochemistry, coagulation, serology and urine test strip results.

8.2 Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters

The above mentioned safety parameters, except MRI and EKG, will be assessed at pre-screening visit, treatment visits, face to face safety visits, Int visit and final visit during Part A, and at treatment visits, face to face safety visits and final visit during Part B.

Brain MRI will be performed only at visits V0, V9, V16, V17, V21 and V25 [Part A] and at V1B (only in the absence of a MRI performed within 6 months (±15 days)), V9B, V16B, V17B, V21B and V22B [Part B]; EKG will be performed only at visits V0, V16, V21, V23 and V25 during Part A and at visits V16B, V21B and V22B during Part B, and will be assessed centrally by the Medical Advisor (except at V22B); and urine test strip will be performed at visits V0, V1, V3, V6, V9, V12, V15, V16, V17, V20, V21, V23 and V25 during Part A and at V3B, V6B, V9B, V12B, V15B, V16B, V17B, V20B, V21B and V22B during Part B.

All these parameters will be available in the eCRF. The parameters will be either entered in the eCRF (and corresponding source documents) by the site staff or electronic records from vendors will be uploaded to the subject's eCRF, as specified in the corresponding manuals and guidelines

8.2.1 Adverse Events

Adverse events, including adverse reactions (AR) occurring at any time between signing of the subject's ICF and the last day of the subject's participation in the clinical trial will be recorded on the appropriate subject's eCRF entry. The recording of adverse events will be elicited by spontaneous reporting by the study individual or by a non-leading inquiry or direct observation by the study staff. It is Investigator's responsibility to ensure that all AEs are appropriately recorded.

8.2.2 Clinical Laboratory Evaluations

Blood tests for hematology, toxicology (reactive protein), biochemistry and coagulation will be carried out at visits V0, V1, V3, V6, V9, V12, V15, V16, V17, V20, V21, V23 and V25 [Part A] and at visits V3B, V6B, V9B, V12B, V15B, V16B, V17B, V20B, V21B and V22B [Part B] or at the time of an early discontinuation (PDV) (see Table 3 and Table 5). Serology, analysis of rheumatology factors and anti-nuclear antibodies will be carried at V0 and V25. Urine test strip will be performed at visits V0, V1, V3, V6, V9, V12, V15, V16, V17, V20, V21, V23 and V25 [Part A] and at visits V3B, V6B, V9B, V12B, V15B, V16B, V17B, V20B,V21B and V22B [Part B] or at the time of an early discontinuation (PDV). Additionally, at baseline visit (V1) the ApoE genotyping. All these clinical laboratory analysis will be centralized at [redacted] and at Araclon-Grifols. For detailed information on clinical laboratory tests see the Laboratory Manual.

Analytical and urine test strip results will be registered in the eCRF. Data will be either entered in the eCRF (and corresponding source documents) by site staff or uploaded from electronical records afterwards to the corresponding section of the subject's eCRF as detailed in laboratory manual and data management guidelines.

The investigator must review the results of laboratory analyses when received, and sign and date them. The Investigator will be required to classify laboratory results out of the normal range reported by the laboratory as clinically significant or not according to his/her criteria. Laboratory results out of the normal range judged by the Investigator as clinically significant will be flagged and recorded in the subject's eCRF as an AE.

All patients with clinically significant abnormal laboratory values should be regularly monitored until these values return to the normal range or until another valid reason other than an adverse event related to the drug is identified.

The results of these clinical laboratory analysis carried out at the screening visit (V0), will determine the inclusion of the patient into the clinical trial. The Medical Advisor's approval is needed in V0, V16 and V16B in order to accept the continuation of the patient in the study.

At Screening Visit (V0), V21, V25 and V21B the presence of several auto-antibodies will be determined within assessment of eligibility and safety.

8.2.3 Vital Signs and body mass index

Vital signs will be measured by a medically certified individual or a nurse according to his/her standard clinical practice. Vital signs will be measured at the following visits: (V0, V1, V3, V4, V6, V7, V9, V10, V12, V13, V15, V16, V17, V18, V20, V21, V23 and V25 [Part A], and V1B, V3B, V4B, V6B, V7B, V9B, V10B, V12B, V13B, V15B, V16B, V17B, V18B, V20B, V21B and V22B [Part B] and PDV, if applicable) The following vital signs will be assessed:

- Temperature
- Blood Pressure (systolic blood pressure [SBP]) and diastolic blood pressure [DBP]),
- Heart Rate
- Respiratory Rate

Body temperature will be measured orally or at the axilla. Measurements of systolic and diastolic blood pressure, heart rate and respiratory rate will be recorded after at least five minutes of lying at rest. The blood pressure measurements will always be taken from the same arm. If there is any suspicion that the measurement is unreliable, blood pressure will be measured again. The value obtained on this second occasion will be deemed final and shall be recorded in the relevant section of the eCRF.

To calculate the body mass index (BMI), height will only be measured during visit 1 (day 0). Body weight (patient in light clothing without shoes) will be recorded at all visits (and PDV, if applicable). The results will be used to calculate body mass index.

Vital signs will be routinely monitored. Results will be recorded in source documents and on the subject eCRF. The Investigator will be required to classify vital signs abnormalities as clinically significant or not according to his/her criteria. Vital signs abnormalities judged by the Investigator as clinically significant will be recorded in the subject's eCRF as AEs.

8.2.4 Physical and Neurological Examinations

A complete physical examination of patients will be carried out at all face to face visits (V0, V1, V3, V4, V6, V7, V9, V10, V12, V13, V15, V16, V17, V18, V20, V21, V23 and V25 [Part A] and V1B, V3B, V4B, V6B, V7B, V9B, V10B, V12B, V13B, V15B, V16B, V17B, V18B, V20B, V21B and V22B [Part B]; and PDV, if applicable). The general patient status will be assessed including examination of neck (including thyroid), eyes, ears, throat, abdomen, skin/mucous membranes, and cardiovascular, respiratory, genito-urinary and musculoskeletal systems.

At each visit, a conventional neurological examination will be carried out, consisting in a brief inspection of cortical functions, cranial nerves, motor function, reflexes, sensory function, gait and posture. A medically certified individual will conduct the physical and neurological examination at all of the face-to-face visits. Results will be recorded in the physical and neurological examination sections of the subject's eCRF.

After the baseline visit (V1), any new finding or worsening of a previous finding from physical and neurological examination will be recorded in the subject's eCRF as an AE.

8.2.5 EKG

An electrocardiogram (EKG) will be performed at visits V0, V16, V21, V23 and V25 during Part A and at visits V16B, V21B and V22B during Part B (and PDV, if applicable). All EKGs, will be assessed centrally by the Medical Advisor. Any new finding or worsening of a previous finding will be recorded in the subject's eCRF as an AE.

8.2.6 Brain MRI

The brain MRI in the AB1601 study is fundamentally a safety protocol designed to identify the occurrence of cerebral complications secondary to the administration of ABvac40. It will be performed at visits V0, V9, V16, V17, V21 and V25 during Part A and at visits V1B (only in the absence of a MRI performed within 6 months (±15 days)), V9B, V16B, V17B, V21B and V22B during Part B, and will be assessed centrally by the MRI Central Reader. Additionally, data from MRI at visits V0, V16, V21, V25, V16B, V21B and V22B will be used for exploratory efficacy analysis (see section 7.2.1). The MRI at the pre-selection visit (V0) will also be used to see if patients who want to enter the study meet the criteria for inclusion and none of the exclusion criteria.

Electronic MRI results will be uploaded to the subject's eCRF. Detailed procedures for acquiring, recording and assessing MRI results are described in the Neuroimaging Management document.

Any new finding or worsening of a previous finding will be recorded in the subject's eCRF as an AE. Particular attention will be paid to the so called Amyloid Imaging Related Abnormalities (ARIA). The spectrum of ARIA includes signal hyperintensities on fluid attenuation inversion recovery sequences thought to represent "vasogenic edema" and/or sulcal effusion (ARIA-E), as well as signal hypointensities on GRE/T2* thought to represent hemosiderin deposits (ARIA-H), including microhemorrhage and superficial siderosis.

A decision tree is set in place for the management of these SAE in case of occurrence (see section 8.3.10).

8.2.7 Patient's Diary and Patient emergency card

Study subjects will be provided with a "Patient Diary" (PD). This will be used to record body temperature, local reactions to the treatment or any other AE. Furthermore, the subject will be required to record in the PD any concomitant medication including changes in the conventional treatment of AD.

The patient/caregiver will be told to come to subsequent visits with the patient diary completed. The researcher will transcribe information recorded by the patient/caregiver in the relevant pages of the eCRF. The patient's original diary will be kept at the trial center along with the original documents.

Upon inclusion in the study, each patient and their caregiver will receive a "patient card" stating that they are participating in a clinical trial, identified by its code, title, and name of the study drug. The name, address and emergency telephone number (available 24 hours) of the responsible investigator will be printed on the card along with a request to contact the investigator in case of emergency. If a patient suffers a serious adverse event and needs to go to hospital, they should go to the nearest hospital possible; the patient and caregiver will be advised to carry the "patient card" with them at all times during the study, as a safety measure.

8.3 Procedures for Eliciting Reports of and for Recording and Reporting Adverse Event and Intercurrent Illnesses

8.3.1 Warnings/Precautions

For complete information on ABvac40 refer to the IB document.

8.3.2 Adverse Event Monitoring

Subjects must be carefully monitored for AEs. This monitoring includes clinical and laboratory tests and physical signs. Adverse events should be assessed in terms of their seriousness, severity, and causal relationship to the IP.

Adverse events will be elicited by spontaneous reporting by the study individual or by a non-leading inquiry or direct observation by the study staff.

8.3.3 Adverse Event Definitions

8.3.3.1 Adverse Events

An AE is defined as any untoward medical occurrence in a subject or clinical investigation subject administered a medicinal product or study treatment and which does not necessarily have a causal relationship with this administration. An AE can therefore be any unfavorable and unintended sign (including any abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

The frequency of adverse events should be investigated using indirect questions at each study visit (e.g., How are you today? How do you feel?). Adverse events can also be detected when the patient directly and spontaneously reports them during a visit or between visits or by physical examination, blood test and other assessments. Abnormal laboratory parameters are only considered as an adverse event if the researcher classifies them as "clinically significant".

Alzheimer’s Disease clinical signs or symptoms and pathological laboratory parameters are not considered adverse events if already present in the first assessment, except when deterioration or increase in frequency is observed.

All adverse events will be documented in a specific form for adverse events. The documentation includes the following information:

- Duration (start and end date),
- Intensity (mild, moderate, severe),
- Possible causal relationship with study treatment (related or not),
- Action taken (no measure taken, adjust dose/temporarily stop study drug, permanently discontinue study drug, administer concomitant medication, provided a nonpharmacological therapy, brief or prolonged hospitalization)
- Assessment of outcome (full recovery, improvement, unchanged, deterioration),
- Meet any of the severity criteria.

8.3.3.2 Suspected Adverse Drug Reactions/Adverse Reactions

A Suspected Adverse Drug Reactions (ADR) includes any noxious and unintended reaction to an investigational medicinal product, regardless of dose administered. Unlike AEs, in the case of an adverse reaction a causal relationship between a medicinal product or study treatment and an AE is at least a reasonable possibility.

8.3.4 Assessment of Causality of Adverse Event

The Investigator is required to provide a causality assessment for each AE reported to the Sponsor. The Sponsor will consider the Investigator's causality assessment and also provide its own assessment.

Causal relationship to the study drug will be established according to medical judgment on whether there is a **reasonable possibility of a causal relationship between the AE and the IMP administration**:

The Investigator must determine and classify the AE causality according to the following categories:

- **Not related:** There is not a reasonable possibility of causal relationship between the AE and the study drug.
- **Related:** There is evidence to suggest a causal relationship between the study drug and the AE.

Criteria to assess the causal relationship should take into account of the following conditions: 1) a plausible temporal sequence from the study drug administration to the AE onset; 2) whether the event follows a known response pattern to the suspected treatment; 3) whether the AE could be reasonably explained by the subject's clinical state, comorbidities, or concomitant medications, as well as 4) the occurrence of improvement on stopping/reducing the treatment (positive dechallenge) and/or reappearance of the event on repeated exposure (positive rechallenge).

8.3.5 Severity of Adverse Event or Suspected Adverse Drug Reaction

AEs and suspected ADRs will be classified depending on their severity according to the following definitions:

Mild: an AE which is well tolerated by the subject, causing minimum degree of malaise and without affecting normal activities.

Moderate: an AE that interferes with the subject's normal activities.

Severe: an AE that prevents the subject from performing their normal activities.

AE and suspected ADR severity gradation must be distinguished from AE and suspected ADR seriousness gradation, which is defined according to event consequence. For example, headache can be mild, moderate or severe but not necessarily serious in all these cases.

The Investigator will be responsible for assessing the AE and suspected ADR intensity during the clinical trial, taking into account current criteria included in this section.

8.3.6 Expectedness of Adverse Event or Suspected Adverse Drug Reaction

An AE or suspected ADR is considered "unexpected" if the nature, seriousness, severity or outcome of the reaction(s) is not consistent with the reference information. The expectedness shall be determined by the Sponsor according to the reference document for any serious ADRs (potentially related SAEs) for expedited safety reporting purposes.

8.3.7 Seriousness of Adverse Event or Suspected Adverse Drug Reaction

An AE or suspected ADR is considered "serious" if, in the view of either the Investigator or Sponsor, it results in any of the following outcomes:

- Death
- Life-threatening AE (life-threatening in the definition of "serious" refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe)
- In-patient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect
- An important medical event (important medical event in the definition of "serious" refers to those events which may not be immediately life-threatening, or result in death, or hospitalization, but from medical and scientific judgment may jeopardize the subject or/and may require medical or surgical intervention to prevent one of the other outcomes listed above).

*Hospitalization is to be considered only hospital stay for equal or more than 24 hours. The following hospitalizations should not be reported as SAEs:

- Hospitalization or prolongation of hospitalization needed for procedures required by the clinical trial protocol.

- Hospitalization or prolongation of hospitalization as part of a routine procedure followed by the center.
- Hospitalization for a survey visit, annual physicals, or social reasons.
- Elective or pre-planned hospitalizations for a pre-existing condition that had not worsened from Baseline (e.g. elective or scheduled surgery arranged prior to start of the study).
- Admissions not associated with an AE (e.g. social hospitalization for purposes of respite care).

This definition permits either the Sponsor or the Investigator to decide whether an event is "serious". If either the Sponsor or the Investigator believes that the event is serious, the event must be considered "serious" and evaluated by the Sponsor for expedited reporting.

8.3.8 Adverse Event Documentation

For subjects receiving at least one dose of the IMP, all AEs and SAEs occurring after the subject has **signed the ICF through the Final Visit (i.e., end of study)** must be fully recorded in the subject's eCRF, and SAE form (if serious) as well as in the medical record. For subjects who do not receive at least one dose of the IMP (e.g. screening failure subjects), all SAEs occurring after the subject has **signed the ICF through the until subject withdrawal** must be fully recorded in the subject's eCRF, and SAE form as well as in the medical record. If no AE has occurred during the study period, this should also be indicated in the eCRF.

It is the responsibility of the Investigator to ensure that AEs are appropriately recorded.

At each visit, AEs will be elicited by asking the individual a non-leading question such as "Do you feel different in any way since the last visit?" Moreover, AEs will also be collected through directly observed events or spontaneously volunteered by the subject. Clearly related signs, symptoms and abnormal diagnostic procedures should preferably be grouped together and recorded as a single diagnosis or syndrome wherever possible.

The following variables must be recorded in the AE eCRF:

- The verbatim term (a diagnosis is preferred)
- Date/time of onset
- Date/time of resolution
- Severity (mild, moderate, severe)
- Causality (unrelated, possibly related, definitely related)*
- Seriousness (yes, no)
- Action taken (with regard to IMP)
- Other action (to treat the event)
- Outcome and sequel (follow-up on AE)

*Causality assessment will be made only when the AE occurs after the subject has received at least one dose of the IMP. An AE occurring before subject's exposure to IMP will be always labeled as "unrelated".

In addition to the Investigator's own description of the AEs, each AE will be encoded according to the Medical Dictionary for Regulatory Activities (MedDRA).

For example, a laboratory test abnormality considered clinically significant, eg, causing the subject to withdraw from the study, requiring treatment or causing apparent clinical manifestations, or judged clinically significant in the context of the subject's medical history by the Investigator, should be reported as an AE. Each event must be described in detail along with start and stop dates, severity, relationship to IMP, action taken and outcome. Each event must be adequately supported by documentation as it appears in the subject's medical or case file.

8.3.9 Reporting of Serious Adverse Events

Any SAE that occurs after **signing the study ICF through the Final Visit (ie, end of study)** must be expeditiously reported whether or not considered attributable to the study drug. Each SAE must be fully recorded in the subject's eCRF and SAE Report Form. In addition, any SAE that occurs greater than *14 months* after the last dose of the investigational product should be reported if the Investigator feels that the event is related to the use of investigational product.

SAEs will be reported using the designated SAE Report Form. When the Investigator becomes aware of a SAE, she/he must submit a completed, signed and dated SAE Report Form (in English) **within 24 hours** to the [redacted] by email/fax. The date of this SAE discovery by the site staff should be documented in the source documents (i.e. medical records).

Each SAE must be followed up until resolution or stabilization. After the initial report, all relevant information for SAE follow-up, and for the outcome, must also be supplied to the Sponsor in a timely manner (within 3 days from its identification or within 24 hours for relevant new information) by means of the SAE Report Form. In addition, the Sponsor or contract research organization (CRO) may request additional information and/or reports.

All SAE Report Forms must be reported to [redacted] by email or fax to:

[redacted]
[redacted]
Fax: [redacted]
e-mail: [redacted]

When required, and according to local law and regulations, SAEs must be reported to the IRB/EC and regulatory authorities.

8.3.10 Special safety procedures

8.3.10.1 Serious adverse events of special interest

Amyloid Imaging Related Abnormalities (either ARIA-E or ARIA-H) and Aseptic Meningo-Encephalo-Myelitis have occurred in clinical trial of anti-β-amyloid immunotherapies and are considered as Serious Adverse Events. They should be reported as previously described.

In addition, a specific Decision Tree is set in place for the management of these SAEs, in case of occurrence see below:

Process for positive ARIA (ARIA-H and ARIA-E) findings

The results of the central MRI safety read will be made available to the Site and Sponsor prior to the next scheduled dose. The dose cannot be administered until the MRI results are received and approval is received from the MRI Central Reader. In the event of a positive ARIA (ARIA-H or/and ARIA-E) finding, the MRI Safety Review Committee, composed by i) the site IP, ii) the MRI central reader and iii) the study Medical Advisor (who will have access to the MRI data and central radiology reports) will review the Central Radiology read together with all other safety data available to the Sponsor determine the appropriate course of action. If there is a positive ARIA finding, then the subject will be asked to attend a follow-up scans at 2, 4, 6 and 12 months after the event in order to consider the ARIA finding resolved or clearly decreased and stabilized (as per the central read). Those scans are classified as "unscheduled" scans.

ARIA-H reporting

Any new micro-hemorrhage (1 or more) found in an MRI is an ARIA-H and needs to be informed to [redacted] Drug Safety either as a new SAE or Follow up SAE (Section 8.3.9), taking account of the following:

- If the total number of new micro-hemorrhage is greater than 3 (at any time during the study) in relation to V0, patients will stop the study medication regardless of number of events presented at screening visit.

- If the total number of new micro-hemorrhage is less than 3 (at any time during the study) in relation to V0, patients will NOT stop the study medication regardless of number of events presented at screening visit.

In addition and in order to consider an ARIA-H as a new event or progression of previous one, we will take in consideration the following:

- Any new micro-hemorrhage (1 or more) showed in the follow up MRIs and/or Protocol MRIs in a subject with previous ARIA-H will be consider a progression of previous ARIA-H if the patient DID NOT TAKE the study medication in between of MRI examinations. In such cases, a Follow up SAE will be completed with all information

(Follow up SAE will be completed, regardless of new findings, after a Follow Up MRI in order to keep [redacted] Drug Safety informed on the progression of patients).

- Any new micro-hemorrhage (1 or more) showed in the follow up MRIs and/or Protocol MRIs will be consider a NEW ARIA-H if the patient took the study medication in between of MRI examinations and in such case, a new SAE Report Form will be completed with all information required.

ARIA Thresholds and ARIA Decision

We propose this to be based in the guidelines published by the Alzheimer Association Research Roundtable Working Group (Sperling et al. 2011) as follow.

1. Patients developing mild ARIA-E or mild ARIA-H (≤3 incident micro-haemorrhages) without clinical symptoms could continue in the study.

2. Patients developing moderate or severe ARIA-E (as assessed by the MRI central reader) without clinical symptoms, or those with ARIA-E accompanied by mild clinical symptoms, will suspend treatment and resume once ARIA (and symptoms, if any) resolved.

For these patients, if ARIA appears at the MRI after the third shot and resolve within two months, they resume for the remaining two shots and booster; if ARIA does not resolve within two months then, they resume at the booster shot (m10) if resolved; otherwise, they permanently discontinue treatment.

3. Patients who develop ARIA-E or ARIA-H (≤3 incident micro-haemorrhages) accompanied by moderate, severe, or serious clinical symptoms, >3 incident micro-haemorrhages, any incident macro-haemorrhage, or >1 incident haemosiderosis at any time during the study will permanently discontinue treatment.

8.3.10.2 Reactions to the vaccine

Reactions to the vaccine may vary from mild to severe reactions (shock or anaphylaxis) that can cause death. If such a severe reaction is suspected, patients will be observed for 24 hours in the study center after administration of the vaccine (overnight hospitalization) and before being discharged vital signs and a thorough examination will be recorded to assess any reaction to the vaccine (see Appendix 1).

8.3.11 Reference safety information

8.3.11.1 Expected Serious Adverse Drug Reactions

Expected serious Adverse Drug Reactions (ADR) to ABvac40 are Amyloid Imaging Related Abnormalities (ARIA) either ARIA-edema (ARIA-E) or ARIA-hemorrhagic (ARIA-H).

ARIA-E is the most frequent SAE occurring in clinical trial involving passive immunotherapies with monoclonal antibodies targeting Aβ peptides. Incidence of ARIAs appeared to depend, among other factors, on the drug product characteristics and dosing but can affect a relevant proportion of the participants (up to 50% in some cases). Although ARIAs have been much less frequently reported after active immunization of patients with vaccines against Aβ peptides (in particular, not cases of ARIA-E or ARIA-H occurred during ABvac40

phase I clinical trial), their occurrence in this phase II is deemed as expected and a specific protocol is set in place to managing this serious Adverse Drug Reaction.

All Expected serious ADRs must be submitted to expedited safety reporting using the designated SAE Report Form and the procedure described in the Clinical Trial Protocol (see section 8.3.9), and msut be accompanied with the corresponding MRI report available.

8.3.11.2 Unexpected Serious Adverse Drug Reactions

All other SAEs considered as potentially related with the investigational medicinal product (IMP), including Aseptic Meningo-Encephalo-Myelitis and systemic reactions to the vaccine (see appendix 1) should be considered as unexpected and submitted to expedited safety reporting using the designated SAE Report Form and the procedure described in the Clinical Trial Protocol (see section 8.3.9).

8.4 Type and Duration of the Follow-Up of Subjects after Adverse Events

In so far as is possible, all individuals will be followed up until the AE or suspected ADR has been resolved. If an AE/suspected ADR/SAE is present when the subject has completed the study, the course of the event must be followed until the final outcome is known, or the event has been stabilized and no further change is expected and the Investigator decides that no further follow-up is necessary.

9 STATISTICS

9.1 Statistical Methods

Unless otherwise specified, descriptive statistics will include the number of observations, number of missing values, mean (95% CI), standard deviation (SD), median, minimum, Q1, Q3 and maximum values for the continuous/quantitative data or absolute and relative frequency counts and percentages for categorical/qualitative data.

Individual patient data will be listed.

Data handling and evaluation procedures will be described in the Statistical Analysis Plan.

9.1.1 Demographic and Baseline Characteristics

Demographic and baseline characteristics will be summarized by treatment in the overall group and according to a-PET status (a-PET+ and a-PET-). For quantitative variables the number of observations, number of missing values, mean (95% CI), SD, median, Q1/Q3 and minimum/maximum will be provided. For qualitative variables, the frequency and percentage will be provided. Demographic variables will be compared between treated and placebo groups as correspond (Mann-Whitney or Chi square).

9.1.2 Efficacy Analysis (Part A)

9.1.2.1 Primary Efficacy Analysis (immunogenicity)

The primary variable for immunogenicity is the average maximal increment (MΔ) of anti-Aβ40 antibody signal (OD in ELISA).

For the comparison of the response to treatment between the verum and placebo group the maximal increment (MΔ) of anti-Aβ40 antibody signal (OD in ELISA) with regard to the pre-treatment visit (either V0 or V1) in each subject will be used as a summary measure. The treatment comparisons will be carried out by one-sided t test or Mann–Whitney U test if variable has non-normal distribution.

The trial will be considered successfully confirmatory regarding efficacy (immunogenicity) of ABvac40 if the average maximal increment (MΔ) of anti-Aβ40 antibody signal (OD in ELISA) in the verum group is >0 OD than the average MΔ in the placebo group. This primary efficacy analysis will be conducted in the overall study population.

- Null hypothesis: The average maximal increment (MΔ) of anti-Aβ40 antibody signal (OD in ELISA) for the treated group is equal to that for placebo.
- Alternative hypothesis: The average maximal increment (MΔ) of anti-Aβ40 antibody signal (OD in ELISA) for the treated group is >0 OD than the average MΔ in the placebo group.

The MΔ of anti-Aβ40 antibody signal between the two treatment groups will be compared using an Analysis of Covariance (ANCOVA) model, using the MΔ as the dependent variable, baseline anti-Aβ40 antibody signal (OD in ELISA) as covariate and the treatment group and

amyloid positivity as fixed effects. In addition, the number and percentage of patients in each visit reaching a value greater or equal than the MΔ will be tabulated by groups for each variable.

Patients in the verum group will be classified as "Positive Responders" when: i) their MΔ of anti-Aβ40 antibody signal is equal or greater than 3 times the standard deviation of the titration ELISA and ii) more than the 50% of the signal in the visit reaching the MΔ is eliminated by pre-adsorption of the sample. The antibody titers, defined as the inverse of the maximal plasma sample dilution which showed an OD increase ≥ 3 SD with regard to the pre-immune sample (either V0 or V1), of "Positive Responder" will be reported.

9.1.2.2 Secondary Efficacy Analysis

The trial will be considered satisfactory if the difference of the average maximal increment (MΔ) of anti-Aβ40 antibody signal (OD in ELISA) in the treated group is > 1.778 OD than the average MΔ in the placebo group; the difference that was reached between both groups in the phase I clinical trial of ABvac40.

The secondary efficacy variables regarding assessment of disease biomarkers and cognition (see section 7.1) will be analyzed descriptively in the overall group and separately in the amyloid-PET negative (verum and placebo) and the amyloid-PET positive (verum and placebo) subjects pertaining to the ITT and PP populations

For the exploratory comparison of the secondary efficacy variables for the characterization of the immune response (section 7.1.2) between the verum and placebo group, the maximal increment (MΔ) with regard to the pre-treatment visit (either V0 or V1) in each subject will be used as a summary measure for each of the following variables: i) the OD signal for plasma peptides and cytokines levels, or Aβ plasma levels as determined by LC-MS, ii) the relative number of cells subsets from peripheral blood mononuclear cells; and iii) the number of cytokine and antibody producing cells. Statistical comparison between groups of treatment will be done for the variable MΔ by means of ANCOVA models, using the MΔ as the dependent variable, baseline as covariate and the treatment group and amyloid positivity as fixed effects. In addition, the number and percentage of patients in each visit reaching a value greater or equal than the MΔ will be tabulated by groups for each variable.

Mixed-model repeated-measures (MMRM) analyses will be performed for all the primary and secondary efficacy variables (section 7.1) to assess between-group differences in change scores from baseline (V0/V1) to Int-6m (V16), V21 and to the final visit (V25). The dependent variable in each MMRM analysis will be "change from baseline". Fixed effects will include baseline scores on outcome measures, age at baseline, group assignment, amyloid positivity, study visit, and treatment-by-visit interaction. Additionally, covariates that are significantly associated with the response measure ($p < 0.15$) and are out of balance at baseline ($p < 0.2$) will be included as fixed effects. Variables considered as potential covariates in each model will be demographic variables, baseline characteristics, general treatment for AD and concomitant medication.

9.1.3 Safety Analysis (Part A)

All the safety variables will be evaluated in the Safety population.

9.1.3.1 Primary Safety Analysis

The primary safety variable is the frequency (%) of Adverse Events (AEs). The primary safety endpoint will be analyzed descriptively in the Safety population. For this primary safety analysis, the amyloid-PET negative and the amyloid-PET positive subjects within each treatment group will be pooled together.

The trial will be considered satisfactory if the pattern of AEs (overall or grouped as neurological, psychiatric or cardiovascular) is consistent with a good safety and tolerability profile of ABvac40 for human administration.

All AEs will be coded using the last version of the MedDRA dictionary where neurological AEs corresponds with the System Organ Class (SOC) Nervous system disorders, psychiatric AEs corresponds with the SOC Psychiatric disorders, and cardiovascular SOC corresponds with Vascular Disorders and Cardiac disorders.

The number and percentage of patients in each treatment group reporting at least 1 occurrence of an AE for each unique SOC and PT will be tabulated. AEs will be also tabulated by severity and by the relationship to study medication in each treatment group as assessed by the investigator. Patients who experienced multiple adverse events with the same PT will be counted once for the summaries, using the worst severity. Specific tables will be provided for AEs of special interest such as neurological, psychiatric and cardiovascular AEs.

As an exploratory analysis, the incidence of AEs (overall or grouped as neurological, psychiatric or cardiovascular) will be compared between the group receiving the active treatment (ABvac40) and the control group receiving placebo. Fisher's exact test will be used for these analyses.

9.1.3.2 Secondary Safety Analysis

The safety and tolerability analysis will be based on the safety population, comprising all subjects who took at least one dose of study medication.

<u>Withdrawal criteria (discontinuation decision):</u>

Withdrawal criteria will be summarized using descriptive statistics.

<u>Number of withdrawn patients due to adverse events (AEs); reason for withdrawal:</u>

The number and percentage of patients (in each treatment group) prematurely discontinuing study treatment due to an AE will be tabulated by SOC and PT. Listings of patients with AEs leading to discontinuation and individual narrative summaries for these cases will be provided.

<u>Serious adverse events (SAEs)</u>

The number and percentage of patients in each treatment group reporting at least 1 occurrence of a SAE for each unique SOC and PT will be tabulated. SAEs will be also tabulated by the relationship to study medication in treatment groups as assessed by the investigator. Listings of patients with SAEs (and deaths) and individual narrative summaries for these cases will be provided.

<u>Physical and neurological examination</u>

Physical findings (normal and abnormal) will be listed for each clinical trial subject. Any clinically significant abnormality developed by individual during the clinical trial and not already present at baseline will be reported as an AE.

Physical and neurological examination data will be summarized in tables.

Concomitant medication

Concomitant medication and concomitant therapy will be summarized as number of patients being treated with each type of medication/therapy classified according to ATC level 3 and World Health Organization (WHO) Drug Dictionary preferred term.

Vital signs and anthropometric data

Vital signs (blood pressure, heart rate, respiratory rate and body temperature) and anthropometric data (body mass index, weight and height) will be summarized together with changes from baseline.

Brain MRI

Brain MRI findings will be listed for each clinical trial subject and summarized in tables.

ECG

Overall interpretation of ECG results (i.e. normal or abnormal and clinical significance) as well as ECG findings will be summarized in tables.

Laboratory safety assessments

For laboratory data, summary statistics will be produced for observed values and for changes from baseline to each visit. In addition, the number of abnormal and clinically significant observations will be tabulated for each treatment group by visit. Abnormal values will be flagged in listings.

Shift tables will show the number of patients who changed from below, within or above the reference range at baseline to below, within or above the reference range at each time of assessment.

For laboratory values which are below the limit of quantification, the value corresponding to the limit of quantification will be used when summarizing data (e.g. if the result is <x.xx then the value x.xx will be used in the statistical analysis). “Overflow” readings in OD signal will be replaced by the maximal signal measurable by the Microplate reader.

9.1.4 Efficacy and Safety Analysis (Part B)

The analyses for Part B will mirror the analyses of Part A, without testing any hypothesis. The detailed statistical analyses will be described in the Statistical Analysis Plan.

9.1.5 Interim Analysis (Int)

During the study, two Int will be performed to assess safety, tolerability and biological activity of the treatment (including data from the collected disease biomarkers at each of them). These analyses will be reviewed by an independent DSMB. The first Int will be carried out once the

first 30 patients have completed the 24-week visit (Int-6m) and the second one once all patients have completed the 24-week visit (Int-6m, eight weeks after the fifth monthly immunization), or in October 2020, whichever occurs first. The double blinding of the study will be maintained throughout these two Int. Additional DSMB reviews may be made *ad hoc* in the event of safety issues.

The DSMB statistical analysis plan (SAP) will provide the technical details of the Int.

. To assure blinding, all Int will be performed by an IS, who is not involved in any other tasks in this study.

9.1.5.1 Data Safety Monitoring Board (DSMB)

In order to assess safety and critical efficacy endpoints during the conduct of the trial, an independent DSMB has been appointed. The DSMB is an independent group consisting of three physicians who are not investigators in the study or other IMP development studies who have collective experience in the conduct and safety monitoring of clinical studies as well as monitoring and evaluation of clinical study safety data in randomized clinical studies in early phase. In addition, an IS will be also part of the DSMB.

The membership of the DSMB and the responsibilities of the DSMB are defined in the DSMB Charter. The Charter, signed by all DSMB members, includes detailed information about DSMB members' responsibilities, timing and purpose of DSMB meetings and Safety/Efficacy endpoints reviews of DSMB meetings, with their final recommendations.

The aims of the DSMB are to safeguard the interest of trial participants, assess the safety and efficacy of the interventions during the trial, and monitor the overall conduct of the clinical trial. Thus, the DSMB will provide recommendations and advice to the Sponsor. The Sponsor has overall executive responsibility over the Study. Any recommendation and/or decision will be taken in the best interest of the participants of this study, in order to safeguard their welfare and safety, and in accordance with CGP (ICH E6) and the latest version of the Declaration of Helsinki.

Schedules meetings of the DSMB will be face-to-face or by teleconference. These meetings will be classified as organizational meeting (kick-off) or formal data evaluation meetings.

At least two formal data evaluation meetings will be scheduled at:

1. Once the first 30 patients have completed the 24-week visit (Visit 16)
2. Once all patients have completed the 24-week visit (Visit 16), or in October 2020, whichever occurs first.

All two formal data evaluation meetings will be closed sessions and will include the safety endpoints as well as the main efficacy endpoints. At the three meetings, the DSMB will review the blinded/unblinded safety and efficacy data.

For the formal data evaluation meetings the IS will produce listings and summaries tables, including, but not limited to:

- Recruitment figures and losses to follow-up (all meetings)
- Demography and ApoE genotyping (all meetings)
- Safety parameters (all meetings):

- Number of withdrawn patients, number of patients who discontinued from treatment and reasons for withdrawal and discontinuation, respectively
- SAEs and AEs
- Rate (%) AEs
- Expected serious adverse drug reactions like Amyloid Related Imaging Abnormalities
- Physical and neurological examination significant abnormal findings
- Concomitant medication
- Vital signs (blood pressure, heart rate, respiratory rate, body temperature) and body mass index (weight, height) values.
- Safety Brain MRI
- Key EKG parameters
- Analytical haematology, toxicology (reactive protein), biochemistry, coagulation, serology and urine test strip results (all data regardless if they are abnormal).

- Average maximal increment (MΔ) of anti-Aβ40 antibody signal (OD in ELISA) (all meetings)
- Aβ40 & Aβ42 in plasma (all meetings)
- RBANS total score (all meetings)
- Results of futility analysis and sample size re-estimation (all meetings)

After each formal data evaluation meetings, the DSMB will provide recommendations to the Sponsor mainly in the following areas:

- Suspension of recruitment pending further safety review, if data received suggest significant adverse effect of the investigational medicinal product.
- Stop the study due to efficacy concerns (futility)*:
  - If, given the data up to the meeting's cut-off date, the futility analysis, based on the primary efficacy analysis (confirmatory) of superiority (meaning that the average maximal increment [MΔ] of specific anti-Aβ40 antibody signal [OD in ELISA] for the treated group is >0 OD to that of placebo group), yields a conditional power of less than 20% the DSMB will recommend to stop the study. Details on the futility analysis will be given in the DSMB SAP.

    **A safety and efficacy reviews will be taken into account in order to make a recommendation*
- Increase the sample size based:
  - If pooled SD for the average maximal increment (MΔ) of anti-Aβ40 antibody signal (OD in ELISA) is higher than the estimated 1.6 (according to the results of the Phase I study), the DSMB will recommend to the sponsor to increase the sample size according to the observed pooled SD, to ensure at least 80% power to detect a treatment superiority with margin of 1.778 in average maximal increment (as considered in the secondary efficacy analysis [satisfactory])

Despite the statistical stopping rules indicated above, these are not formal rules; the recommendation to stop rests on the wise judgment of the DSMB on the basis of the totality of evidence at their disposal, both within the trial and externally. However, the ultimate decision rests with the Sponsor.

Details of the formal data evaluation meetings are included in the DSMB SAP and DSMB Charter.

9.2 Determination of Sample Size

This trial is intended as a confirmatory assay for the primary safety and primary efficacy variables.

For the safety primary endpoint, the sample size may be calculated to ensure ≥95% probability of detecting an adverse event that occurs with a rate of at least 5% within the ABvac40 treated dose cohort. Under these assumptions, using the Hanley´s simple approximation [32], a minimum of 60 patients in the active dose arm will be needed (and 60 in the placebo group).

This sample size (60 patients in the active group and 60 in the placebo group), considering ~40% dropouts along the clinical trial, will imply that approximately 70 patients will complete the study. These 70 patients will have greater than 85% power to detect a treatment increment of 1.778 (the one found in ABvac40 Phase I trial) in maximum change from baseline in anti-Aβ40 antibody signal (OD in ELISA without preadsorption) between the active group and placebo group, using a one-sided T test at a significance level of 0.025 (and considering standard deviations of 2.0 and 1.0 in the active and placebo groups, respectively). The study will not be powered to demonstrate efficacy in clinical or other biomarker outcomes.

Thus, the screening will be continued until 120 individuals diagnosed with a-MCI or vm-AD with amnestic syndrome of the hippocampal type had been recruited into the trial and have received at least one dose of the Verum or placebo, as correspond. A minimum of 70 subjects are expected to complete the study per protocol (35 in each treatment group).

9.3 Level of Significance to Be Used

The statistical tests will be performed with a 2.5% significance level for the primary efficacy (immunogenicity) variable and 5% for the and will be one-sided for the primary variable of biological activity and two-sided for the secondary biological activity/efficacy variables. In addition to the tests, 95% CI will be reported.

9.4 Criteria for Termination of the Study

The Investigator or the Sponsor may terminate this study prematurely for any reasonable cause. The Independent Ethics Committee(s) (IECs) and Competent Authorities (CAs) should be informed promptly.

Conditions that may warrant termination include, but are not limited to:

- The discovery of an unexpected, significant, or unacceptable risk to the subjects enrolled in the study, or potential study subjects
- A decision on the part of the Sponsor to suspend or discontinue development of the IMP

- If after analyzing the study data from Part A (up to month 18 – V23), no clinical differences have been observed between the Placebo and the Verum group according to the following stopping rules, the sponsor could prematurely finalize the study. A least one of the following criteria must have been fulfilled:
    1. For PET negative population: If the lower bound of the 95% exact CI for the proportion of negative PET patients treated with vaccine is greater than the proportion in Placebo group after 12 months (V21).
    2. For PET positive population: If the lower bound of 95% exact CI for the proportion of Reduction of cerebral amyloid plaques of positive PET patients treated with vaccine is greater than the proportion in Placebo group after 12 month (V21).
    3. For PET positive population: If the lower bound of the 95% exact CI for the mean of decline in Clinical Dementia Rating-Sum of Boxes (CDR-SB) in the positive PET patients treated with vaccine is greater than the mean in Placebo group after 18 months (V23).

If the CAs obtains information that raises doubts about the safety or scientific validity of the clinical study, the CAs can suspend or prohibit the study. Before the CAs reaches its decision it shall, except where there is imminent risk, ask the Sponsor and/or the Investigator for their opinion, to be delivered within one week (Directive 2001/20/EC, Article 12, Section 1).

If the study is prematurely terminated or suspended for any reason, the Investigator/institution should promptly inform the study subjects and should assure appropriate therapy and follow-up for the subjects.

9.5 Procedure for Accounting for Missing, Unused, and Spurious Data (if applicable)

In the ITT population, to perform the statistical analyses that uses the end point (the last time-point of each variable), if a subject prematurely withdraws from the study, missing data with respect to each variable will be replaced by the mean value of the corresponding treatment group at the corresponding visit.

9.6 Reporting Deviation(s) from the Statistical Analysis Plan

The detailed statistical analysis methodologies will be documented in the Statistical Analysis Plan. If there are any deviations from the originally planned analyses in the Statistical Analysis Plan, they will be fully described and justified in the protocol amendment(s) and/or final Clinical Study Report.

9.7 Subject Population(s) for Analysis

The Safety population consists of all subjects who received any amount of study drug.

The Intent-to-treat (ITT) population consists of all subjects randomized who received at least one dose of study medication for whom at least one post-baseline anti-Aβ40 antibody signal assessment is available.

There will be two per-protocol (PP) populations:

- Part A: consists of all subjects who have received all doses of study medication (on V1, V4, V7, V10, V13 and V18), have attended the safety visit after booster (V20) and have no major protocol deviations.
- Part B: consist of all subjects who have received all doses of study medication (on V1B, V4B, V7B, V10B, V13B and V18B), have attended the safety visit after booster (V20B) and have no major protocol deviations.

10 DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

The data will be recorded and kept current in eCRFs (and corresponding source documents) by the study site personnel directly responsible for the information and reviewed for completeness by the monitor. Araclon-Grifols personnel or designee can review the records.

In accordance with ICH GCP guidelines, the monitor must have direct access to the investigator's source documentation in order to verify the data recorded in the eCRFs for consistency and to verify adherence to the protocol, and the completeness, consistency, and accuracy of data entered. "Source documentation" includes individual subject files, separate from the eCRFs, which should be maintained and include visit dates, laboratory results, concomitant treatment, vital signs, medical history, examinations, AEs, investigational product dispensing logs, and other notes as appropriate. The investigator agrees to cooperate with the monitor to ensure that any problems noted during the course of these monitoring visits are resolved.

The investigator agrees to allow an auditor appointed by the sponsor as well as representatives of the relevant authorities and the ethics committees to verify, through an audit at the study center, that the study is implemented and documented according to the study protocol, good clinical practice and applicable regulations. The auditor/inspector will have to examine all essential documents and verify from a random sample of data entered into the clinical report forms is correct according to the original data found in clinical records. Therefore, the researcher will provide direct access to the data/documents and locations where trial activities are carried out (e.g., Laboratories, rooms and hospital pharmacy).

11 QUALITY CONTROL AND QUALITY ASSURANCE

Monitoring and auditing procedures defined/agreed by the sponsor will be followed, in order to comply with ICH GCP guidelines. Each center will be visited at regular intervals by a monitor to ensure compliance with the study protocol, ICH GCP and legal aspects. The on-site verification of the eCRF for completeness and clarity will include cross checking with source documents, and clarification of administrative matters. Query verification of data will be described in the Data Management Plan.

Representatives of regulatory authorities or of Araclon-Grifols may conduct audits or inspections or audits of the investigator study site. If the investigator is notified of an audit or inspection by a regulatory authority, the investigator agrees to notify the Araclon-Grifols Representative (eg, CAM, PM, PL) immediately. The investigator agrees to provide to representatives of a Regulatory Agency or Araclon-Grifols access to records, facilities, and personnel for the effective conduct of an audit or inspection.

11.1 **Monitoring**

This study will be monitored regularly by a clinical monitor according to the GCP guidelines of the ICH and appropriate standard operating procedures (SOPs).
Before the study begins, the monitor will review the protocol and eCRFs with the researcher and his team.

It is the investigator's responsibility to ensure that the study is carried out according to the agreed study protocol and that data sent to the sponsor in the eCRF and in all required reports are accurate, complete, legible and on time. Furthermore, it is the investigator's responsibility to ensure that all relevant data such as the patient's medical history, concomitant diseases and medication, date of inclusion in the study, dates of study visits, results of examinations and development if adverse events are documented correctly in the subject's original files.

The investigator will be responsible for making sure that all persons involved in the trial receive training regarding their duties.

The investigator agrees to allow the monitor to personally verify the correct execution and documentation of the study during scheduled visits. The purpose of trial monitoring is to verify that:

- The human rights and welfare are protected.
- The data reported are accurate, complete, and verifiable from original documents.
- The conduct of the trial is consistent with the up-to-date approved protocol and any possible amendments, with GCP and with applicable legal requirements.

To this end, data entered in the eCRF will be compared with data from medical records, etc. In particular, the availability of signed and dated informed consent will be checked. No information from the medical records regarding the identity of the subjects in the study will leave the study center.

For the verification of the original data, as a minimum the following information from patient files will be included:

- Date of patient written informed consent, selection number of,
- Dates of patient visits, adverse events (mild and severe),
- Confirmation of the diagnosis of the indication that is being treated (mild or moderate AD in this case),
- IMP and administration regimen (e.g., Initiation of treatment, dose), concomitant medication,
- Primary variables of biological activity, secondary variables of biological activity.

Print outs of the analytic data, EKGs and MRI data are considered original data.

The investigator and their team will cooperate with the clinical monitor, providing any missing information whenever possible. The investigator will be available to answer any questions that arise during monitoring of the study.

11.2 **Audit and inspection**

The investigator agrees to allow an auditor appointed by the sponsor as well as representatives of the relevant authorities and the ethics committees to verify, through an audit at the study center, that the study is implemented and documented according to the study protocol, good clinical practice and applicable regulations. The auditor/inspector will have to examine all essential documents and verify from a random sample of data entered into the clinical report forms is correct according to the original data found in clinical records. Therefore, the researcher will provide direct access to the data/documents and locations where trial activities are carried out (e.g., Laboratories, rooms and hospital pharmacy).

11.3 **Data Management**

11.3.1 Data Documentation

The clinical report forms are designed for recording assessments by different investigators and they provide accurate advice on definitions of terms. The sponsor or designee will explain in detail how to complete the eCRFs to people involved in data entry.
The data recorded in the eCRFs during the study will be documented under a subject number and the subject will only be identifiable by their subject number. If, as an exception, it is necessary to identify a subject for legal or safety reasons, both the sponsor (including CRO staff) and the investigator are required to maintain the confidentiality of this information.
As a general rule, all information in the eCRF should be traceable to the subject's original documents on file. As a minimum the following information must be available as original data in the patient's medical record kept by the researcher:

- Compliance of the patient to participate in this study (study identified by study number, product under investigation, randomization number), history
- Records of diseases/treatments and concomitant medication, dates of visits,
- Use of study medication, records of adverse events,

- Reasons for premature discontinuation, as appropriate,

All data entered in the eCRF requires that an electronic or written record be defined as original data.
eCRFs are to be filled in immediately after each examination. The investigator will give a reasonable explanation for any missing data. The investigator will sign completed eCRFs to confirm reliability and truthfulness of data.
If the investigator authorizes other people to enter data into the eCRF, this delegation must be documented with the names, positions, signatures and initials of these people.

11.3.2 Data processing

All data will be entered in the eCRF (and corresponding source documents) by investigator or designee according to pre-defined instructions in the Data Entry Manual. Data entry will be performed on an ongoing basis as subjects perform their visits.

[redacted] Electronic Data Capture system (Trial Master) will be used in this study. The EDC system will maintain an audit trail that will document changes together with date and user in the database after the data entry has started.

Laboratories and vendors data will be entered by study staff in the eCRF unless elsewhere specified, in which case electronic files will be uploaded into patients eCRF, as explained in the relevant manuals and detailed in the Data Transfer Specification.

11.3.3 Coding

The following dictionaries should be used for coding:
- Medical history: MedDRA
- Adverse events: MedDRA
- Concomitant medication: WHO-DD (ATC)

11.3.4 Data cleaning

During the initiation phase of the study, a Data Management Plan (DMP) will be developed which will describe in detail how data will be managed.
Automatic checks defined in the Data Validation Plan (DVP) will be generated by the system. Manual queries will also be created, if needed. Investigator or designated site staff will be required to respond to the checks and queries and confirm or correct the data. Query Management will be detailed in the DMP.

11.3.5 Missing and false data

All missing or false data will be investigated. If the investigator cannot provide a reasonable explanation, then the data shall be treated as unresolved queries. All data from the eCRF will be exported into SAS datasets and will be considered for analysis as specified in the statistical analysis plan.

11.3.6 Closing the database

After the study has been completed, the occurrence of any emergency decryption of the code and any violation of the protocol will be determined. Once these actions are completed and the database is declared complete and accurate, it will be closed. Any subsequent changes to the database can only be done through a joint agreement in writing between the Sponsor and [redacted].

12 ETHICS

12.1 Institutional Review Board/Ethics Committee

Documented approval from appropriate IRBs/ECs will be obtained for all participating centers/countries prior to study start, according to ICH GCP guidelines, local laws, regulations and organizations. When necessary, an extension, amendment or renewal of the IRBs/ECs approval must be obtained and also forwarded to the sponsor. The IRBs/ECs must supply to the sponsor, upon request, a list of the IRBs/ECs members involved in the vote and a statement to confirm that the IRBs/ECs is organized and operates according to ICH GCP guidelines and applicable laws and regulations.

12.2 Ethical Conduct of the Study

The procedures set out in this protocol, pertaining to the conduct, evaluation, and documentation of this study, are designed to ensure that the sponsor and investigator abide by ICH GCP guidelines. The study will also be carried out in keeping with applicable local law(s) and regulation(s). This may include an audit by the sponsor representatives and/or an inspection by Regulatory Authority representatives at any time. The investigator must agree to the audit or inspection of study-related records by the sponsor representatives and/or Regulatory Authority representatives, and must allow direct access to source documents to the sponsor and/or Regulatory Authority representatives.

Modifications to the study protocol will not be implemented by either the sponsor or the investigator without agreement by both parties. However, the investigator may implement a deviation from, or a change to, the protocol to eliminate an immediate hazard(s) to the study subjects without prior IRB/EC/Sponsor approval/favorable opinion. As soon as possible, the implemented deviation or change, the reasons for it and if appropriate the proposed protocol amendment should be submitted to the IRB/EC/Sponsor. Any deviations from the protocol must be fully explained and documented by the investigator.

No medical waivers for protocol inclusion/exclusion criteria will be allowed by the sponsor, and that in case the need for a change to the protocol is identified, it will be submitted as a protocol amendment to the competent regulatory authority and/or ethics committee as applicable per regulations.

Direct advertising will be used in this study in order to solicit subject participation. Direct advertising includes, but is not necessarily limited to: newspaper, radio, TV, bulletin boards, posters that are intended for candidate subjects. Any advertisement will be submitted for review and approval to the IRB/EC charged with this responsibility to ensure that the information contained in the advertisement is not misleading and that the procedure for recruiting subjects gives adequate protection for the rights and welfare of the subjects.

12.3 Regulatory Authority Approvals/Authorizations

Regulatory Authority approvals/authorizations/ notifications, where required, must be in place and fully documented prior to study start. Study information including contact information for

investigator sites responsible for conducting the study will be posted on a publicly accessible clinical registry (ies) as required by local law.

12.4 Subject Information and Consent

Subject information and ICF will be provided to investigator sites. Prior to the beginning of the study, the investigator must have the IRB/EC written approval/favorable opinion of the written ICF and any other written information to be provided to subjects. The written approval of the IRB/EC together with the approved subject information/ICF must be filed in the study files and a copy of the documents must also be provided to Sponsor by the investigator site.

Written ICF must be obtained from the patient (or legal representative, if applicable) and from a close relative/caregiver before any study specific procedure takes place. Participation in the study and date of ICF given by the subject and by the close relative/caregiver should be documented appropriately in the subject's files. A signed copy of the subject and close relative/caregiver ICFs will be provided to the subject (or subject's authorized representative) and to the close relative/caregiver, respectively.

12.5 Confidentiality

All records identifying the subject will be kept confidential and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available.

Subject names will not be supplied to the sponsor. Only the subject code number and subject initials will be recorded in the eCRF, and if the subject's name appears on any other document (*i.e.,* pathologist report), it must be removed before a copy of the document is supplied to the sponsor. Study findings stored on a computer will be stored in accordance with local data protection laws. Subjects will be informed in writing that representatives of the sponsor, IRB/EC, or Regulatory Authorities may inspect their medical records and personal health information to verify the information collected, and that all personal information made available for an audit or inspection will be handled in strictest confidence and in accordance with local data protection laws and with the European General Data Protection Regulation (2016/679) (GDPR).

If the results of the study are published, the subject's identity will remain confidential.

The investigator will maintain a list to enable subjects' records to be identified.

12.6 Patient health insurance

During their participation in the study, each subject will be covered by an insurance policy in accordance with the Spanish Medicines Act, which covers any possible risks arising from their participation in the study.
Patients will be informed of the existence of the contract and the obligations that come with it:

- The insured person can only receive other medical treatment during the clinical trial, if the clinical investigator consents to such treatment. Emergency treatment is exempt from this rule.

- The insurer must be informed immediately of any health deterioration that may result from the clinical trial. In case of damages, the subject's signed informed consent form will be presented.

If it is necessary to make an insurance claim, the medical investigator will notify the monitor and sponsor immediately so they can submit the claim to the insurance company.

12.7 **Compensation for the subject**

By participating in this clinical trial, no additional costs will be incurred. A taxi services and a meal voucher will be available for all participants and caregiver throughout the study. In addition, travel by private car, public transport or taxi will be reimbursed. For private car travel, the amount of fuel used shall be reimbursed; for public transport or taxi, the refund can only be made on presentation of the original receipt. Any additional costs related to participation in this study may be refunded upon presentation of the original receipt. For this, patients should contact the staff responsible for the study during visits.

13 DATA HANDLING AND RECORD KEEPING

13.1 Data Handling

The study data will be recorded and kept current in the eCRF by the site study personnel directly responsible for the information. Entries made in the eCRF must be verifiable against source documents. The data in the eCRF will be monitored at the site by Araclon-Grifols representatives at regular intervals and reviewed for completeness and compared with the source documents. Examples of acceptable source documents include individual subject medical records, prospective information gathered on source documentation worksheets, lab reports and other diagnostics pertinent to this study which are separate from the eCRFs. The listing of types of source documents which will be defined in the source data agreement will be filed in TMF.

All adverse events (AEs) and serious adverse events (SAEs) must be recorded. All SAEs must be recorded on the SAE form. The SAE form must be kept in site records with a copy provided to the designated person as detailed in the study file.

13.2 Record Retention

At study completion, all study data will be transferred to Araclon-Grifols according to ICH GCP guidelines, local laws, regulations, and Araclon-Grifols requirements. The study file and all source data should be retained until notification is given by the sponsor for destruction.

An investigator is required by ICH GCP guidelines to retain the study files. If an investigator moves, withdraws from an investigation or retires, the responsibility for maintaining the records may be transferred to another person (*i.e.,* other investigator). Araclon-Grifols must be notified in writing of the person responsible for record retention and the notification will be retained in the sponsor study file and the investigator site file.

14 FINANCING AND INSURANCE

In the event of subject injury as a direct result of either administration of investigational product or any non-standard of care study procedure, Sponsor will pay for the costs of treatment, provided the subject has followed the instructions given by the study doctor and the illness or injury is not due to the natural progression of any conditions existing before the subject participated in the study. Financial compensation for such things as lost wages, disability, or discomfort due to any research-related injury is not available.

Sponsor shall maintain comprehensive general liability insurance or self-insurance in amounts adequate to cover any damage, demand, claim, loss or liability caused or incurred by Sponsor, or as otherwise required by applicable laws and/or regulations.

15 PUBLICATION POLICY

Institution and the Investigator agree that the first publication shall be made in conjunction with the presentation of a joint, multi-center publication of the study results from all appropriates sites. If such a multi-center publication is not submitted within twelve (12) months after conclusion of the study at all sites or after Araclon-Grifols confirms there will be no joint, multi-center publication, then institution and/or Investigator shall have the right, at their discretion, to publish, either in writing or orally, the results of the study performed under the protocol, subject to the conditions outlined below:

- The results of the study will be reported in the publicly accessible registry(ies).
- Institution and/or Investigator shall furnish Araclon-Grifols with a copy of any proposed publication at least thirty (30) days in advance of the date of submission for publication.
- Within said thirty (30) day period, Araclon-Grifols shall:
  - Review such proposed publication for Confidential Information (other than Study results) and for subject information subject to the Health Insurance Portability and Accountability Act of 1996 ("HIPAA") and other applicable privacy laws;
  - Review such proposed publication for the unauthorized use of the name, symbols and/or trademarks of Araclon;
  - By written notice to the Investigator, identify with specificity the text or graphics in such proposed publication that Araclon-Grifols contends contains Confidential Information, protected subject information, or the unauthorized use of Araclon or Grifols' name, symbols and/or trademarks so that the proposed publication may be edited appropriately to remove such text or graphics before publication; and
  - By written request, Araclon-Grifols may delay proposed publications up to sixty (60) days to allow Araclon-Grifols to protect its interests in Araclon-Grifols Inventions described in such publications.
- Institution and/or Investigator shall give Araclon-Grifols the option of receiving an acknowledgment for its sponsorship of the study in all such publications or presentation.

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17 APPENDICES

Appendix 1 Definition of adverse reactions

Local reactions:

They are those that occur in the injection area. The appearance of erythema, pruritus and hyperemia in that area is considered as an effect that may be associated with the administration thereof but it's not considered as an adverse reaction. We will consider such case when an induration (wheal) occurs at the site of inoculation of the extract and it has a diameter which is referred below. Regarding to the moment of occurrence of local adverse reaction, we differentiate between:

• Immediate: local adverse reaction that occurs within 30 minutes after extract administration. It is considered that it happens when the largest diameter of the wheal is larger than 5 cm in adult subjects and 3 cm in pediatric subjects.

• Delayed: local adverse reaction that begins 30 minutes after extract administration. It is considered that it happens when the largest diameter of the wheal exceeds 10cm in adult subjects and 7cm in pediatric subjects.

Systemic reactions:

They are those that occur away from the injection site. As in the case of local reactions, we differentiate between immediate or delayed response considering the occurrence within or after the first 30 minutes following extract administration, respectively. The severity of systemic adverse reaction is usually associated with the speed of beginning of the first symptoms of it.

Currently, systemic adverse reactions are ranked in five grades (1-5), depending on their severity, where grade 1 is the least severe and grade 5 is the most sever. Classification in these grades is based on the affected organ and the intensity of the registered clinical manifestations:

• Grade 1: exclusive implication of one of the following systems/organs: skin, conjunctiva, upper respiratory tract, or other (non-specific).

• Grade 2/3: Involvement of more than one of the systems/organs described above or presence of asthma, gastrointestinal involvement or lower respiratory tract (grade 2 with reduced FEV1 baseline of 40% with good response to rescue bronchodilators; grade 3 with a reduction less than 40% without adequate response to the rescue bronchodilators).

• Grade 4: respiratory failure and/or cardiovascular with hypotension (no need to be associated with loss of consciousness).

• Grade 5: death of the subject.