(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
02 August 2024 (02.08.2024)

WIPO | PCT

(10) International Publication Number
WO 2024/156912 A1

(51) International Patent Classification:
A61K 39/00 (2006.01)

(21) International Application Number:
PCT/EP2024/052002

(22) International Filing Date:
26 January 2024 (26.01.2024)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
23153558.4 26 January 2023 (26.01.2023) EP

(71) Applicant: **AC IMMUNE SA** [CH/CH]; EPFL Innovation Park, Building B, 1015 Lausanne (CH).

(72) Inventors: **STREFFER, Johannes**; c/o AC Immune SA, EPFL Innovation Park, Building B, 1015 Lausanne (CH). **KOSCO-VILBOIS, Marie**; c/o AC Immune SA, EPFL Innovation Park, Building B, 1015 Lausanne (CH). **VUKICEVIC, Marija**; c/o AC Immune SA, EPFL Innovation Park, Building B, 1015 Lausanne (CH).

(74) Agent: **BOULT WADE TENNANT LLP**; Salisbury Square House, 8 Salisbury Square, London EC4Y 8AP (GB).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, MG, MK, MN, MU, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, CV, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SC, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, ME, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*
— *with sequence listing part of description (Rule 5.2(a))*

WO 2024/156912 A1

(54) Title: ANTI-ABETA VACCINE THERAPY

(57) Abstract: A liposomal vaccine composition comprising a !-amyloid (Aβ)-derived peptide antigen displayed on the surface of the liposome that comprises, consists essentially of or consists of amino acids 1-15 of Aβ, a peptide comprising a universal T-cell epitope and an adjuvant comprising monophosphoryl lipid A (MPLA) is used for inducing an anti-Aβ immune response in a human subject without inducing a serious adverse event. The !-amyloid (Aβ)-derived peptide antigen (SEQ ID NO: 1) is administered in an amount of 300-2000 μg. The liposomal vaccine composition is administered intramuscularly or subcutaneously.

ANTI-ABETA VACCINE THERAPY

FIELD OF THE INVENTION

The invention relates to anti-abeta therapeutic vaccines and their use in inducing an anti-Aβ immune response without inducing serious adverse events. Such vaccines are useful for the treatment and prevention of diseases, in particular an amyloid-beta associated disease or condition or a condition characterised by, or associated with, loss of cognitive memory capacity, such as Alzheimer's disease (AD) and Down syndrome (DS), including Down syndrome-related Alzheimer's disease. The vaccines incorporate Aβ-derived peptide B-cell antigens and T-cell epitopes.

BACKGROUND

Alzheimer's Disease (AD) is a devastating, progressive degenerative disorder characterized by loss of cognitive functions, including memory, as well as the loss of ability to perform regular daily activities. AD affects approximately 40 million patients worldwide, with the number increasing rapidly as the population ages. The major neuropathological change in the brain of AD patients is neuronal death, mainly in memory and cognition-related regions (Soto, 1999). One of the most striking pathological features of AD is the abundant presence of amyloid beta (abeta, Abeta, β-amyloid, Aβ) plaques in brains of diseased individuals (Soto, 1999). Aβ plaques are formed by the 39 to 43 amino acid long Aβ peptide, which is in random coil conformation in its natural non-pathological form. During the transition to the pathological state, it transforms mainly into a β-sheet secondary structure, spontaneously aggregating into insoluble deposits.

While the majority of available treatments for AD are considered to be primarily symptomatic in their action, the most effective approach to date involves passive immunization with the use of anti-Abeta monoclonal antibodies administered directly to the patient. Such an approach led to the first authorization of an anti-Abeta monoclonal antibody therapy with the FDA approval of Aduhelm® (aducanumab) in 2021. More recently, the FDA approved LEQEMBI™ (lecanemab), another anti-Abeta monoclonal antibody, in January 2023 via the accelerated approval pathway for the treatment of Alzheimer's disease, and in July 2023 the FDA granted traditional approval for LEQEMBI™ (lecanemab-irmb), for the treatment of Alzheimer's Disease. Several other anti-Abeta monoclonal antibodies are currently in an advanced phase of development (e.g., Eli Lilly applied for full FDA approval of donanemab, in July 2023). Vaccines present the advantage of stimulating the immune system to produce

a pool of slightly different, but very specific antibodies, while the response can be further recalled by additional vaccinations, if needed. However, an active immunization (vaccination) approach against Aβ represents several main challenges. Amyloid beta is a so-called self-antigen, which the human body is constantly exposed to. Therefore, it is quite difficult to break immune tolerance and induce an antibody response against it. In addition, it is quite difficult to induce a strong immune response to a vaccine in elderly and sick people, such as AD patients, due to their weakened immune system and decreased number of immune cells.

Despite these challenges, in a reported initial study, a full-length Aβ1-42 vaccine (AN1792) induced an antibody response and a promising efficacy, with a slower rate of cognitive decline in patients who had received vaccination than in placebo-treated patients (Gilman, 2005). However, 6% of treated patients developed meningoencephalitis, an inflammatory reaction considered to be due to a T-cell-mediated response against full length Aβ1-42 (Orgogozo, 2003). Other anti-amyloid vaccines currently in the clinical development phase include UB-311 from Vaxxinity and Abvac 40 from Araclon Biotech.

Another known anti-Aβ vaccine, ACI-24, contains a sequence of 15-amino acids with complete identity with the human sequence 1-15 of Aβ (WO2007/068411). This peptide antigen is linked to a liposomal carrier with the aim to stimulate antibodies against Aβ, while avoiding meningoencephalitis and hemorrhage (Muhs, 2007, Pihlgren, 2013). The choice of the Aβ1-15 peptide serving as the antigen was based on the rationale that this sequence contains a B-cell epitope, but lacks a strong T-cell reactive site of full-length Aβ1-42 (Monsonego, 2003), the latter being considered to be the cause of the unwanted inflammatory reactions. ACI-24 has been shown to act through a simultaneous activation of a B-cell receptor specific for Aβ1-15 and the Toll-like receptor 4 (TLR4), the latter activated by monophosphoryl lipid A (MPLA) adjuvant present in the ACI-24 vaccine (Pihlgren, 2013). B-cells are activated to proliferate and produce immunoglobulin (Ig) by cross-linking the B-cell surface Ig receptor.

In order to increase antibody production, a second signal can be provided by a T helper cell activated by a T-cell epitope. T-cell epitopes, presented by the major histocompatibility complex (MHC) molecules (in human called human leucocyte antigen (HLA)) on the surface of an antigen-presenting cell (APC), promote the differentiation of cognate T helper cells capable of producing IFNγ and IL-4. Cytokine release and co-stimulatory signals between activated T and B cells increase antibody responses and class-switching. After primary

vaccination, naïve T cells proliferate and differentiate into effector cells. A small fraction of these cells will form the pool of long-lived memory T cells, capable of quickly proliferating upon re-encountering the cognate peptide after vaccine boosting (Sallusto, 2010). So-called "universal" T-cell epitopes are specific to the T-cells that are present in the vast majority of the human population. They commonly originate from antigens to which humans are normally exposed during their lifetime (e.g. tetanus, influenza, etc.). The ability of a T-cell epitope to activate T cells is the result of at least two complementary properties: i) affinity of binding to the HLA groove, meaning the strength of the binding, as well as ii) its capacity to bind different HLA haplotypes in a promiscuous manner, meaning the ability to cover very diverse human populations, with regards to the differences in the expression of HLA molecules.

WO2019/197414 describes a vaccine composition comprising the ACI-24 vaccine modified so as to include a peptide comprising a universal T-cell epitope encapsulated within the liposome. WO2019/197414 reports that immunization of C57BL/6 mice with the ACI-24 vaccines containing a T-cell epitope induced an increase of Aβ1-42 oligomer-specific antibody titers as compared to ACI-24 vaccine (without a T-cell epitope). In preclinical studies the ACI-24 vaccine containing a T-cell epitope has been shown to generate a potent and sustained immune response. Further, induced antibodies showed binding to pyroglutamate abeta, a highly neurotoxic form of Aβ which is a promising target for Alzheimer's therapy (Vukicevic, 2022).

Down syndrome (DS), also known as trisomy 21, is one of the most common causes of intellectual disability, affecting 1 in 800 newborns. This condition most commonly involves triplication of chromosome 21 (Belichenko, 2016). Subjects with DS have characteristic facial features, deficits in the immune and endocrine systems, and delayed cognitive development. Major improvements in medical care and understanding of the condition have not only improved the quality of life for DS subjects, but have also significantly extended their lifespan. DS subjects now have comparable mortality rates up to age 35 to those with other intellectual disabilities. However, after age 35, the mortality rate doubles every 6.4 years for DS subjects versus 9.6 years for non-DS people. An average life expectancy for DS subjects is 60 years, compared to an average of 79 years for the general population in the USA.

A key feature of adult subjects with DS is their increased risk of developing similar clinical symptoms of Alzheimer's Disease (AD), characterized by a decline in specific cognitive domains suggestive of a diagnosis of dementia. Virtually all subjects with DS older than 40

years exhibit neuropathological changes similar to AD, in the form of senile plaque formation and neurofibrillary tangles (Head, 2012). It is well accepted that the neuropathology for AD-like cognitive decline involves the β-amyloid (Aβ) peptide deposition and subsequent plaque formation, neurofibrillary tangles, vascular damage, neuro-inflammation and ultimately neuronal cell death. The gene of the amyloid protein precursor (APP), which encodes the precursor protein of Aβ, resides on chromosome 21. In subjects with DS, the entire or at least a part of chromosome 21 is present in triplicate. Consequently, this leads to three copies of the gene that encodes APP, which results in the generation of an excess of Aβ. An increased Aβ protein production, has been shown to correlate with AD-like symptoms in DS subjects as well as in the general population that develops AD (Head, 2012). These findings show conclusively that lifelong overexpression of wild-type APP causes cognitive decline in subjects with DS, in a similar way to the amyloid cascade hypothesis used to describe subjects with AD. Down syndrome-related Alzheimer's Disease is characterized by the presence of brain neuropathological hallmarks of Alzheimer's Disease (including notably the accumulation of brain amyloid plaques and neurofibrillary tangles) which can lead, when the brain lesions are sufficiently developed, to the appearance of clinical symptoms like cognitive decline and functional impairment.

The decline in cognitive function for DS subjects occurs over the years prior to a dementia diagnosis. Cognitive decline is classified into three categories: mild, moderate, and severe. Mild cognitive decline is often characterized by noticeable memory lapses that impact daily life as well as behavioral changes. Moderate cognitive decline is characterized by increased memory loss that extends farther into the past, significant personality changes caused by agitation and confusion, changes in sleep patterns, and a need for assistance in daily life. Severe cognitive decline can mean losing the ability to communicate, a severe decline in physical capabilities, and a need for full-time help with routine daily tasks. Symptoms such as apraxia and agnosia are reported in 28% of DS subjects by 30 years of age, as well as changes in personality and behavior (Head, 2012). Early Aβ deposition may be related to subtle declines in episodic and/or executive functioning, called mild cognitive impairment (Hartley, 2017). A recent study using positron emission tomography tracer [11C] Pittsburgh compound B (PiB) to measure brain amyloid burden in DS subjects has shown that an increase of global amyloid-β was related to decline in verbal episodic memory, visual episodic memory, executive functioning, and fine motor processing speed. DS subjects who were consistently PiB+ demonstrated worsening of episodic memory, whereas those who were consistently PiB- evidenced stable or improved performance (Hartley, 2017). The

diagnosis of cognitive decline can be difficult in the DS population since it can appear similar to symptoms of intellectual disability, so improved diagnostic methods are being investigated. Compounding the difficulty in diagnosis is that early symptoms are not uniformly exhibited. For example, memory loss is a key early clinical symptom of developing dementia, but this does not hold true in the DS population.

Current treatment for cognitive decline in DS is very limited, with the majority of research focused on dementia or AD. Therapies that have been investigated and shown promise for these indications, such as cholinesterase inhibitors, have so far shown to have poor efficacy in DS subjects experiencing cognitive decline (Prasher, 2002). In contrast to AD, immunotherapies targeting Aβ are not being widely addressed in DS.

WO2013/044147 and Belichenko (2016) describe vaccination of Ts65Dn mice, a model of DS, with a vaccine containing the Aβ 1-15 peptide embedded into liposomes.

DESCRIPTION OF THE INVENTION

The present invention arises from clinical trials of the ACI-24 vaccine comprising an anti-abeta (anti-Aβ) antigen (comprising amino acids 1-15 of the human Aβ sequence), a universal T-cell epitope and MPLA adjuvant in a liposomal formulation (ACI-24.060). The vaccine was able to elicit an anti-abeta antibody response in human subjects with AD (prodromal AD) without inducing serious adverse event (SAE) related to the study treatment (investigational product). More specifically, early interim results from the first cohort of (prodromal AD) patients showed that the vaccine when administered to subjects with prodromal AD at 300 µg or 900 µg of antigen, was able to elicit an anti-Abeta antibody response as soon as at week 6, i.e. 2 weeks after the second injection, along with the following clinical observations:

- Safety and tolerability was considered good;
- No SAE related to study treatment was observed;

Accordingly, the invention provides a method of inducing an anti-Aβ immune response in a human subject without inducing a serious adverse event (i.e. a SAE caused by the treatment), the method comprising administering to the human subject a liposomal vaccine composition comprising:

a. A β-amyloid (Aβ)-derived peptide antigen displayed on the surface of the liposome that comprises, consists essentially of or consists of amino acids 1-15 of Aβ
b. A peptide comprising a universal T-cell epitope
c. An adjuvant comprising monophosphoryl lipid A (MPLA)

wherein the β-amyloid (Aβ)-derived peptide antigen is administered in an amount of 300-2000 µg.

Such methods may also be expressed in the form of a medical use. Accordingly, the invention also provides a liposomal vaccine composition comprising:

a. A β-amyloid (Aβ)-derived peptide antigen displayed on the surface of the liposome that comprises, consists essentially of or consists of amino acids 1-15 of Aβ
b. A peptide comprising a universal T-cell epitope
c. An adjuvant comprising monophosphoryl lipid A (MPLA)

for use in inducing an anti-Aβ immune response in a human subject without inducing a serious adverse event (i.e. a SAE caused by the treatment), wherein the β-amyloid (Aβ)-derived peptide antigen is administered in an amount of 300-2000 µg.

Similarly, the invention provides for use of a liposomal vaccine composition comprising:

a. A β-amyloid (Aβ)-derived peptide antigen displayed on the surface of the liposome that comprises, consists essentially of or consists of amino acids 1-15 of Aβ
b. A peptide comprising a universal T-cell epitope
c. An adjuvant comprising monophosphoryl lipid A (MPLA)

in the manufacture of a medicament for use in inducing an anti-Aβ immune response in a human subject without inducing a serious adverse event (i.e. a SAE caused by the treatment), wherein the β-amyloid (Aβ)-derived peptide antigen is administered in an amount of 300-2000 µg.

All embodiments herein apply to such methods or medical uses, however expressed. Throughout the disclosure reference is made to “liposomal vaccine compositions”. This term may be used interchangeably with “liposomal composition”. The compositions are immunogenic and thus may equally be referred to as “liposomal immunogenic compositions”.

As introduced above and described in further detail herein, it has been demonstrated that the liposomal compositions of the invention are safe for administration to human subjects. The compositions are safe when administered at dosages that generate a beneficial anti-Aβ immune response. Safety is measured (assessed) with reference to the absence of any serious adverse event caused by administration of the liposomal vaccine composition. "Serious adverse event", or "SAE", may be defined as any adverse event or adverse reaction that results in death, is life-threatening, requires hospitalisation or prolongation of existing hospitalisation, results in persistent or significant disability or incapacity, or is a congenital anomaly or birth defect. "Life-threatening" in the definition of a serious adverse event 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. Important adverse events/reactions that are not immediately life-threatening or do not result in death or hospitalisation, but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious. Although interpretation of such events requires medical judgement, the investigators participating in human clinical trials are able to determine whether a serious adverse event has occurred during the clinical trial and whether or not this is related to (induced or caused by) the administration of the liposomal vaccine composition. This determination may be based on whether or not the event is considered to be possibly or likely caused by administration of the liposomal vaccine composition. For the avoidance of doubt, it is possible that a serious adverse event may occur in a given subject which is not related to (induced or caused by) administration of the liposomal vaccine composition. This is not precluded by the invention.

Specific SAEs which are not induced (as demonstrated to date) when the liposomal vaccine compositions of the invention are administered include for example, but are not limited to:

- CNS inflammation or other important unwanted reactions to the vaccine;
- Meningoencephalitis;

As discussed above, in a previous study (Orgogozo, 2003) some patients developed an inflammatory reaction considered to be due to a T-cell-mediated response against full length Aβ1-42. This T-cell-mediated response against full length Aβ1-42 is avoided using the liposomal compositions of the invention, which are based on Aβ1-15. Aβ-specific T-cell activation can be evaluated using enzyme-linked immune absorbent spot (ELISpot), which

is a type of assay that focuses on quantitatively measuring the frequency of cytokine secretion for a single cell.

Amyloid-related imaging abnormalities (ARIA) are abnormal signals seen in neuroimaging of Alzheimer's Disease patients, that may be observed with amyloid-modifying therapies. ARIA-E refers to cerebral edema, involving the breakdown of the tight endothelial junctions of the blood-brain barrier and subsequent accumulation of fluid. ARIA-H refers to cerebral microhaemorrhages (mH), small haemorrhages in the brain, often accompanied by hemosiderosis.

As presented herein, and unless otherwise specified, dosage amounts relate to the per dose administration amount of the β-amyloid (Aβ)-derived peptide antigen in the liposomal vaccine composition. Thus, the dosages are, unless otherwise specified, expressed with reference to tetrapalmitoylated Abeta 1-15 as described herein and also in SEQ ID NO: 1:

SEQ ID NO: 1 - Tetrapalmitoylated Abeta 1-15
H-Lys(palmitoyl)-Lys(palmitoyl)-Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys(palmitoyl)-Lys(palmitoyl)-OH

Where particular values are specified, these values are subject to manufacturing tolerances as would be appreciated by one skilled in the art. Typically, the specified dose covers 15% variation either side of the indicated value. For example, a specified dose of 1000 µg of β-amyloid (Aβ)-derived peptide antigen encompasses from 850 to 1150 µg of β-amyloid (Aβ)-derived peptide antigen. The term “anti-Aβ immune response” refers to the production of anti-Aβ antibodies that bind to Aβ by the human subject in response to administration of the liposomal vaccine composition. The response may thus also be referred to as an anti-Aβ antibody response. The antibodies may comprise antibodies of IgM isotype. The antibodies preferably comprise antibodies of IgG isotype. The antibody response is typically polyclonal. This response can be measured in suitable samples taken from the human subject, such as a serum-containing sample. Thus, the sample may comprise, or be derived from, a blood sample. The antibodies preferably bind to pathological forms of Aβ, defined as forms of Aβ that comprise β-sheet multimers. The antibodies produced may therefore be termed “Aβ-specific” antibodies. The anti-Aβ immune response may be measured by any suitable method, such as an ELISA. For example, the anti-Aβ immune response may be measured by a method in which Aβ, such as Aβ1-42, is coated on a solid support to which is applied

the sample from the human subject. A secondary antibody may be used to detect binding of antibodies from the sample to the immobilized Aβ. Such methods may be quantitative. The secondary antibody may be an anti-Ig antibody, thereby permitting all isotypes to be detected. The secondary antibody may be an anti-IgG antibody. This may permit Aβ-specific IgG titers to be measured.

Thus, according to all aspects of the invention, the β-amyloid (Aβ)-derived peptide antigen (dosage expressed for tetrapalmitoylated Abeta 1-15 as set forth in SEQ ID NO: 1) is administered in an amount of 300-2000 µg. This dosage combines a good safety (including no induced SAE) with the ability to generate an anti-Aβ immune response. According to some embodiments, the β-amyloid (Aβ)-derived peptide antigen is administered in an amount of 300-2000 µg, preferably 300-1600 µg, preferably 300-1000 µg, more preferably 300-900 µg. In certain embodiments, the β-amyloid (Aβ)-derived peptide antigen (dosage expressed for tetrapalmitoylated Abeta 1-15 as set forth in SEQ ID NO: 1) is administered in an amount of 300 µg. In certain embodiments, the β-amyloid (Aβ)-derived peptide antigen (dosage expressed for tetrapalmitoylated Abeta 1-15 as set forth in SEQ ID NO: 1) is administered in an amount of 900 µg. In certain other embodiments, the β-amyloid (Aβ)-derived peptide antigen (dosage expressed for tetrapalmitoylated Abeta 1-15 as set forth in SEQ ID NO: 1) is administered in an amount of 1600 µg. In a preferred embodiment, the β-amyloid (Aβ)-derived peptide antigen of SEQ ID NO: 1 (tetrapalmitoylated Abeta 1-15) is administered in an amount of 300-1600 µg. In one preferred embodiment, the β-amyloid (Aβ)-derived peptide antigen of SEQ ID NO: 1 (tetrapalmitoylated Abeta 1-15) is administered in an amount of 300-900 µg.

As would be readily appreciated by one skilled in the art, dosages may alternatively be expressed with reference to the equivalent amount of Abeta 1-15 alone (i.e. without lysine residues and palmitoylation) as described herein and also in SEQ ID NO: 2:

SEQ ID NO: 2 - Abeta 1-15
H-Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-OH

The Aβ-derived peptide antigen is displayed, at least partially, on the outer surface of the liposome. “Displayed on the surface of the liposome” means that the peptide is presented, at least partially, on the external surface of a(n intact) liposome, as would be understood by one skilled in the art (see e.g. Muhs, 2007, Pihlgren, 2013). This is typically by insertion into,

or otherwise anchoring to, the outer surface of the liposome. Insertion into the outer surface of the liposome may be facilitated through attachment of the Aβ-derived peptide antigen to a moiety that inserts into the outer surface of the liposome. The liposome may be any liposome that is suitable to present the Aβ-derived peptide antigen on the surface. Typically, the moiety comprises a hydrophobic moiety to ensure insertion into the lipid bilayer of a liposome. The moiety may be any suitable moiety but is preferably a fatty acid. Thus, in preferred embodiments, the β-amyloid (Aβ)-derived peptide antigen is lipidated. The fatty acid may comprise a palmitoyl residue. The β-amyloid (Aβ)-derived peptide antigen may therefore be palmitoylated. A preferred construction comprises the Aβ-derived peptide antigen (Aβ(1-15)) attached to two palmitoyl residues in the N and C terminal regions of the peptide. Thus, the peptide antigen is tetrapalmitoylated. This may be facilitated by incorporating two amino acids, such as lysine, residues in the N and C terminal regions of the Aβ-derived peptide antigen. The amino acid, such as lysine, residues are palmitoylated.

In some embodiments, the liposome has a negative surface charge; the liposome is anionic. Preferably, the liposome comprises phospholipids and even more preferably, the phospholipids comprise dimyrsitoylphosphatidyl-choline (DMPC) and dimyrsitoylphosphatidyl-glycerol (DMPG). The liposome may further comprise cholesterol. The molar ratios of these three components may be 9:1:7 in some embodiments.

A most preferred construction therefore comprises the Aβ-derived peptide antigen reconstituted in the liposome. Accordingly, these compositions of the invention may generally be referred to herein as “liposomal vaccine compositions of the invention”.

The Aβ-derived peptide antigen induces a B-cell response in the subject. It is a “B-cell antigen”. B-cells are activated to proliferate and produce immunoglobulin (Ig) by cross-linking the B-cell surface Ig receptor. As already explained, Aβ plaques are formed by the 39 to 43 amino acid long Aβ peptide, which is in random coil conformation in its natural non-pathological form. During the transition to the pathological state, it transforms mainly into a β-sheet secondary structure, spontaneously aggregating into insoluble deposits. The Aβ-derived peptide antigen comprises, consists essentially of or consists of amino acids 1-15 of Aβ, which may be referred to as “Aβ(1-15)” (WO2007/068411, ACI-24). In this context, the term “consists essentially of” means that the Aβ-derived peptide antigen includes the 15 contiguous amino acids 1-15 of Aβ but can include a limited number of additional residues, such as four lysine residues to facilitate palmitoylation.

The Aβ-derived peptide antigen included in the compositions of the invention adopts a secondary structure that replicates a pathological form of Aβ. Preferably, the Aβ-derived peptide antigen adopts a secondary structure comprising a β-sheet conformation. Even more preferably, the Aβ-derived peptide antigen adopts a predominantly β-sheet conformation when displayed on the surface of the liposome.

The Aβ-derived peptide antigen included in the compositions of the invention is a synthetic peptide. In some embodiments, the Aβ-derived peptide antigen is produced by chemical synthesis.

The liposomal vaccine compositions comprise a universal T-cell epitope. The universal T-cell epitope may be, at least in part, encapsulated in the liposome. By “universal T-cell epitope” is meant an epitope that is specific to T-cells that are present in the majority of the human population. They commonly originate from antigens to which humans are normally exposed during their lifetime. Examples include antigens incorporated in routinely administered vaccines. Specific examples are T-cell epitopes included in tetanus, influenza and diphtheria, and also Keyhole limpet hemocyanin (KLH) and Epstein Barr virus (EBV). The “universal” ability of a T-cell epitope to activate T cells is the result of at least two complementary properties: i) affinity of binding to the HLA groove, meaning the strength of the binding, as well as ii) its capacity to bind different HLA haplotypes in a promiscuous manner, meaning the ability to cover very diverse human populations, with regards to the differences in the expression of HLA molecules. The universal T-cell epitopes may bind to a majority of MHC class II alleles present in the human population. The universal T-cell epitopes included in the vaccine compositions of the invention may thus be capable of stimulating a CD4 T-cell response. The universal T-cell epitopes included in the vaccine compositions of the invention may thus be capable of stimulating a helper T-cell response that enhances (Aβ-specific) antibody production by B-cells.

The vaccine composition may comprise two, three or four different universal T-cell epitopes. It is preferred that multiple different universal T-cell epitopes are included in the same peptide. Thus, synthetic peptide constructs containing multiple different universal T-cell epitopes are preferred. In certain embodiments the peptide comprises two, three or four universal T-cell epitopes. Where at least two universal T-cell epitopes are included in a synthetic peptide construct they may be joined by a linker. The linker is used to physically

connect the universal T-cell epitopes to one another in a manner that does not detract from the immunogenicity of the linked epitopes. Suitable linkers for joining amino acids to one another are well known in the art. Preferred linkers are themselves amino acid based linkers, i.e. peptide linkers. They can thus join the universal T-cell epitopes to one another through peptide bonds. The linker is one which enables correct processing of the universal T-cell epitopes. Antigen presentation by MHC class II molecules requires the entry of antigens into the endosomal-lysosomal compartment. These antigens are then processed by proteolytic enzymes, of which the lysosomal cysteine proteases of the papain family constitute an important subset. The generated peptides bind to MHC class II molecules, which are then displayed at the surface of professional antigen presenting cells (APCs) including macrophages, dendritic cells (DCs) and B cells (Lutzner and Kalbacher 2008). Thus, preferably the linker comprises a substrate for a lysosomal cysteine protease of the papain family. The linker may comprise a substrate for one or more of cathepsin S, cathepsin B and cathepsin L. In some embodiments, the linker comprises, consists essentially of, or consists of at least two or at least three amino acids. In some embodiments, the linker comprises, consists essentially of, or consists of the amino acids VVR, TVGLR, KVSVR, PMGAP or PMGLP.

The peptides comprising two universal T-cell epitopes may, therefore, be linear peptides in the format:

[universal T-cell epitope 1]–[linker]-[universal T-cell epitope 2]

The peptides comprising three universal T-cell epitopes may, therefore, be linear peptides in the format:

[universal T-cell epitope 1]–[linker]-[universal T-cell epitope 2]-[linker]-[universal T-cell epitope 3]

The peptides comprising four universal T-cell epitopes may, therefore, be linear peptides in the format:

[universal T-cell epitope 1]–[linker]-[universal T-cell epitope 2]-[linker]-[universal T-cell epitope 3]-[linker]-[universal T-cell epitope 4]

It should be noted that the linkers do not have to be identical between each pair of linked universal T-cell epitopes. Thus, for example, the linker between universal T-cell epitope 1 and universal T-cell epitope 2 could be different from the linker between universal T-cell

epitope 2 and universal T-cell epitope 3. In the case of four universal T-cell epitopes each of the three linkers could be different or two could be the same and the third different (in any order). In some embodiments where multiple linkers are included in the peptide they are all identical.

In some embodiments, the universal T-cell epitopes are derived from diphtheria toxin, tetanus toxin, Epstein Barr Virus, influenza hemagglutinin and/or keyhole limpet hemocyanin. Preferred combinations of universal T-cell epitopes are therefore selected from:

a. A combination of a diphtheria toxin and tetanus toxin universal T-cell epitope
b. A combination of an Epstein Barr Virus and tetanus toxin universal T-cell epitope
c. A combination of an Epstein Barr Virus, tetanus toxin and keyhole limpet hemocyanin universal T-cell epitope; or
d. A combination of an influenza hemagglutinin, diphtheria toxin, tetanus toxin and Epstein Barr Virus universal T-cell epitope

Preferably the liposomal vaccine composition employs a synthetic peptide construct containing multiple different universal T-cell epitopes joined by peptide linkers. Suitable universal T-cell epitopes and peptides comprising multiple universal T-cell epitopes include those described in WO2019/197414, incorporated herein by reference.

The peptide comprising a universal T-cell epitope may be synthesized by solid phase synthesis. In some embodiments, the peptide comprising a universal T-cell epitope may comprise at most 85 amino acids. Optionally the peptide containing at least one T-cell epitope may have a maximum of 80, 75 or 70 amino acids in length. In some embodiments the peptide containing at least one T-cell epitope may comprise at least 10 amino acids. Using a peptide having at least 10 amino acids may help ensure that a sufficiently immunogenic T-cell epitope is generated. Optionally, the peptide containing at least one T-cell epitope may comprise upwards of 10 amino acids. For example, the peptide containing at least one T-cell epitope may comprise at least 20, 30, 40 amino acids. In other embodiments the peptide may comprise between 30 and 60 amino acids. This is based on the preferred minimum length per universal T-cell epitope and the preference for a peptide comprising at least two, three or four T cell epitopes.

Examples of suitable universal T-cell epitopes include peptides comprising, consisting essentially of or consisting of an amino acid sequence selected from SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, and SEQ ID NO.12. The composition of these peptides is explained in more detail with reference to Table 1 below.

Table 1

Name	**Sequence**	**Peptide origin**
SEQ ID NO: 8	PIFFLHHSNTDRLWAI	KLH
SEQ ID NO: 9	IVAQSIALSS	Diphtheria Toxin
SEQ ID NO: 10	SMGVYQILAIYST	Influenza hemagglutinin
SEQ ID NO: 11	LYNLRRGTAL	Epstein Barr Virus
SEQ ID NO: 12	SAGVYQILAIYST	Influenza hemagglutinin

In an embodiment the universal T-cell epitope comprises, consists essentially of, or consists of an amino acid sequence selected from the group SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, and SEQ ID NO.12, or an analogue thereof. Analogues are functionally equivalent sequences, in terms of ability to stimulate T-helper cells, that may comprise one or more modifications compared to the sequences recited. They may comprise one or more (preferably one or two) additions, deletions or substitutions provided function as a universal T-cell epitope is retained. The minimum and maximum lengths of the peptide are set out above and apply *mutatis mutandis* to the analogue. Where the peptide contains more than one T-cell epitope, the minimum length of the peptide is adjusted accordingly to preserve function of each T-cell epitope. In an embodiment the universal T-cell epitope comprises an amino acid sequence selected from the group SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, and SEQ ID NO.12. In an embodiment the universal T-cell epitope consists essentially of or consists of an amino acid sequence selected from the group SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, and SEQ ID NO.12.

Combinations of these peptides can also be included in the vaccine compositions of the invention. The combined peptides are preferably joined by one or more linkers as defined hereinabove.

For example, the peptide comprising a universal T-cell epitope may comprise, consist essentially of, or consist of at least two universal T-cell epitopes, each having an amino acid sequence selected from the group consisting of SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, or an analogue thereof.

For example, the peptide comprising a universal T-cell epitope may comprise two, three or four universal T-cell epitopes, each having amino acid sequence selected from the group consisting of SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12.

In an embodiment the T-cell epitope comprises an amino acid sequence SEQ ID NO: 8. In an embodiment the T-cell epitope comprises an amino acid sequence SEQ ID NO: 9. In an embodiment the T-cell epitope comprises an amino acid sequence SEQ ID NO: 11. In one embodiment the peptide comprising a T-cell epitope comprises an amino acid sequence SEQ ID NO: 10. In another embodiment the peptide comprising a T-cell epitope comprises an amino acid sequence SEQ ID NO: 12.

In one embodiment the peptide comprising a T-cell epitope comprises an amino acid sequence SEQ ID NO: 11 and at least an amino acid sequence SEQ ID NO: 8.

In one embodiment the peptide comprising a T-cell epitope comprises an amino acid sequence SEQ ID NO: 10 and at least one of an amino acid sequence SEQ ID NO: 9 and/or an amino acid sequence SEQ ID NO: 11. In one embodiment the peptide comprising a T-cell epitope comprises an amino acid sequence SEQ ID NO: 12 and at least one of an amino acid sequence SEQ ID NO: 9 and/or an amino acid sequence SEQ ID NO: 11.

In one embodiment the peptide comprising a T-cell epitope comprises an amino acid sequence SEQ ID NO: 10, an amino acid sequence SEQ ID NO: 9, and an amino acid sequence SEQ ID NO: 11. In one embodiment the peptide comprising a T-cell epitope comprises an amino acid sequence SEQ ID NO: 12, an amino acid sequence SEQ ID NO: 9, and an amino acid sequence SEQ ID NO: 11.

The universal T-cell epitopes are preferably joined by one or more linkers, preferably peptides linkers more preferably the one or more linker(s) comprise, consist essentially of, or consist of the amino acids VVR, TVGLR, KVSVR, PMGAP or PMGLP. In one embodiment the linkers comprise, consist essentially of, or consist of the amino acids VVR.

For example, the peptide comprising a universal T-cell epitope may comprise, consist essentially of, or consist of at least two universal T-cell epitopes, each having an amino acid sequence selected from the group consisting of SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, wherein the universal T-cell epitopes are joined by one or more linkers, preferably wherein the one or more linker(s) comprise, consist essentially of, or consist of the amino acids VVR, TVGLR, KVSVR, PMGAP or PMGLP, more preferably wherein the one or more linker(s) comprise, consist essentially of, or consist of the amino acids VVR.

In one embodiment the peptide comprising a T-cell epitope comprises an amino acid sequence SEQ ID NO: 10, an amino acid sequence SEQ ID NO: 9, and an amino acid sequence SEQ ID NO: 11 wherein the universal T-cell epitopes are joined by one or more linkers comprising, consisting essentially of, or consisting of the amino acids VVR, TVGLR, KVSVR, PMGAP or PMGLP, preferably VVR. In one embodiment the peptide comprising a T-cell epitope comprises an amino acid sequence SEQ ID NO: 12, an amino acid sequence SEQ ID NO: 9, and an amino acid sequence SEQ ID NO: 11, wherein the universal T-cell epitopes are joined by one or more linkers comprising, consisting essentially of, or consisting of the amino acids VVR, TVGLR, KVSVR, PMGAP or PMGLP, preferably VVR.

Examples of suitable peptides comprising multiple different universal T-cell epitopes include peptides comprising, consisting essentially of or consisting of an amino acid sequence selected from the group consisting of SEQ ID NO: 3 (SAT42), SEQ ID NO: 4 (SAT43), SEQ ID NO: 5 (SAT44), SEQ ID NO: 6 (SAT47), SEQ ID NO: 7 (SAT 58). The composition of these peptides is explained in more detail with reference to Table 2 below.

Table 2.

Name	Sequence	Peptide design	Peptide origin
SAT42 (SEQ ID NO: 3)	VHHNTEEIVAQSIALSSLMV PMGAPQYIKANSKFIGITEL	SEQ ID NO: 9 + PMGAP + Tetanus toxin	Diphtheria Toxin+Tetanus toxin
SAT43 (SEQ ID NO: 4)	VYGGSKTSLYNLRRGTALA IVVRQYIKANSKFIGITELVV RPIFFLHHSNTDRLWAI	SEQ ID NO: 11 + VVR + Tetanus toxin + VVR + SEQ ID NO: 8	Epstein Barr+ Tetanus+ KLH
SAT44 (SEQ ID NO: 5)	VYGGSKTSLYNLRRGTALA IVVRQYIKANSKFIGITEL	SEQ ID NO: 11 + VVR + Tetanus toxin	Epstein Barr+ Tetanus
SAT47 (SEQ ID NO: 6)	SMGVYQILAIYSTVVRIVAQ SIALSSVVRYIKANSKFIGV VRLYNLRRGTAL	SEQ ID NO: 10 + VVR + SEQ ID NO: 9 + VVR + Tetanus + VVR + SEQ ID NO: 11	Influenza hemagglutinin+ Diphtheria+Tetan us+ Epstein Barr
SAT58 (SEQ ID NO: 7)	SAGVYQILAIYSTVVRIVAQ SIALSSVVRYIKANSKFIGV VRLYNLRRGTAL	SEQ ID NO: 12 + VVR + SEQ ID NO: 9 +VVR+Tetanus+ VVR + SEQ ID NO: 11	Influenza hemagglutinin+ Diphtheria+Tetan us+ Epstein Barr

In an embodiment the peptide comprising a universal T-cell epitope used herein comprises, consists essentially of or consists of an amino acid sequence selected from the group consisting of SEQ ID NO: 5 (SAT44), SEQ ID NO: 6 (SAT47), SEQ ID NO: 7 (SAT 58), SEQ ID NO: 3 (SAT42), SEQ ID NO: 4 (SAT43) or analogues thereof. An analogue of these peptides is as defined herein and accounts for the fact that each peptide includes 2-4 separate (universal) T-cell epitopes. Thus, the minimum length of an analogue here is 20-40 amino acids to ensure that the functionality of each T-cell epitope is preserved (the minimum length per T-cell epitope being 10 amino acids). Peptides or analogues may incorporate alternative linker sequences as described herein. Preferably the peptide comprising a universal T-cell epitope used herein comprises, consists essentially of or consists of an amino acid sequence selected from SEQ ID NO: 5 (SAT44), SEQ ID NO: 6 (SAT47), SEQ ID NO: 7 (SAT 58), or analogues thereof. In one embodiment the peptide comprising a universal T-cell epitope used herein comprises, consists essentially of or

consists of an amino acid sequence selected from SEQ ID NO: 6 (SAT47) and SEQ ID NO: 7 (SAT 58). In one embodiment the peptide comprising a universal T-cell epitope used herein comprises, consists essentially of or consists of the amino acid sequence SEQ ID NO: 7 (SAT 58).

In an embodiment the peptide comprising a universal T-cell epitope may be administered in an amount of 40 µg to 700 µg. Typically, the specified dose covers 30% variation either side of the indicated value. The peptide comprising a universal T-cell epitope may be included in the compositions at a dose that correlates with the dose of the β-amyloid (Aβ)-derived peptide antigen. Thus, for example, a liposomal vaccine composition in which the β-amyloid (Aβ)-derived peptide antigen (dosage expressed for tetrapalmitoylated Abeta 1-15 as set forth in SEQ ID NO: 1) is administered in an amount of 900 µg (which may be +/- 15% in view of manufacturing tolerances) may comprise a peptide comprising a universal T-cell epitope administered in an amount of 270 µg (which may be +/- 30% in view of manufacturing tolerances) up to an amount of 360 µg (which may be +/- 30% in view of manufacturing tolerances). Similarly, a liposomal vaccine composition in which the β-amyloid (Aβ)-derived peptide antigen (dosage expressed for tetrapalmitoylated Abeta 1-15 as set forth in SEQ ID NO: 1) is administered in an amount of 300 µg (which may be +/- 15% in view of manufacturing tolerances) may comprise a peptide comprising a universal T-cell epitope adjuvant administered in an amount of 90 µg (which may be +/- 30% in view of manufacturing tolerances) up to an amount of 120 µg (which may be +/- 30% in view of manufacturing tolerances). Similarly, a liposomal vaccine composition in which the β-amyloid (Aβ)-derived peptide antigen (dosage expressed for tetrapalmitoylated Abeta 1-15 as set forth in SEQ ID NO: 1) is administered in an amount of 1600 µg (which may be +/- 15% in view of manufacturing tolerances) may comprise a peptide comprising a universal T-cell epitope adjuvant administered in an amount of 470 µg (which may be +/- 30% in view of manufacturing tolerances) up to an amount of 650 µg (which may be +/- 30% in view of manufacturing tolerances).

The liposomal vaccine compositions comprise an adjuvant that comprises monophosphoryl lipid A (MPLA) (“MPLA adjuvant”). Lipid A based adjuvants derive from lipopolysaccharide (they are chemically modified to reduce toxicity) and have been proven to be safe and effective. The MPLA adjuvant used herein is preferably a synthetic monophosphoryl lipid A (MPLA). As defined herein, the term MPLA encompasses MPLA-derivatives such as Monophosphoryl Hexa-acyl Lipid A, 3-Deacyl (Synthetic) (3D-(6-acyl) PHAD®), PHAD®

(Phosphorylated HexaAcyl Disaccharide) and MPL. The MPLA adjuvant may be a Toll-like receptor (TLR) agonist, in particular a TLR4 agonist. The purpose of the adjuvant(s) is to increase or stimulate the immune response in the subject. Preferably, the at least one MPLA adjuvant forms part of a liposome; it may form part of the lipid bilayer. The MPLA adjuvant may be, at least in part, displayed on the outer surface of the liposome; this may be as a consequence of the adjuvant forming part of at least the outer layer of the lipid bilayer. The liposome may effectively function as an adjuvant with the addition of monophosphoryl lipid A (MPLA). The MPLA adjuvant typically forms part of the outer layer of the liposome. The MPLA is typically added during liposomal formation (as explained further herein). Preferred liposomes thus comprise dimyrsitoylphosphatidyl-choline (DMPC), dimyrsitoylphosphatidyl-glycerol (DMPG), cholesterol and MPLA. The molar ratios of these four components may be 9:1:7:0.05 in some embodiments.

In some embodiments of the invention, the compositions of the invention comprise two different adjuvants. Additional adjuvants that may be employed according to the invention include aluminium hydroxide (Alum) and/or CpG amongst others. One or more MPLA adjuvants forming part of a liposome may be combined with an encapsulated adjuvant in some embodiments. In other embodiments, one or more MPLA adjuvants forming part of a liposome may be mixed with a further adjuvant (such as Alum or CpG) when forming the liposomes.

The MPLA adjuvant may be administered in an amount of 15-600 µg. Typically, the specified dose covers 40% variation either side of the indicated value. The MPLA adjuvant may be included in the compositions at a dose that correlates with the dose of the β-amyloid (Aβ)-derived peptide antigen. Thus, for example, a liposomal vaccine composition in which the β-amyloid (Aβ)-derived peptide antigen (dosage expressed for tetrapalmitoylated Abeta 1-15 as set forth in SEQ ID NO: 1) is administered in an amount of 900 µg (which may be between +/- 15% in view of manufacturing tolerances) may comprise an MPLA adjuvant administered in an amount of 200 µg (which may be +/- 40% in view of manufacturing tolerances) up to an amount of 270 µg (which may be +/- 40% in view of manufacturing tolerances). Similarly, a liposomal vaccine composition in which the β-amyloid (Aβ)-derived peptide antigen (dosage expressed for tetrapalmitoylated Abeta 1-15 as set forth in SEQ ID NO: 1) is administered in an amount of 300 µg (which may be +/- 15% in view of manufacturing tolerances) may comprise an MPLA adjuvant administered in an amount of 65 µg (which may be +/- 40% in view of manufacturing tolerances) or up to an amount of 90

µg (which may be +/- 40% in view of manufacturing tolerances). Similarly, a liposomal vaccine composition in which the β-amyloid (Aβ)-derived peptide antigen (dosage expressed for tetrapalmitoylated Abeta 1-15 as set forth in SEQ ID NO: 1) is administered in an amount of 1600 µg (which may be +/- 15% in view of manufacturing tolerances) may comprise an MPLA adjuvant administered in an amount of 360 µg (which may be +/- 40% in view of manufacturing tolerances) or up to an amount of 480 µg (which may be +/- 40% in view of manufacturing tolerances). This dosage contributes to the safety and efficacy (in terms of the ability to generate an anti-Aβ immune response) of the liposomal vaccine composition. According to some embodiments, the MPLA adjuvant is administered in an amount of 15-600 µg, preferably 40-450 µg. As presented herein, where particular values are specified, these values are subject to manufacturing tolerances as would be appreciated by one skilled in the art.

In one embodiment the invention provides a liposomal vaccine composition comprising:

a. A β-amyloid (Aβ)-derived peptide antigen displayed on the surface of the liposome that comprises, consists essentially of or consists of amino acids 1-15 of Aβ; wherein the β-amyloid (Aβ)-derived peptide antigen is tetrapalmitoylated Abeta 1-15 as set forth in SEQ ID NO: 1;
b. A peptide comprising a universal T-cell epitope comprising, consisting of, or essentially consisting of the amino acid sequence of SEQ ID NO: 7 (SAT 58), or an analogue thereof, and
c. an adjuvant comprising monophosphoryl lipid A (MPLA), preferably 3D-(6-acyl) PHAD®

for use in inducing an anti-Aβ immune response in a human subject without inducing a serious adverse event, wherein the β-amyloid (Aβ)-derived peptide antigen is administered in an amount of 300-2000 µg, preferably in an amount of 300-1600 µg, such as in an amount of 300 µg, 900 µg or 1600 µg, preferably in an amount of 300 µg or 900 µg.

In another aspect the invention provides a liposomal vaccine composition comprising:

a. A β-amyloid (Aβ)-derived peptide antigen displayed on the surface of the liposome that comprises, consists essentially of or consists of amino acids 1-15 of Aβ, wherein the β-amyloid (Aβ)-derived peptide antigen is tetrapalmitoylated Abeta 1-15 as set forth in SEQ ID NO: 1;

b. A peptide comprising a universal T-cell epitope comprising, consisting of, or essentially consisting of the amino acid sequence of SEQ ID NO: 7 (SAT 58), or an analogue thereof
c. an adjuvant comprising monophosphoryl lipid A (MPLA), preferably 3D-(6-acyl) PHAD®.

In one preferred embodiment the vaccine composition comprises the β-amyloid (Aβ)-derived peptide antigen in an amount of 300 µg, the peptide comprising a universal T-cell epitope in an amount of between 90 and 120 µg, and the MPLA adjuvant in an amount of between 65 and 90 µg.

In another preferred embodiment the liposomal vaccine composition comprises the β-amyloid (Aβ)-derived peptide antigen in an amount of 900 µg, the peptide comprising a universal T-cell epitope in an amount of between 270 and 360 µg, and the MPLA in an amount of between 200 and 270 µg.

In another preferred embodiment the liposomal vaccine composition comprises the β-amyloid (Aβ)-derived peptide antigen in an amount of 1600 µg, the peptide comprising a universal T-cell epitope in an amount of between 470 and 650 µg, and the MPLA in an amount of between 360 and 480 µg.

The liposomal vaccine compositions of the invention may be synthesised through known means. See for example WO2005/081872, WO2012/020124, WO2012/055933 and WO2013/044147, WO2019/197414, each of which is hereby incorporated by reference.

The liposomal vaccine compositions may be administered to the subject by any appropriate route of administration. As the skilled person would be aware, vaccine compositions may be administered by topical, oral, rectal, nasal or parenteral (such as intravenous, intradermal, subcutaneous, or intramuscular) routes. In addition, vaccine compositions may be incorporated into sustained release matrices such as biodegradable polymers, the polymers being implanted in the vicinity of, or in close proximity to, where delivery is desired. However, in preferred embodiments, the vaccine composition is administered by injection, most preferably intramuscularly or subcutaneously. Typical volumes of the injectable dosage forms of the liposomal vaccine compositions are between 0.01 to 10 ml, such as 0.75 to 2.5 ml.

The liposomal vaccine compositions may be administered a single time to the subject to generate a protective immune response. However, generally, the liposomal vaccine compositions are administered multiple times to the same subject. Thus, so-called prime-boost regimens may be employed according to the invention. Administration of the vaccine is typically separated by an intervening period of at least 1 week and often around 1-12 months. Safety and efficacy (in terms of the ability to generate an anti-Aβ immune response) has been confirmed for the liposomal vaccine compositions when administered regularly over a long period of time. In some embodiments, the liposomal vaccine composition is administered at a first time and is administered at a second time 1 to 4 weeks later. The liposomal vaccine composition may be administered 2, 3, 4, 5, 6, 7, 8, 9, 10 or more times provided a suitable period of time is allowed between administrations. The liposomal vaccine composition may be administered 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 times over the course of a 12 month period provided a suitable period of time is allowed between administrations. The liposomal vaccine composition may be administered indefinitely provided a suitable period of time is allowed between administrations. A suitable period of time is typically at least 1 week and often around 1-12 months. The period of time may be based on monitoring of the individual subject. Monitoring may comprise monitoring the disease status of the subject and/or monitoring levels of immune response of the subject over time. Tests (e.g. MMSE, amyloid PET-scan or anti-Aβ immune response) are described herein that allow the course of disease to be followed. In prophylactic applications, the liposomal vaccine compositions may be administered less frequently compared to therapeutic methods, and may be administered according to a regular schedule. Monitoring may be employed in the context of prophylactic methods. For example, in subjects with a predisposition to developing an amyloid-beta associated disease or condition or a condition characterised by, or associated with, loss of cognitive memory capacity. Suitable tests and biomarkers are described herein and include monitoring brain Abeta levels using amyloid PET-scan (which may be absent in early prevention), monitoring AD progression biomarkers such as, but not limited to, Tau, phosphorylated Tau (eg., pTau 217, pTau 181) and Abeta levels (Aβ1–42 and Aβ1–40) in blood and/or CSF, Neurofilament light Chain (NfL) , Glial fibrillary acidic protein (GFAP), in blood and/or CSF, measuring efficacy notably, but not limited to, on clinical/cognitive/function parameters and measuring immune response in serum and/or CSF including, but not limited to anti-Abeta1-42 IgM titers and/or anti-Abeta1-42 IgG titers in blood and/or CSF.

Where time periods for a vaccination regimen are described herein, the initial administration of the liposomal vaccine composition is considered time zero (0). In some embodiments, the liposomal vaccine composition is administered every 4-12 weeks for a period of at least 48 weeks. For example, the liposomal vaccine composition may be administered in five separate administrations at weeks 0, 4, 12, 24 and 48. In another embodiment the liposomal vaccine composition may be administered in six separate administrations at weeks 0, 4, 8 and 12, 24, 48 and 74.

According to all administration regimes, the liposomal vaccine composition may be additionally administered as required at a later time point(s). Typically this is after the completion of the initial administration schedule ("the schedule"). It may thus be referred to as a "booster" administration. Such a further administration may occur at a suitable time point after completion of the initial administration schedule; such as 4, 12, 24, 26, 36, or 48 weeks after the final administration according to the schedule or longer such as 1, 2, 2.5, 3, 3.25, 3.5, 4, 5 or more years after the final administration according to the schedule.

The liposomal vaccine compositions may be administered such that an induced anti-Aβ immune response is obtained following two administrations of the liposomal vaccine. Preferably, the liposomal vaccine compositions may be administered at a first time and administered at a second time 2 to 6 weeks later, preferably wherein the second administration is 4 weeks after the first administration.

In a preferred embodiment, the liposomal vaccine compositions may be administered at a third time 6 to 10 weeks after the second administration, preferably wherein the third administration is 8 weeks after the second administration..

As already indicated, the liposomal vaccine compositions induce an anti-Aβ immune response in a human subject without inducing a serious adverse event. In some embodiments, administration of the liposomal vaccine composition results in a reduction in the amount of Aβ associated plaques in the brain of the subject. This may be measured by methods known in the art, such as a PET scan (using appropriate amyloid tracers, e.g. as discussed herein). The liposomal vaccine compositions may be administered to human subjects in order to treat, prevent, induce a protective immune response against or alleviate the symptoms associated with an amyloid-beta associated disease or condition or a condition characterised by, or associated with, loss of cognitive memory capacity. The liposomal

vaccine compositions may thus be administered for both prophylactic and therapeutic purposes in human subjects.

The amyloid-beta associated disease or condition may be a neurological disorder such as (and in particular) Alzheimer's Disease (AD). Other examples of amyloid-beta associated diseases or conditions according to the invention include mild cognitive impairment (MCI), Down syndrome (DS), including Down syndrome-related Alzheimer's disease, cardiac amyloidosis, cerebral amyloid angiopathy (CAA), multiple sclerosis, Parkinson's disease, Lewy body dementia, ALS (amyotrophic lateral sclerosis), Adult Onset Diabetes, inclusion body myositis (IBM), ocular amyloidosis, glaucoma, macular degeneration, lattice dystrophy and optic neuritis. Many of these conditions are characterized by, or associated with, loss of cognitive memory capacity. Conditions characterized by, or associated with, loss of cognitive memory capacity according to the invention therefore include AD, mild cognitive impairment (MCI), Down syndrome, including Down syndrome-related Alzheimer's disease, cardiac amyloidosis, cerebral amyloid angiopathy (CAA), multiple sclerosis, Parkinson's disease, Lewy body dementia, ALS (amyotrophic lateral sclerosis) and inclusion body myositis (IBM).

Thus, the invention is directed to treatment and prevention of an amyloid-beta associated disease or condition or a condition characterized by, or associated with, loss of cognitive memory capacity, comprising administering the vaccine of the invention. The amyloid-beta associated disease or condition or a condition characterized by, or associated with, loss of cognitive memory capacity, includes Alzheimer's Disease, mild cognitive impairment (MCI), Down syndrome (DS), including Down syndrome-related Alzheimer's disease, cardiac amyloidosis, cerebral amyloid angiopathy (CAA), multiple sclerosis, Parkinson's disease, Lewy body dementia, ALS (amyotrophic lateral sclerosis), Adult Onset Diabetes, inclusion body myositis (IBM), ocular amyloidosis, glaucoma, macular degeneration, lattice dystrophy and optic neuritis, preferably Alzheimer's disease (AD), Down syndrome (DS) and Down syndrome-related Alzheimer's disease.

For AD, it has been observed that intervention may be most effective as early as possible in the development of cognitive impairment. Thus, prophylactic administration may be advantageous, particularly in the presence of other risk factors. In such embodiments, the human subject, prior to treatment, may display an absence of cognitive impairment consistent with a Mini Mental State Examination (MMSE) score of around 30. For the avoidance of doubt, this score indicates no cognitive impairment. The Mini Mental State

Examination (MMSE) (Folstein 1975) is well-known in the field; it is the most commonly used test for complaints of problems with memory or other mental abilities and is used by clinicians to help detect cognitive impairment and to help assess its progression and severity. It consists of a series of questions and tests, each of which scores points if answered correctly. The MMSE tests a number of different mental abilities, including a person's memory, attention and language. The score is from 0 to 30 with 30 being the best possible and 0 being the worst possible score.

In addition, administration to human subjects with early AD may also be beneficial. In some embodiments, the human subject, prior to treatment, displays cognitive impairment consistent with a Mini Mental State Examination (MMSE) score of at least 18 (so 18-30), such as 18-28, preferably at least 20 (so 20-30), such as 20-28. In some embodiments, the human subject is suffering from AD, in particular early AD. Such subjects may display cognitive impairment consistent with a MMSE score of at least 20. Early AD includes prodromal AD (also referred to as mild cognitive impairment due to AD) and mild AD. In some embodiments, the human subject is suffering from prodromal AD. In some embodiments, the human subject is suffering from mild AD. Such subjects may display cognitive impairment consistent with a MMSE score of 20-28. In other embodiments, the subject is not suffering from severe (late stage) AD. In further embodiments, the human subject is suffering from early AD, mild AD, mild to moderate AD, moderate AD- or not severe AD. Such subjects may display cognitive impairment consistent with a MMSE score of at least 12. The global score of Clinical Dementia Rating scale (CDR-GS) may also be used to define the severity of dementia in subjects with AD using a 0–5 point scale, (0 = absent; 0.5 = questionable; 1= present, but mild; 2 = moderate; 3 = severe; 4 = profound; 5 = terminal). Generally, a CDR-GS score of 0 is associated with preclinical AD (asymptomatic stage of AD), a score of 0.5 is associated with prodromal AD (MCI due to AD) and a score of 1 is associated with mild AD dementia.

In specific embodiments, the human subject is suffering from mild to moderate AD. Such subjects may display cognitive impairment consistent with a MMSE score of at 12-28. In specific embodiments, the human subject is suffering from moderate AD. Such subjects may display cognitive impairment consistent with a MMSE score of 12-19.

Other factors that may be included when selecting subjects for treatment include age. For example, the subject may be over 40 years of age.

As already discussed, a key feature of adult subjects with DS is their increased risk of developing (AD pathology along with) similar clinical symptoms of Alzheimer's Disease (AD), characterized by a decline in specific cognitive domains suggestive of a diagnosis of dementia in the most advanced stage. Virtually all subjects with DS older than 40 years exhibit neuropathological changes similar to AD, in the form of senile plaque formation and neurofibrillary tangles (Head, 2012). Thus, when reference is made herein to treating, preventing, inducing a protective immune response against, slowing down progression of symptoms, or alleviate the symptoms associated with DS specifically, it is intended to relate to AD-like symptoms in DS subjects. Preventive treatment may be applied to those subjects without evidence of beta amyloid plaque formation and neurofibrillary tangles. As already discussed, a study using positron emission tomography tracer [11C] Pittsburgh compound B (PiB) to measure brain amyloid burden in DS subjects has shown that an increase of global amyloid-β was related to decline in verbal episodic memory, visual episodic memory, executive functioning, and fine motor processing speed. DS subjects who were consistently PiB+ demonstrated worsening of episodic memory, whereas those who were consistently PiB- evidenced stable or improved performance (Hartley, 2017). Thus, preventive treatment may be applied to those subjects who are PiB- or shown to be amyloid negative by using other amyloid-PET tracers (e.g. florbetaben). Conversely, therapeutic treatment may be applied to those subjects with evidence of beta amyloid plaque formation and neurofibrillary tangles and/or who are PiB+ or shown to be amyloid positive by using other amyloid-PET tracers (e.g, florbetaben). DS is a population at increased risk for AD-like disease. It offers opportunities for exploring effective treatments for AD that will benefit both the DS and general populations. Homogeneity in pathogenesis, age-related disease onset and absence of other dementias powerfully enable prevention trials of AD-like symptoms in DS. A focus in DS subjects is prevention therapy. Biomarker endpoints of Alzheimer pathology may be adopted to monitor the therapy. Examples include, but are not limited to, Abeta levels, total tau, phosphorylated Tau proteins, soluble amyloid precursor protein alpha (sAPPα), soluble amyloid precursor protein beta (sAPPß), Orexin-A, Neurofilament light chain (NfL), GFAP, inflammatory cytokines, angiogenic proteins and vascular injury markers in plasma and/or in CSF, TLR-4 expression may be adopted to monitor the therapy. PET-scan imaging may also be employed, such as using positron emission tomography tracer [11C] Pittsburgh compound B (PiB), Florbetapir or florbetaben, to measure brain amyloid burden in DS subjects (Hartley, 2017), and potentially Tau positron emission tomography tracers such as flortaucipir or PI-2620. Free, total and complexed IgG titers may be measured. Free, total and complexed IgM titers may be measured. Clinical efficacy may be measured notably by

using Clinical Global Impression of Change (CGIC) and/or by cognition tests (e.g., Cambridge Neuropsychological Test Automated Battery (CANTAB) motor control, reaction time, paired associative learning, Cued Recall Test (CRT) or its modified version (mCRT), Cambridge Cognitive Examination – Down Syndrome (CAMCOG-DS), modified Selective Reminding Test (SRT), NEuroPSYchological Assessment-II – Train and Car Subtest (NEPSY-II), Kaufman Brief Intelligence Test 2 (KBIT-2)) ; Brief Praxis Test (BPT4), behavior (e.g. by Vineland Adaptive Behavior Scale (VABS), Neuropsychiatric Inventory (NPI) and by assessing the progression to dementia (eg., Dementia Screening Questionnaire for Individuals with Intellectual Disabilities (DSQIID)).

In human subjects with DS, assessment by MMSE may not be appropriate. Similarly, the age considerations may be different (e.g. due to shorter life expectancy). Male or female subjects with DS may be treated at any age, in particular prophylactically. As already mentioned preventive treatments may be applied to subjects without evidence of beta amyloid plaque formation and neurofibrillary tangles. Conversely, therapeutic treatment may be applied to those subjects with evidence of beta amyloid plaque formation and neurofibrillary tangles. Human subjects with DS may be in the pre-clinical stage of AD, with no amyloid-related cognitive decline. The treated subjects may be 50 years old or less, such as 45, 40, 35, 30 or 25 years or less. Human subjects with DS amenable to treatment may be identified as having mild to moderate intellectual disability using the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) classification. DSM-5 is the 2013 update to the Diagnostic and Statistical Manual of Mental Disorders, the taxonomic and diagnostic tool published by the American Psychiatric Association (APA). In the United States, the DSM serves as the principal authority for psychiatric diagnoses.

Human subjects amenable to treatment may be identified as PET-scan positive for Aβ deposits according to some embodiments. Such Aβ deposits are found in patients with early AD (mild cognitive impairment due to AD and mild AD) and also in more advanced stages of AD, such as moderate AD. For example, florbetaben positron emission tomography (PET) may be employed to investigate amyloid load in the brain. Human subjects amenable to treatment may be identified as PET-scan positive for tau aggregates according to some embodiments. For example, positron emission tomography (PET) with a tau tracer, such as PI-2620, may be employed to investigate tau accumulation in the brain.

Human subjects amenable to treatment may be identified on the basis of CDR score. The Clinical Dementia Rating scale or CDR scale is a numeric scale used to quantify the severity of symptoms of AD (i.e. its 'stage'). The system was developed at Washington University School of Medicine (Hughes et al 1982) and involves a qualified health professional assessing the human subject's cognitive and functional performance in six areas via a semi-structure interview: memory, orientation, judgment and problem solving, community affairs, home and hobbies, and personal care. Scores in each of these may be combined to obtain a composite score ranging from 0 (no symptoms) to 3 (severe), referred to as the sum of boxes (CDR-SB). The CDR-SB score may therefore range from 0 to 18 points. A CDR-SB score of 0 may identify the subject as normal. Such subjects may be amenable to prophylactic treatment, potentially in the presence of other risk factors. A CDR-SB score of 0.5-2.5 may identify a subject with prodromal AD or MCI. A CDR-SB score of 2.5-4.0 may identify a subject with very mild AD. A CDR-SB score of 4.5-9.0 may identify a subject with mild AD. A CDR-SB score of 9.5-15.5 may identify a subject with moderate AD. A CDR-SB score of 16.0-18.0 may identify a subject with severe AD. See O'Bryant et al., Arch Neurol. 2010;67(6):746-749. doi:10.1001/archneurol.2010.115. As already mentioned, administration to human subjects with early stage disease (cognitive impairment or AD) may also be beneficial. Thus, in some embodiments, the human subject, prior to treatment, displays cognitive impairment consistent with a CDR-SB score of no more than 15.5 such as 0.5-15.5, or no more than 9.0, such as 0.5-9.0.

In specific embodiments, the human subject is suffering from not severe AD. The subject suffering from not severe AD may suffer from prodromal AD, early AD, mild AD, mild to moderate AD or moderate AD. Such subjects may display cognitive impairment consistent with a MMSE score of at 12-28. Such subjects suffering from not severe AD may have a CDR-SB score of less than 16.0. The global score of Clinical Dementia Rating scale (CDR-GS) may also be used to define the severity of dementia in subjects with AD using a 0–5 point scale, (0 = absent; 0.5 = questionable; 1= present, but mild; 2 = moderate; 3 = severe; 4 = profound; 5 = terminal). Generally, a CDR-GS score of 0 is associated with preclinical AD (asymptomatic stage of AD), a score of 0.5 is associated with prodromal AD (MCI due to AD), a score of 1 is associated with mild AD dementia and a score of 2 is associated with moderate dementia.

The liposomal vaccine compositions may be administered to a human subject receiving at least one additional therapy. Preferably, the at least one additional therapy may be selected from an acetylcholinesterase inhibitor (ACHEI) and/or memantine.

DESCRIPTION OF THE FIGURES

Figure 1. Shows anti-Abeta1-42 IgG titers in serum of monkeys immunized with ACI-24EE (Group 1, black circles) or ACI-24.060 (Group 2, squares)

Figure 2. Shows anti-Abeta1-42 IgG titers in CSF of individual monkeys immunized with ACI-24EE (Group 1, circles) or ACI-24.060 (Group 2, squares) at predose and at necropsy (Day 183)

Figure 3. Shows images of labelling of Abeta plaques in brain section from subjects with AD, incubated with monkey sera from monkeys immunized with ACI-24.060 and prior to vaccination (Figure 3a Pool of monkey sera predose, Figure 3b Pool of monkey sera at day 92)

Figure 4. Shows resulting OD values for the anti-pyroglutamate Abeta3-42 IgG ELISA with serially diluted sera of monkeys immunized with ACI-24.060 (grey triangles) and donanemab (black squares).

Figure 5. Shows resulting ECL values for the anti-Abeta1-42 oligomers IgG MSD with serially diluted sera of monkey immunized with ACI-24.060 (grey triangles) and lecanemab (black squares).

Table of abbreviations

AD	Alzheimer's Disease
ARIA-E	Amyloid-related imaging abnormalities - vasogenic edema
ARIA-H	Amyloid-related imaging abnormalities - microhemorrhages, superficial siderosis
Aβ	Amyloid beta (abeta)
BPT	Brief Praxis Test
CANTAB	Cambridge Neuropsychological Test Automated Battery
CDR	Clinical Dementia Rating scale
CDR-SB	Clinical Dementia Rating scale - Sum of Boxes
CGIC	Clinical Global Impression of Change
CNS	Central Nervous System
CSF	Cerebrospinal Fluid

DMPC	1,2-Dimyristoyl-sn-glycero-3-phosphocholine
DMPG	1,2-Dimyristoyl-sn-glycero-3-phosphorylglycerol
DSMB	Data and Safety Monitoring Board
ECG	Electrocardiogram
ELISA	Enzyme-linked immunosorbent assay
ELISPOT	Enzyme-linked immune absorbent spot
GABA	Gamma-Aminobutyric acid
GLP	Good Laboratory Practice
HE	Hematoxylin and eosin
IFN	Interferon
IL	interleukin
i.m.	intramuscular
Ig	Immunoglobulin
MMSE	Mini-Mental State Examination
MPLA	Monophosphoryl Lipid A
MRI	Magnetic Resonance Imaging
NIA-AA	National Institute on Aging – Alzheimer's Association
NINCDS-ADRDA	National Institute of Neurological and Communicative Diseases and Stroke - Alzheimer's Disease and Related Disorders Association
NOAEL	No Observed Adverse Effet Level
NPI	Neuropsychiatric Inventory
Pal1-15	Tetrapalmitoylated Aβ1-15
PBS	Phosphate buffered saline
PET	Positron Emission Tomography
s.c.	Subcutaneous
SAE	Serious Adverse Event
sAPPα	Soluble amyloid precursor protein alpha
sAPPβ	Soluble amyloid precursor protein beta
SSRI/SNRI	Selective serotonin reuptake inhibitor /Serotonin–norepinephrine reuptake inhibitor
SGOT	serum glutamic-oxaloacetic transaminase
SGPT	Serum glutamic pyruvic transaminase
TLR4	Toll-like receptor 4
TSH	Thyroid-stimulating hormone

VABS	Vineland Adaptive Behavior Scale

The invention will be further understood with reference to the following non-limiting examples:

Definitions:

The MMSE (Folstein 1975) is a widely used test of overall cognitive function, assessing memory, orientation and praxis in a short series of tests. The score is from 0 to 30 with 30 being the best possible and 0 being the worst possible score.

The Clinical Dementia Rating Scale (Hughes et al 1982) is a global rating of the function (it is not only purely functioning since cognition is also being checked with memory) of Alzheimer patients assessed in six categories: memory, orientation, judgement and problem solving, community affairs, home and hobbies and personal care. It is based on a semi-structured interview conducted with the patient and caregiver, by a rater without access to the results of the cognitive tests described above. Each category has scores from 0 (no symptoms) to 3 (severe) and the sum of these items (Sum of Boxes) may therefore range from 0 to 18 points.

Early AD patients include Mild Cognitive Impairment (MCI) due to AD and mild AD.

According to the National Institute on Aging - Alzheimer Association (NIA-AA) criteria, Mild Cognitive Impairment due to Alzheimer's Disease requires evidence of intra-individual decline, manifested by a change in cognition from previously attained levels, as noted by self- or informant report and/or the judgment of a clinician, impaired cognition in at least one domain (but not necessarily episodic memory) relative to age-and education-matched normative values (impairment in more than one cognitive domain is permissible), a preserved independence in functional abilities, no dementia, and a clinical presentation consistent with the phenotype of AD in the absence of other potentially dementing disorders.

Probable AD dementia according to NIA-AA criteria meets criteria for dementia and in addition, has the following main characteristics: insidious onset (symptoms have a gradual onset over months to years, not sudden over hours or days), clear-cut history of worsening of cognition by report or observation; and the initial and most prominent cognitive deficits are evident on history and examination in one of the following categories: Amnestic presentation

(it is the most common syndromic presentation of AD dementia. The deficits should include impairment in learning and recall of recently learned information). There should also be evidence of cognitive dysfunction in at least one other cognitive domain); Non-amnestic presentations: Language presentation (the most prominent deficits are in word-finding, but deficits in other cognitive domains should be present); Visuospatial presentation: (the most prominent deficits are in spatial cognition, including object agnosia, impaired face recognition, simultanagnosia, and alexia; deficits in other cognitive domains should be present); Executive dysfunction (the most prominent deficits are impaired reasoning, judgment, and problem solving. Deficits in other cognitive domains should be present).

Early AD patients are patients with the MMSE score of at least 20 (equal or above 20). They include patients with Mild Cognitive Impairment due to AD and patients with mild AD.

Mild AD patients are patients with the MMSE score of 20 to 28.

Mild-to moderate AD patients are patients with the MMSE score of 12 to 28.

Moderate AD patients are patients with the MMSE score of 12 to 19.

EXAMPLES

Example 1: Preparation of liposomal vaccine composition ACI-24.060

Step 1: T-cell epitope peptide synthesis and purification

SAT58 T-cell epitope containing peptides were manufactured by linear solid phase peptide synthesis (SPPS) on 2- Chlorotrityl resin using standard Fmoc chemistry. Standard coupling procedure was performed using 3.0 equivalent of amino acid (SAT58 peptide) and coupling reagent in the presence of 3.0 equivalent of base in DMF for at least 1 hour at room temperature. For difficult coupling sequences double coupling was implemented with extended reaction time. After the completion of the amino acid coupling, an acetylation capping step was introduced using 5.0 equivalent of Ac20 (acetic anhydride) in pyridine to avoid the undesired peptide chain elongation. The resin was washed with DMF and Fmoc group was removed by using 20% piperidine in DMF for 5 min. After finishing the SPPS, global deprotection and peptide cleavage from the resin was done using standard cleavage cocktail (TFA/TIS/water/ TBMTP) for a minimum of 2 hours at room temperature. The crude

product was subsequently precipitated with 10-fold excess volume of cold isopropyl ether/heptane, washed with IPE, and the solid was filtered off by using a glass frit and dried under vacuum. The crude peptide was purified on reversed phase C18 column using a gradient of solvent A (water, 0.1 % TFA) and solvent B (acetonitrile, 0.1% TFA) on a preparative HPLC system. The HPLC fractions containing desired peptide with purity above 90% were pooled together diluted in water and performed an ion exchange. The desired ion exchange fractions were lyophilized to provide a powder. The identity and purity of final peptide was characterized and confirmed by HPLC-MS analysis.

Preparation of the ACI-24.060 vaccine (Crossflow injection)

The lipids (DMPG, DMPC, cholesterol and 3D-(6-acyl) PHAD™ (Avanti Polar Lipids, USA)) were dissolved in 96% EtOH (ethanol) in a heating cabinet at 60°C. After complete dissolution of the lipids, the solution was filtered through a 0.2 µm pore size filter into the injection system which was heated to 60°C. In detail, the appropriate amount of (SAT58) was dispersed in EtOH at room temperature by the aid of sonication (EtOH concentration is typically 2% v/v of final SAT58 solution) to form a peptide slurry which was solubilized by dilution with His-Sucrose buffer (10 mM Histidine, 250 mM Sucrose). The SAT58 solution was filtered through a 0.2 µm pore size filter into the injection vessel which was then heated up to 40°C. Liposomes are formed at the site of injection when the lipid/EtOH solution and the SAT58 solution mixes. Immediately after liposome formation there was an online dilution step with 10 mM Histidine, 250 mM Sucrose in order to decrease the EtOH concentration. The intermediate liposomes were extruded through 100 nm pore size polycarbonate membranes at RT. Ultra-/diafiltration (UDF) using a hollow fiber membrane (MWCO: 500 kD) was performed to remove EtOH and the buffer was exchanged to PBS pH 6.9. SAT58 liposomes were then diluted using the dispersion buffer (PBS pH 6.9) to a total lipid concentration of 1 mg/mL and warmed up to 35°C. The Pal1-15 was dissolved in a 10% w/v solution of beta-OG in 10 mM Na_2HPO_4 pH 11.4 buffer at 60°C and was further diluted with the same buffer to a final concentration of 1 mg/mL. After mixing of these two solutions using a crossflow injection module, the liposomal suspension was further incubated at 35°C under stirring to allow complete insertion of Pal1-15. A second UDF step using a hollow fiber membrane (MWCO: 500 kD) was performed to remove beta-OG and to exchange buffer to 10 mM Histidine, 250 mM Sucrose. The product was filtered through a 0.2 µm Acrodisc mPES syringe filters. Final product was prepared by appropriate dilution using 10 mM histidine / 250 mM sucrose buffer and was sterile filtered through a 0.2 µm sterilization grade

filter, followed by aseptic filling into sterile glass vials, which were sealed and stored at 2 – 8 °C.

Example 2. Safety and Efficacy in humans in Phase 1b/2 AD trial

Study objective:

The overall objective of this Phase 1b/2, multicenter, adaptive, double-blind, randomized, placebo-controlled study (ACI-24-AD-DS-2102) is to assess the safety, tolerability, immunogenicity, and pharmacodynamic effects of ACI-24.060 in subjects with prodromal Alzheimer's disease and in adults with Down syndrome. The study is conducted in 2 separate parts: Study part 1 is conducted in subjects with prodromal Alzheimer's disease (AD). Study part 2 will be conducted in subjects with Down syndrome (DS).

Study part 1 in prodromal AD:

Primary Objective:

To assess the safety and tolerability of ACI-24.060.

Secondary Objective:

- To assess the anti-amyloid β (Aβ) antibody response generated by ACI-24.060 in serum.

Exploratory Objectives:

- To explore the effect of ACI-24.060 on brain amyloid levels using positron emission tomography (PET) scan.
- To explore the effect of ACI-24.060 on brain Tau levels using PET imaging.
- To explore the effect of ACI-24.060 on behaviour, cognition, and clinical function.
- To explore the correlation between the magnitude of anti-Aβ antibody response and changes in biomarkers including amyloid PET, volumetric magnetic resonance imaging (MRI) and advanced MRI sequences, and behaviour, cognition, and clinical function.
- To explore the effect of baseline demographic factors including subject's age, apolipoprotein E (ApoE) genotype, and sex on the effect of ACI-24.060 on study parameters.
- To explore the effect of ACI-24.060 on changes in MRI (volumetric and advanced MRI sequences).
- To explore biomarkers related to AD and immunogenicity related to ACI-24.060 in any collected study biofluid.

Study part 2 in Down syndrome:

Primary Objectives:

- To assess the safety and tolerability of ACI-24.060.
- To assess the anti-Aβ antibody response generated by ACI-24.060 in serum.

Secondary Objectives:

- To assess the pharmacodynamic effect of ACI-24.060 on amyloid-related fluid biomarkers.
- To assess the effect of ACI-24.060 on brain amyloid levels using PET scan.

Exploratory Objectives:

- To assess the effect of ACI-24.060 on behaviour, cognition, and clinical function.
- To explore the impact of baseline demographic factors including subject's age, ApoE genotype, and sex on the effect of ACI-24.060 on study parameters.
- To explore the correlation between the magnitude of anti-Aβ antibody response and changes in biomarkers including amyloid PET, volumetric MRI, and behaviour, cognition, and clinical function.
- To explore the effect of ACI-24.060 on changes in MRI (volumetric and advanced MRI sequences).
- To explore the effect of ACI-24.060 on brain Tau levels with tau-PET imaging.
- To explore the impact of the clinical status of AD in DS at baseline on the effect of ACI 24.060 on study parameters.
- To explore the effects of level of intellectual disability at baseline on the impact of ACI 24.060 on study parameters.
- To explore biomarkers related to AD in DS, and immunogenicity related to ACI-24.060 in any collected study biofluid.

Study Part 1:

Study part 1 is conducted in subjects with prodromal AD to assess the effect of study treatment (ACI- 24.060 or placebo) administered over 48 weeks.

Interim Analyses (IAs):

Multiple IAs of safety, tolerability, and immunogenicity data are conducted in each cohort, either expanded or not, in some or all AD subjects, at different predefined study timepoints, during the treatment period and during the follow-up period. The first IA on safety and tolerability was performed once data collection had been completed for the first 4 subjects, 2 weeks after the second study drug injection (i.e., at W6).

Study Part 2:
Study part 2 will be conducted in up to 88 non-demented adult subjects with DS and with confirmed presence of amyloid pathology by PET scan to assess the effect of study treatment (ACI-24.060 or placebo) administered over 74 weeks.

Interim Analyses:
IAs of safety, tolerability, and immunogenicity data may be conducted at different predefined study timepoints, during the treatment period and during the follow-up period. The first IA on safety and tolerability will be performed from 2 weeks after the second study medication injection (i.e., at W6) onwards.

Number of subjects:
Study Part 1:
A total of up to 88 AD subjects may be randomized in study part 1, with the potential for up to 4 cohorts (AD1 to AD4) to be initiated. Each cohort will initially include 4 AD subjects with a 3:1 active treatment/placebo ratio. Any AD cohort may be expanded
Study Part 2:
A total of up to 88 subjects with DS may be randomized in study part 2, with the potential for up to 3 cohorts (DS1 to DS3) to be initiated. Each cohort will initially include 4 to 8 DS subjects with a 3:1 active treatment/placebo ratio. Seventy-two subjects with DS will be randomized in one of the three cohorts with a 2:1 overall active treatment/placebo ratio: 48 subjects will receive ACI-24.060 and 24 subjects will receive placebo. This takes into consideration an anticipated approximately 15% drop-out rate..
Study population:
Study Part 1:
The study population is prodromal AD (mild cognitive impairment [MCI] due to AD) male and female subjects, 50 to 85 years of age, with confirmed presence of amyloid pathology by PET scan.

Study Part 2:
The study population is non-demented male and female subjects with DS, 35 to 50 years of age with confirmed presence of amyloid pathology by PET scan.

Inclusion Criteria:
Study Part 1
1. Age ≥50 and ≤85 years at screening.

2. Diagnosis of prodromal AD: MCI due to AD according to National Institute on Aging Alzheimer's Association (NIA-AA) criteria.

3. PET scan at screening consistent with the presence of amyloid pathology.

4. Clinical Dementia Rating (CDR)-Global Score of 0.5.

5. Subjects who in the opinion of the investigator are able to understand the details of the study and to provide written informed consent.

6. Subjects either not taking any marketed treatment for AD or receiving a stable dose of an acetylcholinesterase inhibitor (ACHEI) and/or memantine for at least 2 months prior to baseline.

7. Subjects cared for by a reliable spouse, informant, or study partner to assure compliance, assist with clinical assessments, and report safety issues, and spouse, informant or study partner consents to serve in this role.

8. Females who are either post-menopausal for at least 1 year and/or surgically sterilized, or females of childbearing potential or not post-menopausal must have a negative blood pregnancy test at screening and be willing to use highly effective methods of contraception from the screening visit until the end of their participation. Male participants in the trial with female partners of childbearing potential are required to use barrier methods of contraception (condoms with spermicide) in addition to contraceptive measures used by female partners during the whole study duration.

9. Subjects and study partners must be sufficiently proficient in the official language(s) of the country they are living in and able to comply with all study procedures, including lumbar punctures.

Study Part 2

1. Age ≥35 and ≤50 years at screening (subjects with DS with age ≥35 and ≤39 years may be considered on the condition that there is prior evidence of amyloid results compatible with AD pathology at PET-scan and/or in biofluids).

2. Male or female subjects with DS with a cytogenetic diagnosis being either trisomy 21 or complete unbalanced translocation of the chromosome 21.

3. PET scan at screening consistent with the presence of amyloid pathology.

4. Subjects, their legal representatives (if applicable) and/or their study partners, in the opinion of the investigator, are able to understand the details of the study and to provide written informed consent before starting any study-related activities.

5. In the opinion of the investigator, subjects, their legal representatives (if applicable), and/or their study partners or informants are able to fully participate in the study, are

sufficiently proficient in the official languages(s) of the country they are living in, and are capable of reliably completing study assessments.

6. Females who are either post-menopausal for at least 1 year and/or surgically sterilized, or females of childbearing potential or not post-menopausal must have a negative blood pregnancy test at screening and be willing to use highly effective methods of contraception from the screening visit until the end of their participation. Male participants in the trial with female partners of childbearing potential are required to use barrier methods of contraception (condoms with spermicide) in addition to contraceptive measures used by female partners during the whole study duration.

7. Mild to moderate intellectual disability as per Diagnostic and Statistical Manual of Mental Disorders (DSM-5) classification.

8. Subjects must have a study partner who has direct and regular contact, at least 10 hours per week, with the subject and who is able to provide reliable answers to questions related to the subject, according to the study investigator.

Dosage and duration of treatment:
The route of administration of study drug will be intramuscular injections.

Study Part 1:
The initial dose of ACI-24.060 tested is 300 µg. In case of dose escalation, the next higher dose will not be increased more than 3-fold, i.e 900 µg. The maximum dose of ACI-24.060 that is being tested is 1600 µg.
Randomized AD subjects will receive 5 study drug injections (ACI-24.060 or placebo) at W0, W4, W12, W24, and W48. In order to optimize the immunogenicity, the administration regimen may need to be revised either with the same or with a different study drug dose.
For each study subject, the treatment period will be followed by a 26-week follow-up period.
The overall study participation will be approximately 80 weeks in AD subjects (up to 6 weeks of screening; 48 weeks of treatment; 26 weeks of follow-up).
Study Part 2:
Randomized subjects with DS will receive their first 5 injections (ACI-24.060 or placebo) according to the same schedule administered in part 1, at W0, W4, W12, W24, W48, plus an additional injection at W74 at a dose already shown to be safe and immunogenic in AD subjects in part 1 of the study.
The dose level of ACI-24.060 may need to be adjusted in the DS study population based on immunogenicity and safety/tolerability IA data from the first 4 to 8 subjects with DS.

The initial dose of ACI-24.060 to be tested is a dose already shown to be safe and immunogenic in AD subjects in part 1 of the study (i.e., ACI-24.060 300µg). The maximum dose of ACI-24.060 that is being tested is 1600 µg.
For each study subject, the treatment period will be followed by a 26-week follow-up period. The overall study participation will be approximately 106 weeks in subjects with DS (up to 6 weeks of screening; 74 weeks of treatment; 26 weeks of follow-up).

As of 19 January 2024, 43 AD subjects have been randomized in the study, 8 of them have received either ACI-24.060 300 micrograms or placebo (AD1 cohort), 29 subjects have received either ACI-24.060 900 micrograms or placebo (AD2 cohort that has been expanded) and 6 subjects have received either ACI-24.060 1600 micrograms or placebo (AD3 cohort).

The safety and the tolerability in the study have been good to date, with most adverse events being of mild severity, no serious adverse event (considered to be possibly or likely related to the study vaccine), no adverse event leading to study withdrawal, and no abnormalities at MRIs performed after study treatment initiation, especially no reported case of ARIA-E. As of 19 January 2024 two serious adverse events (SAEs) have been reported in one subject in the AD2 cohort receiving either ACI-24.060 900 micrograms or placebo. The subject was hospitalized for a nephrectomy in order to remove a clear cell renal tumor. The subject did also present a complete auriculo-ventricular block during the induction of anaesthesia necessitating the installation of a pacemaker. The causal relationship of these events according to the site investigator is considered unrelated or unlikely related to the study vaccine.

Data from interim analyses performed to date have shown that ACI-24.060, administered at doses of 300µg and 900µg, could elicit an anti-Abeta antibody response in subjects as soon as at week 6, i.e. 2 weeks after the second injection. The data show that ACI-24.060 vaccination has been safe and well-tolerated to-date.

Example 3. Chronic Toxicology study:

The safety and immunogenicity of ACI-24.060 has been evaluated in non human primates (NHPs), specifically cynomolgus monkeys. NHPs are the relevant species for the evaluation of immunogenicity of vaccines, due to their evolutionally proximity with the human immune system.

3.1 Study design

Nine healthy cynomolgus monkeys (Macaca fascicularis) of each sex (for a total of 18) were involved in the study. The animals were 2 to 3 years old and weighed 2.7 to 4.4 kg (males) and 2.8 to 3.4 kg (females). Monkeys were allocated to two groups as detailed in Table 3.

Table 3: Study design

Group number	Vaccine	Target Dose Level[b] (µg/injection)	Dose volume (mL/animal)	Animals/group		Necropsy after	
				Males	Females	26 weeks	30 weeks
1/ACI-24EE[a]	ACI-24EE	0	2 x 2mL	4	4	2M / 2F	2M / 2F
2/ACI-24.060	ACI-24.060	1600	2 x 2mL	5	5	3M / 3F	2M / 2F

[a] ACI-24EE: liposomal vaccine without palmitoylated Abeta 1-15 peptide (Pal 1-15), SAT58 and 3D-6A-MPLA

[b]The target dose level for ACI-24.060 was 1600 µg/injection (dosage expressed for tetrapalmitoylated Abeta 1-15 as set forth in SEQ ID NO: 1). Based on the analytical Pal1-15 content, the actual injected dose level was 1712 µg/injection.

The two groups were immunized seven times by the intramuscular (i.m.) route on Days 1, 29, 57, 85, 113, 141 and 169 with either ACI-24EE (placebo, group 1) or ACI-24.060 (group 2).

3.2 Toxicity evaluation:

Assessment of safety was based on clinical observations, food consumption, postdose observations, body weights, injection site evaluations, ophthalmology, ECG collections, blood pressure recordings, clinical pathology (hematology, clinical chemistry, coagulation and urinalysis), and blood immunophenotyping. Complete necropsies were performed on all animals, with a recording of macroscopic abnormalities made and the preservation of selected tissues followed by a full microscopic evaluation.

No ACI-24.060-related clinical observations, body weight alterations, ophthalmology findings, ECG effects, blood pressure alterations, or clinical pathology findings were noted. Slight to moderate reversible erythema or edema was occasionally noted at the injection sites for individual animals in the control or ACI-24.060-treated group.

Treatment with ACI-24.060 at 1712 µg /injection had no effect on absolute or relative counts of T helper and cytotoxic T cells, NK cells, activated NK cells, B cells and monocytes in peripheral blood.

No ACI-24.060-related findings were noted at the terminal or recovery sacrifice.

The dose level of 1712 µg of ACI-24.060 vaccine was locally and systemically well tolerated. As the dose evaluated in this study did not show any ACI-24.060-related findings, ACI-24.060 was considered to be safe and well tolerated at a dose of 1712 µg. The above demonstrate safety and tolerability of chronic administration of ACI-24.060 in cynomologus monkeys.

3.3 Immunogenicity results in serum:

Assessment of immunogenicity, i.e., the level of Abeta specific antibodies produced post vaccination, was conducted on serum samples collected once before immunization and 1 and 3 weeks after each immunization (on Days 8, 22, 36, 50, 64, 78, 92, 106, 120, 134, 148, 162, 176 for all animals, and 190, 204 and 211 for the recovery animals).

Anti-Abeta1-42 IgG titers were analysed on a sample collected at each timepoint by an enzyme-linked immune sorbent assay (ELISA) using a validated method. Briefly, Abeta 1-42 peptide film was immobilized on 96-well micro titer plates for 12 or 72h at 2°-8°C. After washing and blocking, plates were incubated with the samples for one (1) hour at 37 °C, allowing any anti-Abeta 1-42 IgG antibodies present in serum to bind. After incubation, the plates were washed to remove non-reactive serum components. The antibody/antigen complex was detected via a secondary anti-human IgG antibody conjugated to horseradish peroxidase. 3,3',5,5' tetramethylbenzidine (TMB) substrate was added to the wells of the plate and the reaction was stopped using H2SO4. The absorbance is measured at 450 nm and 630 nm using a spectrophotometer. The absorbance at 450 nm was corrected with the absorbance at 630 nm (A450 – A630) and the corrected absorbance is proportional to the amount of anti-Abeta 1-42 IgG antibodies in the sample. The antibody concentration in the sample was calculated from a calibration curve, which was fitted using four-parameter logistic (4PL) plotting.

Anti-Abeta1-42 IgM titers were analysed on a sample collected at pre-vaccination and 1 week after each immunization and at one time point for the recovery animals by ELISA with a qualified method. Briefly, Abeta 1-42 peptide film was immobilized on 96-well micro titer plates for 12 or 72h at 2°-8°C. After washing and blocking, plates were incubated with samples for one (1) hour at 37 °C, allowing any anti-Abeta 1-42 IgM antibodies present in serum to bind. After incubation, the plates were washed to remove non-reactive serum components. The antibody/antigen complex was detected via a secondary anti-human IgM antibody conjugated to horseradish peroxidase. 3,3',5,5' tetramethylbenzidine (TMB) substrate was added to the wells of the plate and the reaction was stopped using H2SO4. The absorbance was measured at 450 nm and 630 nm using a spectrophotometer. The

absorbance at 450 nm was corrected with the absorbance at 630 nm (A450 – A630) and the corrected absorbance was proportional to the amount of anti-Abeta 1-42 IgM antibodies in the sample. The antibody concentration in the sample was calculated from a calibration curve, which is fitted using four-parameter logistic (4PL) plotting.

The resulting anti-Abeta1-42 IgG titers in serum of monkeys immunized with ACI-24EE (Group 1, black circles) or ACI-24.060 (Group 2, squares) are shown in Figure 1. Data are expressed as the geometric mean ±95% confidence interval (CI), with n=8 for the placebo group and n=10 for the ACI-24.060 immunized group. Arrows indicate the immunization day. **** indicates Significance at <0.0001 level. Results showed that animals immunized with ACI-24.060 developed a robust anti-Abeta1-42 IgG response after two immunizations, significantly higher as compared to the placebo group. Furthermore, the anti-Abeta1-42 IgG titers were boosted after each immunization and titers maintained up to 6 weeks, i.e., the last sample collected, after the last immunization.

ACI-24.060 developed a robust anti-Abeta1-42 IgG response after two immunizations, and anti-Abeta1-42 IgM response after third immunization. Anti-Abeta1-42 IgG titers were also detected in CSF of animals immunized with ACI-24.060.

3.4 Immunogenicity results in Cerebrospinal Fluid (CSF):

CSF samples were collected from all animals via lumbar puncture before the vaccination (predose) or from *cisterna magna* at necropsy (on Day 183 for dosing phase animals and on Day 211 for recovery animals). Results of the dosing phase animals only are shown.

Anti-Abeta1-42 IgG titers in the CSF were analysed by Meso Scale Discovery (MSD) . MSD 96-well small spot streptavidin microplates were saturated with PBS-Blocker A 5% overnight at 4°C. After washing, plates were coated with Abeta1-42 biotinylated peptide film and incubated for one hour at 37°C on a shaker at 450 rpm. After washing, samples were added to the plate and incubated for two (2) hours at 37 °C with shaking allowing any anti-Abeta1-42 IgG antibodies present in the sample to bind. Plates were then washed and incubated for one hour at 37°C on a shaker with a Sulfo-TAG labelled anti-human IgG (plus IgG1 depleted) antibody. After washing, plates were fixed with PBS- Formaldehyde 1% for 15 minutes at room temperature and with shaking. After washing step, MSD read buffer 2x was added and plates were read immediately on the MesoScale instrument. The antibody concentration in the sample was calculated from a standard curve using a four-parameter logistic fit with 1/y2 weighting using the MSD software.

The resulting anti-Abeta1-42 IgG titers in CSF of individual monkeys immunized with ACI-24EE (Group 1, circles) or ACI-24.060 (Group 2, squares) at predose and at necropsy (Day 183) are shown in Figure 2. Data are expressed as individual anti-Abeta1-42 IgG titers and geometric mean with 95% CI in AU/mL (one data of ACI-24.060 group Day 183 excluded because of %CV>35%). * indicates significance at <0.05 level. As seen from Figure 3, the anti-Abeta1-42 IgG titers detected in the CSF of animals post immunization with ACI-24.060 were significantly higher as compared to predose, despite the high intra-group variability. No anti-Abeta 1-42 IgG titers were observed in the placebo group.

3.5 Tissue cross reactivity

A human tissue cross reactivity (TCR) study was performed to evaluate the potential cross reactivity of sera from cynomolgus monkeys immunized with ACI-24.060 on a panel of human frozen tissue and blood smears from three unrelated donors, using immunohistochemical (IHC) techniques. Human Alzheimer's disease patient brain sections were used as positive control in the assay. Briefly, frozen sections were dried and fixed in zinc formalin. After rinsing, endogenous peroxidase activity was blocked in a solution of methanol supplemented with 3% H2O2. Then, sections were washed and incubated with reaction buffer. The slides were uploaded into the Discovery XT2 system and a semi-automated IHC staining was performed: sections were incubated with the sera for 1h at 37°C, and then incubated with an anti monkey specific secondary antibody. Detection was performed with Omni Map kit, Ventana following Manufacturer recommendations. The following list (Table 4) of frozen tissue, organs or blood smears from three unrelated human donors were used:

Table 4

Adrenal	Kidney (glomerulus, tubule)	Skin
Bladder (urinary)	Liver	Spinal Cord
Blood Cells [a]	Lung	Spleen
Blood Vessels (endothelium) [b]	Lymph Node	Striated Muscle (skeletal)
Bone Marrow	Ovary	Testis
Brain – cerebellum	Pancreas	Thymus
Brain – cerebral cortex	Parathyroid	Thyroid
Breast (mammary gland)	Peripheral Nerve	Tonsil
Eye	Pituitary	Ureter
Fallopian Tube (oviduct)	Placenta	Uterus – cervix
Gastrointestinal (GI) Tract [c]	Prostate	Uterus – endometrium
Heart	Salivary Gland	

Pooled monkey sera from monkeys receiving 4 immunizations (i.e., Day 92) with ACI-24.060 at dose of 1083 ug/mL were tested. Pooled sera from nonimmunized monkeys (i.e., predose) was used as the background level control.

The serum from immunized monkeys specifically labelled the Abeta plaques contained in the brain sections obtained from patients with AD, while no labelling was observed when incubating the sections with sera from monkeys prior to vaccination (predose) (Figure 3a Pool of monkey sera Predose, Figure 3b Pool of monkey sera at day 92). No off-target cross-reactivity was observed on a panel of more than 30 frozen human tissues, demonstrating selectivity for Abeta.

3.6 Comparison of ACI-24.060 generated antibody titers against pyroglutamate-Abeta in NHP to the clinically validated monoclonal antibody donanemab

Assessment of antibodies against pyroglutamate Abeta produced post vaccination, was conducted on six NHP serum samples collected at Day 176 (3 weeks after the seventh immunization) along with the monoclonal antibody donanemab (source Thermo Fisher).

Anti-pyroglutamate Abeta3-42 IgG titers were determined by an enzyme-linked immune sorbent assay (ELISA). Briefly, pyroglutamate Abeta 3-42 peptide film was immobilized on 96-well plates overnight at 2°-8°C. After washing and blocking, plates were incubated with serially diluted serum samples or donanemab for two (2) hour at 37 °C, allowing any anti-pyroglutamate Abeta3-42 IgG antibodies present in the sample to bind. After incubation, the plates were washed to remove non-reactive components. The antibody/antigen complex was detected via a secondary anti-human IgG antibody conjugated to horseradish peroxidase. After a final wash, plates were incubated for one 1 hour with a substrate solution of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS). The absorbance is measured at 450 nm (optical density, OD). The anti-PyroGluAbeta3-42 antibodies concentration in NHP sera were back-calculated against donanemab, as calibration curve, using an unweighted four-parameter logistic (4PL) regression model with the BioTek Gen5 software (Agilent, version 3.12). Results were expressed in µg/mL and as the mean of the back-calculated concentrations.

The resulting OD values for the anti-pyroglutamate Abeta3-42 IgG ELISA are shown in Figure 4. Six NHP sera collected after 7 injections with ACI-24.060 (Day 176) were tested in parallel with donanemab. Eight 2-fold dilutions from an initial 100-fold dilution or 0.25µg/mL for the sera (upper x-axis) or donanemab (lower x-axis) respectively, were plotted against OD values shown on y-axis.

Binding activity of the ACI-24.060 vaccine-induced antibodies to pyroglutamate-Abeta3-42 in NHP was in the range 11-48 µg/mL of donanemab, which level was associated with Abeta plaque clearance in human AD patients (Gueorguieva, I. et al 2023).

3.7 Comparison of ACI-24.060 generated antibody titers against Abeta oligomers in NHP to the clinically validated monoclonal antibody lecanemab

Assessment of antibodies against Abeta1-42 oligomers produced post vaccination, was conducted on six NHP serum samples collected at Day 176 (3 weeks after the seventh immunization) along with the monoclonal antibody lecanemab (source MedChemExpress).

Anti-Abeta1-42 oligomers IgG titers were determined by Meso Scale Discovery (MSD). Briefly, MSD 96-well small spot standard plates were coated with Abeta 1-42 oligomers overnight at 2-8 °C. After washing and blocking, the serially diluted serum samples and lecanemab were added to the plate and incubated for two (2) hours at 37 °C with shaking, allowing any anti-Abeta1-42 oligomers IgG antibodies present in the sample to bind. After incubation, the plates were washed to remove non-reactive components and incubated for one hour at 37°C on a shaker with a Sulfo-TAG labelled anti-human IgG (plus IgG1 depleted) antibody. After a final washing step, MSD read buffer 2x was added and plates were read immediately on the MesoScale instrument. The intensity of the emitted light (expressed as ECL values) measured by the MSD reader is proportional to the quantity of anti Abeta1-42 oligomers IgG present in the samples. The anti-Abeta1-42 oligomers antibodies concentration in NHP sera were back-calculated against lecanemab, as calibration curve, using a four-parameter logistic fit with 1/y2 weighting using the MSD software. Results were expressed in µg/mL and as the mean of the back-calculated concentrations.

The resulting ECL values for the anti-Abeta1-42 oligomers IgG MSD are shown in Figure 5. Six NHP sera collected after 7 injections with ACI-24.060 (Day 176) were tested in parallel with lecanemab. Eight 2-fold dilutions from an initial 800-fold dilution or 0.03 µg/mL for the sera (upper x-axis) or lecanemab (lower x-axis) respectively, were plotted against ECL values shown on y-axis.

Binding activity of the ACI-24.060 vaccine-induced antibodies to Abeta1-42 oligomers in NHP was in the range of 18-26 µg/mL of lecanemab. In addition, by using adapted protocol of Soderberg et al 2022, it was determined that ACI-24.060 induced antibodies in NHPs with

>1000-fold stronger recognition of Abeta oligomers than monomers. This demonstrated specificity for pathological oligomeric Abeta is similar to that previously shown for lecanemab.

Example 4: Initial predictions of ACI-24.060 Amyloid PET lowering based on NHP data

Quantitative clinical pharmacology models have been developed to describe amyloid lowering evoked by Abeta targeting mAbs such as donanemab. , Gueorguieva, I. et al (2023) describes a donanemab population pharmacokinetic (PK) model with linked pharmacodynamic (PD) models for amyloid plaque reduction and ARIA-E (amyloid-related imaging abnormalities with edema or effusions) occurrence informed by patient-level data from the TRAILBLAZER-ALZ study. The amyloid reduction PD model is an indirect response model in which Amyloid plaque burden (as measured by Amyloid PET in centiloid) is modelled as a plaque compartment with specific rates of synthesis and degradation. The treatment effect of serum donanemab on Amyloid PET was modelled as an increase in the plaque degradation rate. According to Gueorguieva, I. et al (2023) serum donanemab concentrations above 4.43 ug/mL were associated with amyloid plaque reduction and "greater exposure, within the investigated dose range did not further influence the amount of amyloid removed".

As shown in Example 3.7 above, immunization of NHPs with ACI-24.060 generated IgGs which bind to and recognize pyroglu-Abeta with activity equivalent to that achieved with 11 - 48 ug/mL donanemab. This concentration range is comparable to estimated donanemab minimum (C_{min}) and average (C_{avg}) serum exposures achieved in the TRAILBLAZER-ALZ study during the first 3-months of dosing, 8 ug/mL and 40 ug/mL, respectively, (Table 5), and is above the reported threshold serum donanemab concentration reported to drive Amyloid PET lowering, 4.43 ug/mL .

Table 5: Estimated donanemab serum exposures achieved in the TRAILBLAZER-ALZ study.

mAb	Dosing Regimen	C_{max} (ug/mL)	C_{min} (ug/mL)	C_{avg} (ug/mL)
Donanemab	700 mg IV Q4W	186	8	40
Donanemab	1400 mg IV Q4W	388	15	80

Estimated donanemab maximum (C_{max}), minimum (C_{min}) and average (C_{avg}) serum exposures derived from Figure 1 of Gueorguieva, I. et al (2023). Exposures achieved

during the first 3-months of dosing (700 mg IV Q4W) are summarized in the second row and subsequent exposures achieved during the subsequent dosing period (1400 mg IV Q4W) are summarized in the third row.

These results indicate that ACI-24.060 has the potential to lower Amyloid plaques in human AD subjects.

REFERENCE LIST

Belichenko PV, Madani R, Rey-Bellet L, et al. An Anti-β-Amyloid Vaccine for Treating Cognitive Deficits in a Mouse Model of Down Syndrome. PLOS ONE. 2016;11(3):e0152471.

Folstein MF, Folstein SE, McHugh PR (1975) "Mini-Mental State": a practical method for grading the cognitive state of patients for the clinician J Psychiatr Res 12: 189 – 198

Gilman S., Koller M., Black R.S., Jenkins L., Griffith S.G., Fox N.C., Eisner L., Kirby L., Boada Rovira M., Forette F., Orgogozo J.M., Clinical effect of Aβ immunization (AN1792) in patients with AD in an interrupted trial. Neurology 64, 1553-1562 (2005).

Gueorguieva, I. et al. Donanemab Population Pharmacokinetics, Amyloid Plaque Reduction, and Safety in Participants with Alzheimer's Disease. Clinical Pharmacology & Therapeutics, Vol 113, Number 6, 1258 – 1267, (2023).

Hartley SL, Handen BL, Devenny D, et al. Cognitive decline and brain amyloid-β accumulation across 3 years in adults with Down syndrome. Neurobiology of aging. 2017;58:68-76.

Head E, Powell D, Gold BT, Schmitt FA. Alzheimer's Disease in Down Syndrome. European journal of neurodegenerative disease. 2012;1(3):353-364.

Hughes CP, Berg L, Danzinger WL et al (1982) A new clinical scale for the staging of dementia. Am J Psychiatry; 140: 566 – 572

Monsonego A., Weiner H.L., Immunotherapeutic approaches to Alzheimer's disease. Science. 31;302(5646):834-8 (2003).

Muhs A., Hickman D.T., Pihlgren M., Chuard N., Giriens V., Meerschman C., van der Auwera I., van Leuven F., Sugawara M., Weingertner M.-C., Bechinger B., Greferath R., Kolonko N., Nagel-Steger L., Riesner D., Brady R.O., Pfeifer A., Nicolau C., Liposomal vaccines with conformation-specific amyloid peptide antigens define immune response and efficacy in APP transgenic mice. PNAS, 104 23:9810-9815 (2007).

Nasreddine ZS, Phillips NA, et al. The Montreal Cognitive Assessment, MoCA: A brief screening tool for mild cognitive impairment. J Am Geriatr Soc. 2005;53:695-699.

Orgogozo J.M., Gilman S., Dartigues J.F., Laurent B., Puel M., Kirby L.C., Jouanny P., Dubois B., Eisner L., Flitman S., Michel B.F., Boada M., Frank A., Hock C., Subacute meningoencephalitis in a subset of patients with AD after Abet42 immunization. Neurology 61: 46-54 (2003).

Prasher VP, Huxley A, Haque MS (2002) A 24-week, doubleblind, placebo-controlled trial of donepezil in patients with Down syndrome and Alzheimer's disease—pilot study. Int J Geriatr Psychiatry 17(3):270–278 (PMID: 11921156)

Pihlgren M., Silva A.B., Madani R., Giriens V., Waeckerle-Men Y., Fettelschoss A., Hickman D.T., López-Deber M.P., Ndao D.M., Vukicevic M., Buccarello A.L., Gafner V., Chuard N., Reis P., Piorkowska K., Pfeifer A., Kündig T.M., Muhs A., Johansen P., TLR4- and TRIF-dependent stimulation of B lymphocytes by peptide liposomes enables T cell-independent isotype switch in mice. Blood. Jan 3;121(1):85-94 (2013).

Sallusto F., Lanzavecchia A., Araki K., Ahmed R., From vaccines to memory and back. Immunity. Oct 29;33(4):451-63 (2010).

Söderberg L., Johannesson M., Nygren P., Laudon H., Eriksson F., Osswald G., Möller C., Lannfelt L.; Lecanemab, Aducanumab, and Gantenerumab — Binding Profiles to Different Forms of Amyloid-Beta Might Explain Efficacy and Side Effects in Clinical Trials for Alzheimer's Disease, Neurotherapeutics (2023) 20:195–206.

M. Vukicevic, E. Fiorini, S. Siegert, R. Carpintero, M. Rincon, P. Lopez-Deber, N. Piot, M. Ayer, I. Rentero, C. Babolin, S. Bravo-Veyrat, V. Giriens, C. Morici, M. Beuzelin, A. Gesbert, S. Rivot, S. Depretti, P. Donati, J. Streffer, A. Pfeifer, M. H. Kosco-Vilbois, An amyloid beta vaccine that safely drives immunity to a key pathological species in Alzheimer's disease: pyroglutamate amyloid beta, Brain Communications, Volume 4, Issue 1, 2022, fcac022

Soto C., Plaque busters: strategies to inhibit amyloid formation in Alzheimer's disease. Molecular Medicine Today (vol 5), August 1999.

Winblad B., Graf A., Riviere M.E., Andreasen N., Ryan J.M., Active immunotherapy options for Alzheimer's disease. Alzheimers Res Ther. 2014 Jan 30;6(1):7.

Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs. All publications and patents specifically mentioned herein are incorporated by reference in their entirety for all purposes in connection with the invention.

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims. Moreover, all aspects and embodiments of the invention described herein are considered to be broadly applicable and combinable with any and all other consistent embodiments, including those taken from other aspects of the invention (including in isolation) as appropriate.

CLAIMS:

1. A liposomal vaccine composition comprising:
    a. A β-amyloid (Aβ)-derived peptide antigen displayed on the surface of the liposome that comprises, consists essentially of or consists of amino acids 1-15 of Aβ;
    b. A peptide comprising a universal T-cell epitope, and
    c. an adjuvant comprising monophosphoryl lipid A (MPLA)

    for use in inducing an anti-Aβ immune response in a human subject without inducing a serious adverse event, wherein the β-amyloid (Aβ)-derived peptide antigen is administered in an amount of 300-2000 µg.
2. The liposomal vaccine composition for use according to claim 1 wherein the β-amyloid (Aβ)-derived peptide antigen is administered in an amount of 300-1600 µg, 300-1000 µg, or 300-900 µg.
3. The liposomal vaccine composition for use according to claim 1 or 2, wherein the peptide comprising a universal T-cell epitope is, at least partially, encapsulated in the liposome.
4. The liposomal vaccine composition for use according to any one of the above claims, wherein the peptide comprising a universal T-cell epitope comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, and SEQ ID NO: 12, or an analogue thereof.
5. The liposomal vaccine composition for use according to any one of the above claims, wherein the peptide comprising a universal T-cell epitope comprises at least two universal T-cell epitopes, each having an amino acid sequence selected from the group consisting of SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11 or SEQ ID NO: 12, wherein the universal T-cell epitopes are joined by one or more linkers, and wherein the one or more linker(s) comprise, consist essentially of, or consist of the amino acids VVR, TVGLR, KVSVR, PMGAP or PMGLP, preferably VVR.
6. The liposomal vaccine composition for use according to any one of the above claims, wherein the peptide comprising a universal T-cell epitope comprises, consists essentially of or consists of an amino acid sequence selected from the group consisting of SEQ ID NO: 5 (SAT44), SEQ ID NO: 6 (SAT47), SEQ ID NO: 7 (SAT 58), SEQ ID NO: 3 (SAT42), and SEQ ID NO: 4 (SAT43), or an analogue thereof.
7. The liposomal vaccine composition for use according to any one of the above claims, wherein the peptide comprising a universal T-cell epitope comprises, consists essentially

of or consists of the amino acid sequence of SEQ ID NO: 7 (SAT 58), or an analogue thereof.

8. The liposomal vaccine composition for use of according to any one of the above claims, wherein the β-amyloid (Aβ)-derived peptide antigen is administered in an amount of 300 µg.
9. The liposomal vaccine composition for use according to claim 8, wherein the peptide comprising a universal T-cell epitope is administered in an amount of between 90 and 120 µg.
10. The liposomal vaccine composition for use according to claim 8 or 9 wherein the MPLA is administered in an amount of between 65 and 90 µg.
11. The liposomal vaccine composition for use according to any one of claims 8 to 10 wherein the β-amyloid (Aβ)-derived peptide antigen is administered in an amount of 300 µg, the peptide comprising a universal T-cell epitope is administered in an amount of between 90 and 120 µg and the MPLA adjuvant is administered in an amount of between 65 and 90 µg.
12. The liposomal vaccine composition for use of according to any one of claims 1 to 7 wherein the β-amyloid (Aβ)-derived peptide antigen is administered in an amount of 900 µg.
13. The liposomal vaccine composition for use according to claim 12, wherein the peptide comprising a universal T-cell epitope is administered in an amount of between 270 and 360 µg
14. The liposomal vaccine composition for use according to claim 12 or 13 wherein the MPLA is administered in an amount of between 200 and 270 µg.
15. The liposomal vaccine composition for use according to any one of claims 12 to 14 wherein the β-amyloid (Aβ)-derived peptide antigen is administered in an amount of 900 µg, the peptide comprising a universal T-cell epitope is administered in an amount of between 270 and 360 µg and the MPLA is administered in an amount of between 200 and 270 µg.
16. The liposomal vaccine composition for use of according to any one of claims 1 to 7 wherein the β-amyloid (Aβ)-derived peptide antigen is administered in an amount of 1600 µg.
17. The liposomal vaccine composition for use according to claim 16, wherein the peptide comprising a universal T-cell epitope is administered in an amount of between 470 and 650 µg.

18. The liposomal vaccine composition for use according to claim 16 or 17 wherein the MPLA is administered in an amount of between 360 and 480 µg.
19. The liposomal vaccine composition for use according to any one of claims 16 to 18 wherein the β-amyloid (Aβ)-derived peptide antigen is administered in an amount of 1600 µg, the peptide comprising a universal T-cell epitope is administered in an amount of between 470 and 650 µg and the MPLA is administered in an amount of between 360 and 480 µg.
20. The liposomal vaccine composition for use according to any one of the above claims wherein the β-amyloid (Aβ)-derived peptide antigen is lipidated.
21. The liposomal vaccine composition for use according to any one of the above claims wherein the β-amyloid (Aβ)-derived peptide antigen is tetrapalmitoylated.
22. The liposomal vaccine composition for use according to any one of the above claims wherein the adjuvant forms part of the outer layer of the liposome, optionally wherein the adjuvant is, at least in part, displayed on the surface of the liposome.
23. The liposomal vaccine composition for use according to any one of the above claims wherein the monophosphoryl lipid A (MPLA) comprises synthetic monophosphoryl lipid A (MPLA).
24. The liposomal vaccine composition for use according to any one of the above claims wherein the monophosphoryl lipid A (MPLA) comprises monophosphoryl Hexa-acyl Lipid A, 3-Deacyl (Synthetic) (3D-(6-acyl) PHAD®) and/or Phosphorylated HexaAcyl Disaccharide (PHAD®).
25. The liposomal vaccine composition for use according to any one of the above claims wherein the liposome comprises phospholipids.
26. The liposomal vaccine composition for use according to claim 25 wherein the phospholipids comprise dimyrsitoylphosphatidyl-choline (DMPC) and dimyrsitoylphosphatidyl-glycerol (DMPG).
27. The liposomal vaccine composition for use according to any one of the above claims wherein the liposome comprises cholesterol.
28. The liposomal vaccine composition for use according to claim 27 wherein the molar ratio of dimyrsitoylphosphatidyl-choline (DMPC): dimyrsitoylphosphatidyl-glycerol (DMPG): cholesterol is 9:1:7.
29. The liposomal vaccine composition for use according to claim 28 wherein the molar ratio of dimyrsitoylphosphatidyl-choline (DMPC): dimyrsitoylphosphatidyl-glycerol (DMPG): cholesterol: MPLA is 9:1:7:0.05.

30. The liposomal vaccine composition for use according to any one of the above claims wherein the liposomal vaccine composition is administered by injection.
31. The liposomal vaccine composition for use according to any one of the above claims wherein the liposomal vaccine composition is administered intramuscularly or subcutaneously.
32. The liposomal vaccine composition for use according to claim 31 wherein the liposomal vaccine composition is administered intramuscularly.
33. The liposomal vaccine composition for use according to claim 32 wherein the liposomal vaccine composition is administered subcutaneously.
34. The liposomal vaccine composition for use according to any one of the above claims wherein the liposomal vaccine composition is administered at a first time and is administered at a second time 1 to 4 weeks later.
35. The liposomal vaccine composition for use according to any one claims 1 to 33 wherein the liposomal vaccine composition is administered every 4-12 weeks for a period of at least 48 weeks or at least 74 weeks.
36. The liposomal vaccine composition for use according to any one of claims 1 to 33 wherein the liposomal vaccine composition is administered at a first time and is administered at a second time 2 to 6 weeks later, preferably wherein the second administration is 4 weeks after the first administration.
37. The liposomal vaccine composition for use according to any one of the above claims, wherein the induced anti-Aβ immune response is obtained following two administrations of the liposomal vaccine.
38. The liposomal vaccine composition for use according to any one of claims 34 to 37, wherein the liposomal vaccine composition is administered at a third time 6-10 weeks after the second administration of the vaccine composition, preferably wherein the third administration is 8 weeks after the second administration.
39. The liposomal vaccine composition for use according to any one of claims 34 to 38, further comprising a booster administration at a subsequent time point.
40. The liposomal vaccine composition for use according to any one of the above claims, wherein administration of the liposomal vaccine composition results in a reduction in the amount of Aβ associated plaques in the brain of the subject.
41. The liposomal vaccine composition for use according to any one of the above claims, wherein the induced anti-Aβ immune response is for treatment, prevention, induction of a protective immune response against or alleviating the symptoms associated with an amyloid-beta associated disease or condition in the human subject.

42. The liposomal vaccine composition for use according to claim 41 wherein the amyloid-beta associated disease or condition is selected from Alzheimer's Disease, mild cognitive impairment (MCI), Down syndrome (DS), including Down syndrome-related Alzheimer's disease, cardiac amyloidosis, cerebral amyloid angiopathy (CAA), multiple sclerosis, Parkinson's disease, Lewy body dementia, ALS (amyotrophic lateral sclerosis), Adult Onset Diabetes, inclusion body myositis (IBM), ocular amyloidosis, glaucoma, macular degeneration, lattice dystrophy and optic neuritis.
43. The liposomal vaccine composition for use according to claim 42 wherein the amyloid-beta associated disease or condition is Alzheimer's Disease.
44. The liposomal vaccine composition for use according to claim 43 wherein the Alzheimer's Disease is early Alzheimer's Disease.
45. The liposomal vaccine composition for use according to claim 44 wherein the early Alzheimer's Disease includes prodromal Alzheimer's Disease, mild cognitive impairment due to Alzheimer's Disease and mild Alzheimer's Disease.
46. The liposomal vaccine composition for use according to claim 43 wherein the Alzheimer's Disease is prodromal Alzheimer's Disease.
47. The liposomal vaccine composition for use according to claim 43 wherein the Alzheimer's Disease is mild Alzheimer's Disease.
48. The liposomal vaccine composition for use according to claim 43 wherein the Alzheimer's Disease is mild-to-moderate Alzheimer's Disease.
49. The liposomal vaccine composition for use according to claim 43 wherein the Alzheimer's Disease is moderate Alzheimer's Disease.
50. The liposomal vaccine composition for use according to claim 43 wherein the Alzheimer's Disease is not severe Alzheimer's Disease.
51. The liposomal vaccine composition for use according to claim 42 wherein the amyloid-beta associated disease or condition is Down Syndrome.
52. The liposomal vaccine composition for use according to claim 42 wherein the amyloid-beta associated disease or condition is Down syndrome-related Alzheimer's disease.
53. The liposomal vaccine composition for use according to any one of the above claims wherein the human subject, prior to treatment, displays cognitive function consistent with a Mini Mental State Examination (MMSE) score of at least 18, such as 18-28, or at least 20, such as 20-28.
54. The liposomal vaccine composition for use according to any one of the above claims wherein the β-amyloid (Aβ)-derived peptide antigen is tetrapalmitoylated Abeta 1-15 as set forth in SEQ ID NO: 1.

55. The liposomal vaccine composition for use according to any one of the above claims wherein the human subject is receiving at least one additional therapy selected from an acetylcholinesterase inhibitor (ACHEI) and/or memantine.

56. A liposomal vaccine composition comprising:

    a. A β-amyloid (Aβ)-derived peptide antigen displayed on the surface of the liposome that comprises, consists essentially of or consists of amino acids 1-15 of Aβ; wherein the β-amyloid (Aβ)-derived peptide antigen is tetrapalmitoylated Abeta 1-15 as set forth in SEQ ID NO: 1;
    b. A peptide comprising a universal T-cell epitope comprising, consisting of, or essentially consisting of the amino acid sequence of SEQ ID NO: 7 (SAT 58), or an analogue thereof, and
    c. an adjuvant comprising monophosphoryl lipid A (MPLA), preferably 3D-(6-acyl) PHAD®

for use in inducing an anti-Aβ immune response in a human subject without inducing a serious adverse event, wherein the β-amyloid (Aβ)-derived peptide antigen is administered in an amount of 300-2000 µg, preferably in an amount of 300-1600 µg, preferably in an amount of 300 µg, 900 µg or 1600 µg.

57. A liposomal vaccine composition comprising:

    a. A β-amyloid (Aβ)-derived peptide antigen displayed on the surface of the liposome that comprises, consists essentially of or consists of amino acids 1-15 of Aβ, wherein the β-amyloid (Aβ)-derived peptide antigen is tetrapalmitoylated Abeta 1-15 as set forth in SEQ ID NO: 1;
    b. A peptide comprising a universal T-cell epitope comprising, consisting of, or essentially consisting of the amino acid sequence of SEQ ID NO: 7 (SAT 58), or an analogue thereof
    c. an adjuvant comprising monophosphoryl lipid A (MPLA), preferably 3D-(6-acyl) PHAD®.

58. The liposomal vaccine composition according to claim 57 wherein the vaccine composition comprises the β-amyloid (Aβ)-derived peptide antigen in an amount of 300 µg, the peptide comprising a universal T-cell epitope in an amount of between 90 and 120 µg, and the MPLA adjuvant in an amount of between 65 and 90 µg.

59. The liposomal vaccine composition according to claim 57 wherein the vaccine composition comprises the β-amyloid (Aβ)-derived peptide antigen in an amount of 900 µg, the peptide comprising a universal T-cell epitope in an amount of between 270 and 360 µg, and the MPLA in an amount of between 200 and 270 µg.

60. The liposomal vaccine composition according to claim 57 wherein the vaccine composition comprises the β-amyloid (Aβ)-derived peptide antigen in an amount of 1600 µg, the peptide comprising a universal T-cell epitope in an amount of between 470 and 650 µg, and the MPLA in an amount of between 360 and 480 µg.

5

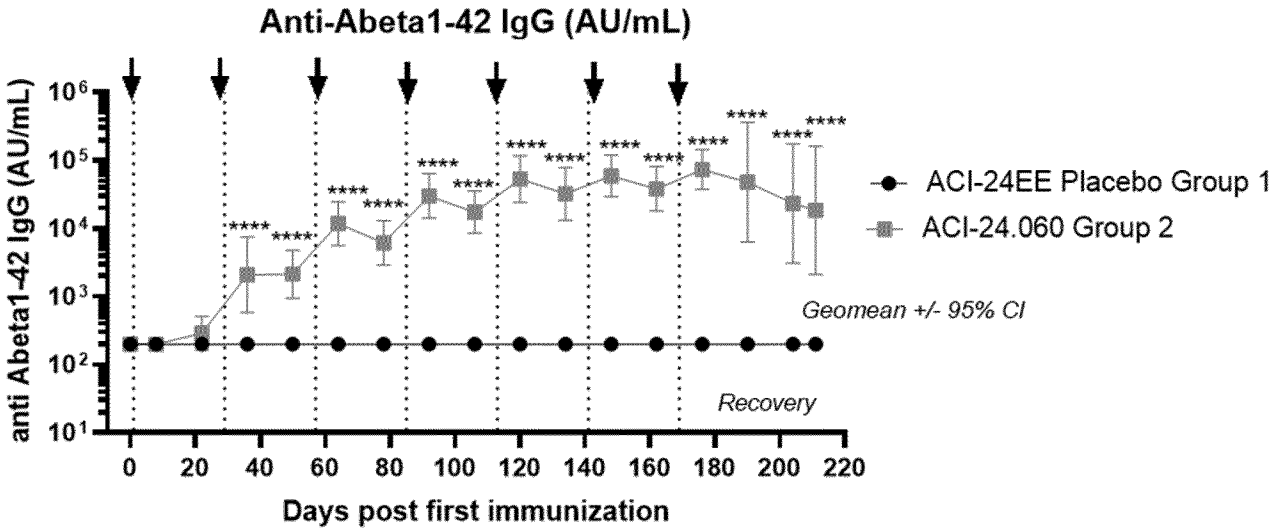

Figure 1

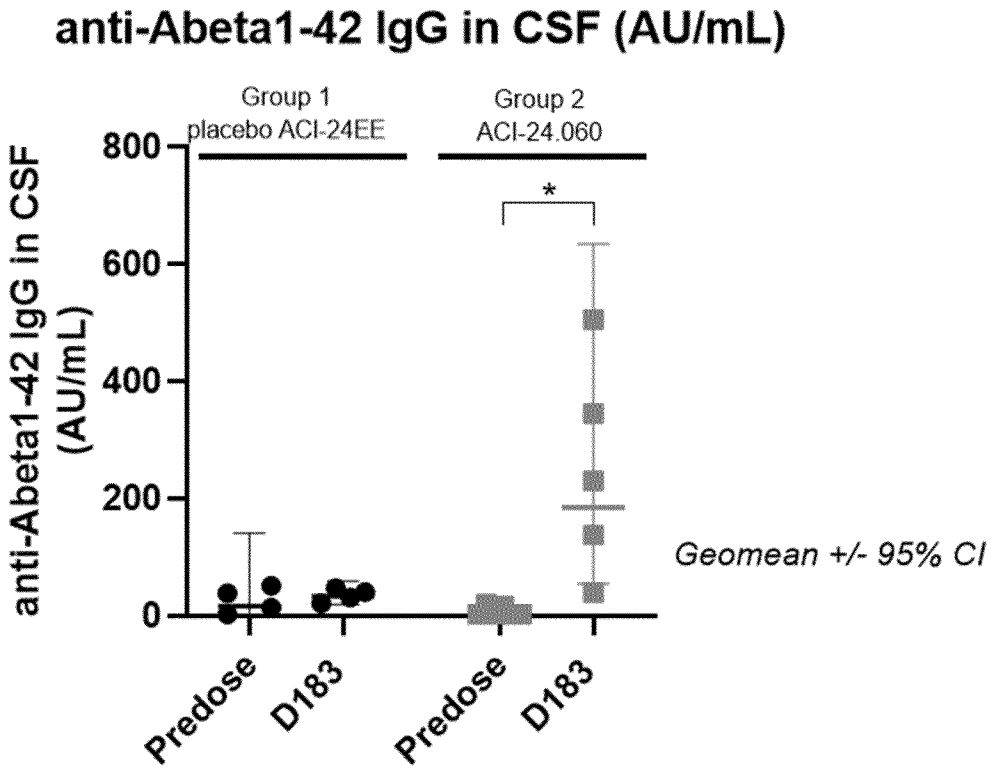

Figure 2

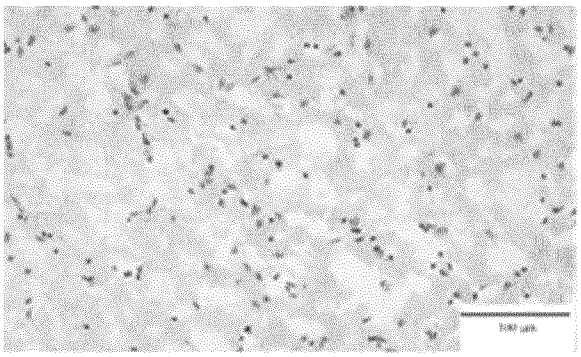

Figure 3a

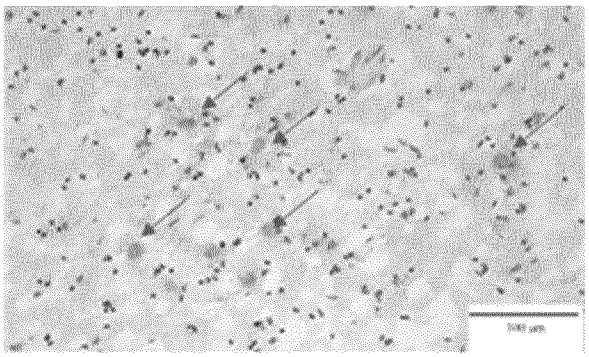

Figure 3b

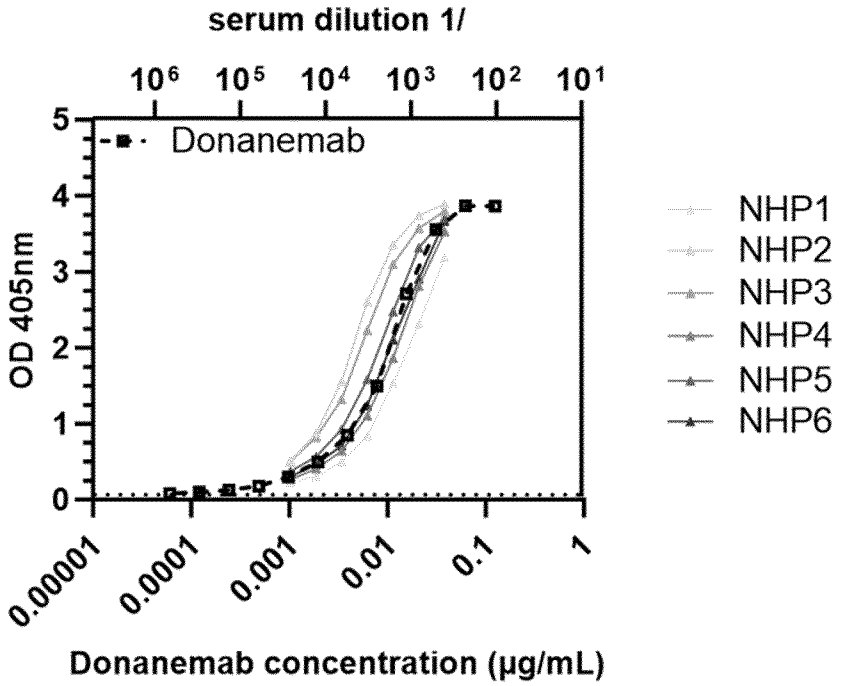

Figure 4

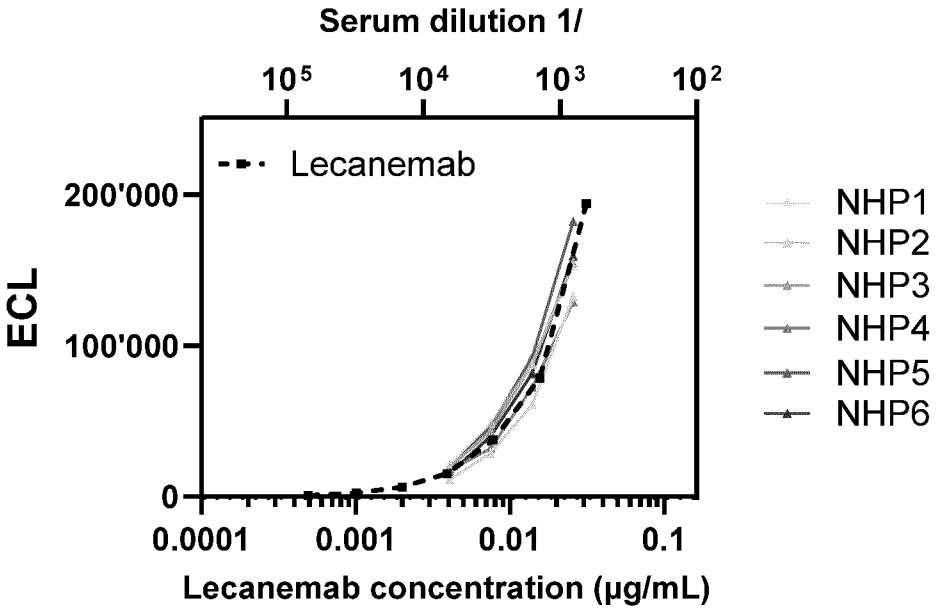
Serum dilution 1/
10⁵
10⁴
10³
10²
Lecanemab
200'000
100'000
0
ECL
0.0001
0.001
0.01
0.1
Lecanemab concentration (µg/mL)
NHP1
NHP2
NHP3
NHP4
NHP5
NHP6

Figure 5

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2024/052002

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2022/226447 A1 (PFEIFER ANDREA [CH] ET AL) 21 July 2022 (2022-07-21) claim 1	1-60
A	US 10 828 351 B2 (AC IMMUNE SA [CH]) 10 November 2020 (2020-11-10) claim 1; table 3	1-60

-/--

[x] Further documents are listed in the continuation of Box C.

[x] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
8 May 2024	21/05/2024

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Patti, Gabriele

2

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Clinicaltrials.Gov: "A Study to Assess the Effects of ACI-24.060 in Alzheimer's Disease and in Down Syndrome (ABATE Study) NCT05462106 ¦", , 18 January 2023 (2023-01-18), pages 1-14, XP093154386, Retrieved from the Internet: URL:https://clinicaltrials.gov/study/NCT05462106?tab=history&a=3 the whole document -----	1-60
X	Michael Rafii: "Safety, Tolerability, and Immunogenicity of the ACI-24 Vaccine in Adults With Down Syndrome - PMC", , 29 June 2022 (2022-06-29), pages 1-22, XP093154046, Retrieved from the Internet: URL:https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9086937/?report=printable abstract -----	1-60

INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP2024/052002

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed.

   b. [ ] furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)).

      [ ] accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. [ ] With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2024/052002

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2022226447 A1	21-07-2022	AU 2020277682 A1	23-12-2021
		BR 112021023209 A2	18-01-2022
		CA 3138145 A1	26-11-2020
		CL 2021003051 A1	22-07-2022
		CN 113853214 A	28-12-2021
		EP 3972633 A1	30-03-2022
		IL 288252 A	01-01-2022
		JP 2022533422 A	22-07-2022
		KR 20220010552 A	25-01-2022
		SG 11202112329R A	30-12-2021
		TW 202110425 A	16-03-2021
		US 2022226447 A1	21-07-2022
		WO 2020234405 A1	26-11-2020
US 10828351 B2	10-11-2020	AU 2019253193 A1	26-11-2020
		BR 112020020660 A2	02-03-2021
		CA 3095983 A1	17-10-2019
		CL 2020002586 A1	15-01-2021
		CO 2020013977 A2	29-01-2021
		CR 20200533 A	22-02-2021
		EP 3773712 A1	17-02-2021
		IL 277851 A	30-11-2020
		JP 2021521149 A	26-08-2021
		KR 20200143422 A	23-12-2020
		MA 52180 A	17-02-2021
		PE 20201344 A1	25-11-2020
		PH 12020551670 A1	07-06-2021
		SG 11202009917P A	27-11-2020
		TW 202003031 A	16-01-2020
		US 2019307867 A1	10-10-2019
		US 2021093700 A1	01-04-2021
		WO 2019197414 A1	17-10-2019