

Liposomal construct

Field of the invention

The invention relates to a liposomal construct suitable for use in vaccine compositions, to liposomal vaccine compositions and the manufacturing thereof.

Background of the invention

The immune system is a complex interactive system of the body that involves a wide variety of components, including cells, and cellular factors, which interact with stimuli from both inside the body and outside the body. One of the better-known aspects of the immune system is its ability to respond to foreign antigens presented by invading organisms, cellular changes within the body, or from vaccination.

The immune system response to antigens involves both humoral responses and cellular responses. Humoral immune responses are mediated by non-cellular factors that are released by cells and which may or may not be found free in the plasma or intracellular fluids. A major component of a humoral response of the immune system is mediated by antibodies produced by B lymphocytes. Cell mediated immune responses result from the interactions of cells, including antigen presenting cells and B lymphocytes (B cells) and T 15 lymphocytes (T cells). Stimulating immune responses through vaccination has been shown to be an effective strategy to treat or prevent a wide variety of diseases and disorders in animals and humans, including infectious diseases, allergies, neurodegenerative diseases, cancer.

Liposomal vaccines have received an increased interest over recent years. The idea to use liposomes as vehicles for the presentation of antigens was tested more than 30 years ago (Allison and Gregoriadis, Nature, 1974, 252, 252). For example, it was shown that diphtheria toxoid incorporated in liposomes is more immunogenic than its free form. Antigens presented via liposomes can induce humoral as well as cellular immune responses. Liposomes are artificial vesicles, mostly made of (phospho)lipids and may contain drugs or soluble antigens in their internal, aqueous volume or amphipathic antigens, such as membrane proteins, incorporated in the bilayer. Antigens from many microorganisms and tumor cells have been incorporated into such liposomes with a detailed characterization and in vivo testing. Clinical studies with antigen containing liposomes have indicated that they are safe and generally induce no severe adverse effects (Kersten and Crommelin, Biochimica et Biophysica Acta 1995, 1241, 117- 138).

WO2005/081872 describes supramolecular antigenic constructs comprising antigenic peptides which are PEGylated at each terminus and reconstructed in liposomes, resulting in enhanced peptide presentation. WO2010/106127 describes an antigenic composition having a modified antigen presented in a highly repetitive array on the surface of a liposome. The antigenic composition is suitable for inducing a T-cell independent immune response upon treatment of a disease. WO2012/055933 relates to liposome-based antigenic constructs having an antigenic peptide of interest modified through hydrophobic moieties and a method of preparing the liposome-based construct.

WO2012/020124 describes the method and constructs that allow to control peptide conformation by modulation of the peptide lipidation pattern, spacer and liposome composition, or via co-administration with small molecules.

WO2019/197414 pertains to a liposomal vaccine composition comprising an amyloid-beta (Abeta)-derived peptide antigen displayed on the surface of the liposome, a peptide comprising a T-cell epitope and an adjuvant. The vaccine composition is disclosed for use in the treating, preventing, or alleviating the symptoms associated with an Abeta associated disease.

However, challenges still exist in the technical field concerning not just the targeting and treating of diseases but also pertaining to vaccine manufacturing and stability of the product. Vaccine instability can be caused by light, heat, oxidation, radiation, changes in the environment or reactions with other components in the vaccine mixture. This can lead to a product that gradually loses potency or degrades such that it is no longer suitable for use.

Following the recent pandemic, it has become apparent that faster means of producing new vaccines are necessary to counter fast mutating virus. There is a need for "plug and play" technologies that can facilitate the rapid development of new vaccines, allowing switching of antigen of a vaccine composition, e.g., as a virus mutates.

It would be desirable to address and/or mitigate at least some of the abovementioned issues.

Summary of the invention

In a first aspect the invention provides a liposomal construct. The liposomal construct comprises a liposome, at least one adjuvant and at least one peptide containing at least one T-cell epitope. The at least one T-cell epitope may comprise, consist of, or consist essentially of, the amino acid sequence of SEQ ID NO: 4 or an analogue thereof. The liposome of the

liposomal construct may be an at least partly spherical vesicle and may comprise at least one lipid bilayer delimiting a core.

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

The liposomal construct of the invention comprises a peptide containing at least one T-cell epitope.

Preferably the peptide containing at least one T-cell epitope is, at least partly, encapsulated within the liposome.

In some embodiments the peptide may additionally be, at least partly, displayed on the inner and/or outer surface(s) of the lipid bilayer of the liposome and/or the peptide may be at least partly integrated within at least one layer of the lipid bilayer of the liposome. The peptide may be associated with the lipid bilayer of the liposome. For example, the peptide may be associated with the liposome in such a manner that it is displayed on the surface of the liposome. For example, the peptide may associate with the liposome through electrostatic interaction between the peptide and the lipid bilayer of the liposome.

In a preferred embodiment the peptide containing at least one T-cell epitope is encapsulated within the liposome. In another preferred embodiment the peptide is, at least partially, encapsulated within the liposome and additionally, is at least partly displayed on the inner and/or outer surface of the liposome.

The peptide included in the liposomal construct of the invention contains at least one T-cell epitope. This peptide is therefore able to activate T-cells and specifically T-helper cells. Each of the at least one T-cell epitopes is typically a universal T-cell epitope. 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 liposomal constructs and liposomal vaccine compositions of the invention may thus be capable of stimulating a CD4 T-cell response. The universal T-cell epitopes included in the liposomal constructs and liposomal vaccine compositions of the invention may thus be capable of stimulating a helper T-cell response that enhances antibody production by B-cells, for example in response to an antigenic peptide displayed on the surface of the liposome as described further herein (e.g. Abeta amino acids 1-15).

The peptide containing at least one T-cell epitope comprises an epitope originating from influenza hemagglutinin that has been modified to replace the methionine at position 2 of SEQ ID NO: 2 with alanine (SEQ ID NO: 4), or an analogue of SEQ ID NO: 4 as defined herein. In some embodiments, the peptide containing at least one T-cell epitope may additionally comprise one or more epitopes originating from one or more of: tetanus, influenza and diphtheria, and also Keyhole limpet hemocyanin (KLH) and Epstein Barr virus (EBV).

In a preferred embodiment, the peptide containing at least one T-cell epitope may additionally comprise an epitope derived from tetanus toxin.

The peptide containing at least one T-cell epitope may be synthesized by solid phase synthesis. In some embodiments, the peptide containing at least one 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.

In some embodiments, the peptide containing at least one T-cell epitope comprises the amino acid sequence of SEQ ID NO: 4. Alternatively, the peptide containing at least one T-cell epitope may consist essentially of or may consist of the amino acid sequence of SEQ ID NO: 4.

In some embodiments, the peptide containing at least one T-cell epitope may comprise at least two T-cell epitopes. At least one of the at least two T-cell epitopes may comprise, consist of, or consist essentially of the amino acid sequence of SEQ ID NO: 4.

An analogue is a functionally equivalent sequence, in terms of ability to stimulate T-helper cells, that may comprise one or more modifications compared to the sequences recited. In the context of SEQ ID NO: 4, the analogue must additionally retain the alanine at position 2 of the sequence. The analogue should not contain any methionine residues. The modifications 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 analogue 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 a preferred embodiment, the peptide containing at least one T-cell epitope comprises a first epitope comprising, consisting of or consisting essentially, of the amino acid sequence of SEQ ID NO: 4 or an analogue thereof, and may further comprise a further T-cell epitope comprising, consisting of or consisting essentially of the amino acid sequence SEQ ID NO: 1 or SEQ ID NO: 3, or an analogue of SEQ ID NO:1 or SEQ ID NO: 3. In this context, an analogue of SEQ ID NO: 1 or SEQ ID NO: 3 is as defined above, namely a functionally equivalent sequence, in terms of ability to stimulate T-helper cells, that may comprise one or more modifications compared to the sequences recited. The modifications may comprise one or more (preferably one or two) additions, deletions or substitutions provided function as a (universal) T-cell epitope is retained. The modifications do not introduce any methionine residues into the peptide. The minimum and maximum lengths of the peptide analogue are set out above and apply *mutatis mutandis* to the analogue.

In a preferred embodiment the peptide containing at least one T-cell epitope comprises at least three T-cell epitopes. At least one of the at least three T-cell epitopes comprises, consists of, or consists essentially of SEQ ID NO: 4, or an analogue thereof. In a preferred embodiment the at least three T-cell epitopes may additionally comprise, consist of, or consist essentially of amino acid sequences that are selected from the amino acid sequences of SEQ ID NO: 1; and/or SEQ ID NO: 3, or analogues thereof.

The peptide may therefore comprise at least three T-cell epitopes wherein the at least three T-cell epitopes comprise, consist of, or consist essentially of amino acid sequences of SEQ ID NO: 4, SEQ ID NO: 1 and SEQ ID NO:3, or analogues of those sequences as defined herein.

In a preferred embodiment, the at least three T-cell epitopes comprise, consist of, or consist essentially of SEQ ID NO: 1, SEQ ID NO: 3 and SEQ ID NO: 4. In a particularly preferred embodiment, the peptide containing at least one T-cell epitope comprises, consists essentially of, or consists of the amino acid sequence of SEQ ID NO: 6, or an analogue thereof. An analogue of SEQ ID NO: 6 is as defined above, taking into consideration that the peptide includes 4 separate (universal) T-cell epitopes. Thus, the minimum length of an analogue here is 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).

In some embodiments the peptide containing at least one T-cell epitope may comprise one or more linkers. The linkers may be configured to join one or more T-cell epitopes of the peptide to each other. In other words, the peptide may comprise multiple T-cell epitopes connected to one another by linkers.

The linker is used to physically connect the T-cell epitopes to one another in a manner that does not detract from the immunogenicity of each of the linked epitopes. Preferred linkers are themselves amino acid-based linkers, i.e., peptide linkers. They can thus join the T-cell epitopes to one another through peptide bonds.

Therefore, the peptide containing at least one T-cell epitope may further comprise one or more linkers. For example, a peptide having at least two T-cell epitopes may comprise at least one linker to join the two T-cell epitopes.

In some preferred embodiments the peptide containing at least one T-cell epitope comprises at least three T-cell epitopes linked by at least two linkers. The peptide may thus be a linear peptide in which the terminal epitopes are not connected to one another.

The linker preferably 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. The linkers may comprise, 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.

In a preferred embodiment at least one linker comprises, consists of, or consists essentially of the amino acid sequence VVR. In a preferred embodiment all the linkers comprised in the peptide containing at least one T-cell epitope comprise, consist of or consists essentially of the amino acid sequence VVR.

In a preferred embodiment, the peptide containing at least one T-cell epitope may comprise the amino acid sequence of SEQ ID NO: 6.

In some embodiments, the peptide having a universal T-cell epitope may consist of, or consist essentially of, the amino acid sequence of SEQ ID NO: 6.

The peptide comprising at least three T-cell epitopes and having the amino acid sequence of SEQ ID NO: 6 may also be referred to interchangeably herein as SAT58 peptide.

Advantageously, the peptide comprising a T-cell epitope as described herein is stable to oxidation. The stability of the peptide comprising a T-cell epitope to oxidation advantageously provides a reduction, or absence, of T-cell epitope peptide oxidation degradation products, that can have negative effects on the toxicology profile and/or shelf life of a liposomal construct or liposomal vaccine. Accordingly, the present invention advantageously enables the provision of stable liposomal constructs and vaccine compositions, as demonstrated in the examples included herein.

Reducing oxidation of the T-cell epitope containing peptide reduces the production of unwanted degradation products in a product in which the liposomal construct is used, e.g. a liposomal vaccine, thereby removing or minimising risks of toxicity from peptide degradation products. Reducing oxidation of the T-cell epitope containing peptide may prolong the shelf-life of the liposomal construct or of a product in which the liposomal construct is used. For example, use of the liposomal construct in a vaccine composition may reduce the risk of oxidation during storage of the vaccine and thus prolong the shelf-life and usability of the vaccine.

These and additional advantages and benefits of the liposomal construct of the invention are described with reference to the Examples hereinbelow.

The liposomal construct comprises at least one adjuvant. Optionally, the liposomal construct may comprise multiple adjuvants. In some embodiments the adjuvant(s) may form part of the liposome, for example, the adjuvant may form part of the lipid bilayer of the liposome. Optionally the adjuvants may be at least in part integrated within the lipid bilayer of the liposome. In some embodiments the adjuvant or adjuvants may be, at least in part, displayed on the surface of the liposome and/or may be at least in part integrated in the lipid bilayer of the liposome.

The adjuvant serves to enhance the immune response when used in pharmaceutical compositions, e.g. vaccines. Adjuvants typically stimulate the immune system so as to induce a stronger and/or longer lasting immune response.

Optionally, the adjuvant(s) of the liposomal construct may be selected from one or more of: monophosphoryl lipid A (MPLA); diphosphoryl lipid A (DPLA); Alum; Pam2CSK4; Pam3CSK4; Pam3CAG; saponins; CpG; lipidated CpG, such as CpG-Cholesterol; cationic lipids; phosphorothioated PS-CpG-ODNs; CpG oligodeoxynucleotides (CpG-ODN); CpG-A; CpG-B or CpG-C.

Further adjuvants that may be used according to the invention are: aluminium phosphate or hydroxide (Al(OH)₃, AlPO₄), salts of calcium, iron, zirconium, QuilA, QS-21, trehalose dimycolate (TDM), lipoteichoic acid (purified from *Staphylococcus aureus*), DDAB (dimethyldioctadecylammonium (bromide salt)), MF59, L18-MDP & B30-MDP (hydrophobic muramyl-dipeptide derivatives), C12-iE-DAP (diamino-pimelic acid).

In a preferred embodiment the at least one adjuvant may be a lipid-based adjuvant(s).

In some embodiments the adjuvant may be a Toll-like receptor (TLR) agonist, in particular a TLR4 agonist or a TLR9 agonist.

As used herein, the term "toll-like receptor 4 agonist" refers to any compound that acts as an agonist of TLR4. Examples of TLR4 agonist useful for the invention include, but not limited to, monophosphoryl lipid A (MPLA). MPLA useful for the invention can be obtained using methods known in the art, or from a commercial source, such as 3D-(6-acyl) PHAD®, PHAD®, PHAD®-504, 3D-PHAD® from Avanti Polar Lipids (Alabaster, Alabama, USA) or MPL™ from various commercial sources. According to particular embodiments, the toll-like receptor 4 agonist is MPLA.

Monophosphoryl lipid A or MPLA refers to a modified form of lipid A, which is the biologically active part of Gram-negative bacterial lipopolysaccharide (LPS) endotoxin. MPLA provides the immunostimulatory activity but is less toxic than LPS.

MPLA term encompasses MPLA-derivatives such as Monophosphoryl Hexa-acyl Lipid A, 3-Deacyl (Synthetic) (3D-(6-acyl) PHAD®), PHAD® (Phosphorylated HexaAcyl Disaccharide), PHAD®-504, 3D-PHAD® from Avanti Polar Lipids (Alabaster, Alabama, USA) or MPL. Thus, according to particular embodiments, the compositions further comprise MPLA. The MPLA is typically added during liposomal formation (as explained further herein). In one embodiment, the adjuvant(s) of the liposomal construct may be 3D-(6-acyl) PHAD®.

For example, a preferred liposomal construct of some embodiments may comprise dimyristoylphosphatidyl-choline (DMPC), dimyristoylphosphatidyl-glycerol (DMPG), cholesterol and MPLA. The molar ratios of these four components may be 9:1:7:0.05 in some embodiments.

As used herein, the term "toll-like receptor 9 agonist" (TLR9 agonist) refers to any compound that acts as an agonist of TLR9. Examples of suitable TLR9 agonist include, but not limited to, CpG oligonucleotides. As used herein, the term "CpG oligonucleotide", "CpG oligodeoxynucleotide" or "CpG ODN" refers to an oligonucleotide comprising at least one CpG motif. As used herein, "oligonucleotide," "oligodeoxynucleotide" or "ODN" refers to a polynucleotide formed from a plurality of linked nucleotide units. Such oligonucleotides can be obtained from existing nucleic acid sources or can be produced by synthetic methods. As used herein, the term "CpG motif" refers to a nucleotide sequence which contains unmethylated cytosine-phosphate-guanine (CpG) dinucleotides (i.e., a cytosine (C) followed by a guanine (G)) linked by a phosphate bond or a phosphodiester backbone or other internucleotide linkages. Examples of synthetic CpG oligonucleotides include, but are not limited to, CpG2006 (also known as CpG 7909), CpG 1018, CpG2395, CpG2216 or CpG2336.

In one embodiment, the adjuvant(s) of the liposomal construct may comprise CpG.

In an embodiment the CpG oligonucleotide may be covalently linked to the lipid bilayer of the liposome through a covalent linkage.

In another aspect the present invention relates to a liposomal vaccine composition comprising a liposomal construct of the invention and at least one antigenic peptide. Throughout the disclosure reference is made to "liposomal vaccine compositions". This term may be used interchangeably with "liposomal composition", "liposomal vaccine" and "vaccine composition". The compositions are immunogenic and thus may equally be referred to as "liposomal immunogenic compositions".

The antigenic peptide is understood to encompass any peptide capable, upon administration to a mammal, particularly a human, of inducing an immune response in said mammal. The antigenic peptide may be derived from a foreign (external) antigen, e.g. a virus or an allergen, or may be derived from a self-antigen, e.g. tumour antigen, cytokines such as, for example, IL-17, IL-27, or proteins such as Abeta, Tau, α -syn, involved in proteinopathies.

The antigenic peptide is displayed, at least partly, on the surface of the liposome. Thus, the liposome may serve to carry the antigenic peptide. Optionally the antigenic peptide may be at least partly connected to the lipid bilayer of the liposome. For example, the antigenic peptide

may be at least partly inserted within, or anchored to, the lipid bilayer of the liposome and/or form part of the outer surface of the liposome.

The antigenic peptide is displayed on the 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. The antigenic peptide may be modified through at least one lipophilic or hydrophobic moiety to facilitate this. Optionally the antigenic peptide may be modified through multiple lipophilic or hydrophobic moieties. For example, the antigenic peptide may comprise two, three or four lipophilic or hydrophobic moieties. The lipophilic or hydrophobic moieties may connect the antigen molecule to the liposome. The one or more lipophilic or hydrophobic moieties may insert at least partly into the outer surface of the liposome, i.e. into the lipid bilayer of the liposome. The one or more lipophilic or hydrophobic moieties are preferably hydrophobic moieties for ease of insertion into the lipid bilayer. In some embodiments, the one or more moieties may be one or more of: a fatty acid, a triglyceride, diglyceride, steroid, sphingolipid, glycolipid, or a phospholipid.

The at least one lipophilic or hydrophobic moiety may facilitate connecting of the antigenic peptide into the lipid bilayer of the liposome. The moiety or moieties may act as an anchor for the peptide in the liposome bilayer and may allow for the antigenic peptide to be positioned and/or stabilized in close proximity to the liposome surface.

Preferably, the at least one lipophilic or hydrophobic moiety is a fatty acid. The fatty acid may comprise a carbon backbone of at least 3 carbon atoms. Optionally, the fatty acid may comprise a carbon backbone having: 4, 6, 8, 12 or 14 atoms.

In some embodiments, the fatty acid may comprise a carbon backbone having up to 24 carbon atoms. The fatty acid may have at least 14 carbon atoms. Alternatively, the fatty acid may comprise a carbon backbone having at least 16 carbon atoms.

Hydrophobic moieties may include, but are not limited to: palmitic acid, stearic acid, myristic acid, lauric acid, oleic acid, linoleic acid, and ltnolenic acid, cholesterol or 1 ,2-dtstearoyl- sn-glycero-3-phosphatidylethanolamine (DSPE).

In a preferred embodiment the moiety or moieties may comprise a palmitoyl residue. Thus, the antigenic peptide may be palmitoylated, i.e. mono-palmitoylated or multi-palmitoylated. For example, the antigen molecule may be modified by at least two palmitoyl residues. Alternatively, the antigen molecule may be modified by at least four palmitoyl residues, i.e.

tetrapalmitoylated. The antigenic peptide can include additional residues, such as lysine residues to facilitate palmitoylation. Those residues are typically found at the N and/or C terminus of the peptide. In some embodiments, there may be 1-4 lysine residues added to the N and/or C terminus. In one embodiment, a preferred construction comprises the antigenic peptide attached to two palmitoyl residues in the N and/or C terminal regions of the peptide. Thus, the antigenic peptide is dipalmitoylated or tetrapalmitoylated. This may be facilitated by incorporating two lysine residues in the N and/or C terminal regions of the peptide antigen, whereby the lysine residues are palmitoylated.

In some embodiments, two different antigenic peptides are introduced into the liposome. The two different antigenic peptides may originate from the same protein or from different proteins. Where the proteins are different they are typically both disease targets for the same condition.

The liposomal construct of the present invention is applicable to a variety of antigenic peptides and can ultimately be employed in therapeutic formulations and vaccines for diseases and disorders including, but not limited to, neurodegenerative diseases, cancer, autoimmune diseases and infectious disease.

In some embodiments, the antigenic peptide may be derived from a foreign antigen. The term "foreign antigen", as used herein, refers to any molecule that is not naturally produced by an individual (human). In general, the immune system of the individual will recognise the foreign antigen and an immune response will be generated against the antigen. For examples, the foreign antigen may be a viral antigen, or an allergen. Examples of viral antigens include, but are not limited to, antigens from rhinoviruses, coronaviruses, enteroviruses, adenoviruses, parainfluenza viruses and respiratory syncytial viruses. The antigen may be a viral fusion protein in some embodiments.

In some embodiments the antigenic peptide may be a self-antigen. The term "self-antigen", as used herein, refers to any peptide derived from an antigen naturally produced by an individual. In general, the immune system of the individual is tolerant to self-antigen molecules and therefore no immune reaction occurs. In some cases, the immune system is not tolerant to the self-antigens and auto-immune diseases may occur.

The use of a self-antigen allows the targeting of a molecule to which the immune system is tolerant and therefore may help to induce an immune response that otherwise would not occur.

In a preferred embodiment the antigenic peptide is a self-antigen peptide.

In some embodiments, the antigenic peptide is a peptide derived from an amyloid protein. In some embodiments the antigenic peptide may be derived from an amyloid-like protein. Amyloid-like proteins include but are not limited to prion protein, Tau protein, alpha-synuclein (a-syn), huntingtin, amylin or Abeta.

In preferred embodiments, the self-antigen peptide may be derived from at least one protein selected from: Abeta, tau protein; α -synuclein, huntingtin, prion protein, or amylin. Therefore, the self-antigen peptide may be an Abeta peptide, an alpha-synuclein peptide, a tau peptide, a huntingtin peptide, a prion peptide, or an amylin peptide. As such, the antigen peptide may target one or more of: an Abeta associated disease or disorder, a tau associated disease or disorder, an alpha-synuclein associated disease or disorder, a Huntington associated disease or disorder, a prion associated disease or disorder, an amylin associated disease or disorder.

In some embodiments the liposomal construct or liposomal vaccine composition according to the invention may be used therapeutically, i.e. as a medicament. The liposomal construct or liposomal vaccine composition according to the invention may be used in therapy, for example for preventing, treating, or alleviating a foreign-antigen disease or disorder, such as an infectious disease or self-antigen disease or disorder, or symptoms thereof.

In an embodiment the self-antigen associated disease or disorder may be a neurological or neurodegenerative disease or disorder.

In a preferred embodiment, the self-antigen peptide may be an Abeta peptide or a fragment thereof. The Amyloid-beta derived peptide antigen may comprise amino acids 1-15 of amyloid-beta. Alternatively, the amyloid-beta derived peptide antigen may consist of or consist essentially of amino acids 1-15 of Abeta. In some embodiments the liposomal construct or liposomal vaccine composition according to the invention may be used for preventing, treating, or alleviating symptoms of an Abeta associated disease or disorder. For example, the Abeta associated disease or disorder may include Alzheimer's Disease, mild cognitive impairment (MCI), Down syndrome (OS), 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.

Thus, in a preferred aspect, the invention provides a liposomal vaccine composition comprising:

A liposome

An MPLA adjuvant

A peptide which comprises, consists essentially of, or consists of the amino acid sequence of SEQ ID NO: 6; and

An amyloid-beta derived peptide antigen displayed on the surface of the liposome comprising, consisting of or consisting essentially of amino acids 1-15 of Abeta.

In preferred embodiments, the liposomal vaccine composition comprises:

A liposome

An MPLA adjuvant integrated in the liposome

A peptide which consists essentially of, or consists of the amino acid sequence of SEQ ID NO: 6; and

An amyloid-beta derived peptide antigen consisting of or consisting essentially of amino acids 1-15 of Abeta, wherein the antigen is palmitoylated and the palmitic acid residues integrate in the outer layer of the liposome.

In preferred embodiments, the liposomal vaccine composition comprises:

A liposome

An MPLA adjuvant integrated in the liposome

A peptide which consists of the amino acid sequence of SEQ ID NO: 6; and

An amyloid-beta derived peptide antigen consisting of amino acids 1-15 of Abeta, wherein the antigen is tetrapalmitoylated via N and C terminal lysine residues (2 at each end) added to the peptide and the palmitic acid residues integrate in the outer layer of the liposome.

Tetrapalmitoylated Abeta 1-15 is described below as SEQ ID NO: 7:

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

Abeta 1-15 is described below as SEQ ID NO: 8:

H-Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-OH

In a preferred embodiment the peptide which comprises, consists essentially of, or consists of, and preferably consists of, the amino acid sequence of SEQ ID NO: 6 is, at least partially, encapsulated within the liposome.

Preferably, the liposome comprises phospholipids and even more preferably the phospholipids comprise dimyristoylphosphatidyl-choline (DMPC) and dimyristoylphosphatidyl-glycerol (DMPG). The liposome may further comprise cholesterol. The molar ratio of these three components may be 9:1:7 in some embodiments. A more specifically preferred liposomal construct of the invention comprises dimyristoylphosphatidyl-choline (DMPC), dimyristoylphosphatidyl-glycerol (DMPG), cholesterol and MPLA. The molar ratios of these four components may be 9:1:7:0.05.

A preferred liposomal vaccine composition of the invention is described and referred to herein as ACI-24.060.

In another preferred embodiment, the self-antigen peptide may be an α -synuclein (a-syn) derived peptide or a fragment thereof. The term α -synuclein derived peptide is intended to encompass both native α -synuclein and variants thereof. Examples of suitable variants include, but are not limited to, the a-synuclein derived antigenic peptides described in WO2022/029181, incorporated herein by reference. In particular, variants of the a-syn peptide from amino acids 111-124 of the full-length human a-syn amino acid sequence (GILEDMPVDPDNEA (SEQ ID NO: 10)). Such variant peptides are able to elicit a strong anti-a-syn antibody response and the induced antibodies show high cross-reactivity with human a-syn even though these peptides have a sequence which is different from the native sequence. Thus, in one embodiment, the α -synuclein derived peptide antigen may comprise the amino acid sequence of SEQ ID NO: 9 (GG-KESMPVDPDNEA), or a version lacking the two glycine residues. SEQ ID NO: 9 is a variant of the a-syn peptide from amino acids 111-124 of the full-length human a-syn amino acid sequence (GILEDMPVDPDNEA (SEQ ID NO: 10)). In another embodiment, the α -synuclein derived peptide antigen may comprise the amino acid sequence of SEQ ID NO: 10.

The native sequence of human alpha-synuclein is:

MDVFMKGLSKAKEGVVAAAETKQGVAAAGKTKEGVLYVGSKTKEGVVHGVATVAEKT
EQVTNVGGAVVTGVTAVAQKTVEGAGSIAAATGFVKKDQLGKNEEGAPQEGILEDMPVDP
DNEAYEMPSEEGYQDYEP (SEQ ID NO: 11).

In a preferred embodiment, the α -synuclein derived antigenic peptide described herein may be attached to one or two palmitoyl residues in the N terminal region and/or one or two

palmitoyl residues in the C terminal region of the peptide. Thus, the antigenic peptide is mono- or di-palmitoylated on one or optionally both of the terminal regions of the peptide. This may be facilitated by incorporating lysine residues in the N and/or C terminal regions of the alpha-synuclein-derived peptide antigen, wherein the lysine residues are palmitoylated. In one embodiment the antigenic peptide may be di-palmitoylated via N or C terminal lysine residues, wherein the lysine residues are palmitoylated. In another embodiment the antigenic peptide may be tetrapalmitoylated via N and C terminal lysine residues (2 at each end). In a preferred embodiment, the α -synuclein derived peptide antigen may comprise, consist of, or consist essentially of, α -synuclein derived antigenic peptide having the amino acid sequence SEQ ID NO: 9 di-palmitoylated via two lysine residues at the N-terminal region.

In some embodiments the liposomal construct or liposomal vaccine composition according to the invention may be used for preventing, treating, or alleviating symptoms of an α -synuclein associated disease or disorder.

Thus, in one aspect, the invention provides a liposomal vaccine composition comprising:

- a liposome

- an MPLA adjuvant

- CpG-Cholesterol

- a peptide which comprises, consists essentially of, or consists of the amino acid sequence of SEQ ID NO: 4 or SEQ ID NO: 6; and

- an α -synuclein derived antigenic peptide displayed on the surface of the liposome comprising, consisting essentially of or consisting of the amino acid sequence of SEQ ID NO: 9 or SEQ ID NO: 10.

In preferred embodiments, the liposomal vaccine composition comprises:

- a liposome

- an MPLA adjuvant integrated in the liposome

- CpG-Cholesterol integrated in the liposome

- a peptide which consists essentially of, or consists of the amino acid sequence of SEQ ID NO: 6; and

an α -synuclein derived antigenic peptide displayed on the surface of the liposome consisting essentially of the amino acid sequence of SEQ ID NO: 9, wherein the antigenic peptide is palmitoylated and the palmitic acid residues integrate in the outer layer of the liposome.

In a preferred embodiment, the liposomal vaccine composition comprises:

- a liposome

- an MPLA adjuvant integrated in the liposome

- CpG-Cholesterol integrated in the liposome

- a peptide which consists of the amino acid sequence of SEQ ID NO: 6; and

an α -synuclein derived peptide antigen displayed on the surface of the liposome comprising or consisting essentially of the amino acid sequence of SEQ ID NO: 9, wherein the antigenic peptide comprises one or two palmitic acid residues in the N terminal region or one or two palmitic acid residues in the C terminal region of the peptide and the palmitic acid residues integrate in the outer layer of the liposome.

In a preferred embodiment the α -synuclein derived peptide antigen displayed on the surface of the liposome comprises or consists essentially of the amino acid sequence of SEQ ID NO: 9, wherein the antigenic peptide comprises two palmitic acid residues in the N terminal region of the peptide, and the palmitic acid residues integrate in the outer layer of the liposome. Preferably, the palmitoylation is via lysine residues added to the peptide, as discussed herein.

Preferably, the liposome comprises phospholipids and cholesterol. The phospholipids may comprise dimyristoylphosphatidyl-choline (DMPC) and dimyristoylphosphatidyl-glycerol (DMPG). In some preferred embodiments the Abeta-associated disease may be Alzheimer's disease. Additionally or alternatively, the Abeta-associated disease may be Down syndrome.

In another embodiment, the self-antigen peptide may be a tau protein derived peptide or a fragment thereof. In some embodiments the liposomal construct or liposomal vaccine composition according to the invention may be used for preventing, treating, or alleviating symptoms of a Tau protein associated disease or disorder.

Exemplary Tau associated diseases or disorders include: Alzheimer's Disease, Parkinson's Disease, Creutzfeldt-Jacob disease, Dementia pugilistica, Down's Syndrome, Gerstmann Straussler- Scheinker disease, inclusion body myositis, prion protein cerebral amyloid

angiopathy, traumatic brain injury, amyotrophic lateral sclerosis, parkinsonism-dementia complex of Guam, Non-Guamanian motor neuron disease with neurofibrillary tangles, argyrophilic grain dementia, corticobasal degeneration, Dementia Lewy Amyotrophic Lateral sclerosis, diffuse neurofibrillary tangles with calcification, frontotemporal dementia, preferably frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17), frontotemporal lobar dementia, Hallevorden-Spatz disease, multiple system atrophy, Niemann-Pick disease type C, Pick's disease, progressive subcortical gliosis, progressive supranuclear palsy, Subacute sclerosing panencephalitis, Tangle only dementia, Postencephalitic Parkinsonism, Myotonic dystrophy, chronic traumatic encephalopathy (CTE), Primary age-related tauopathy (PART), or Lewy body dementia (LBD).

In another embodiment, the self-antigen peptide may be an a-syn protein derived peptide or a fragment thereof. In some embodiments the liposomal construct or liposomal vaccine composition according to the invention may be used for preventing, treating, or alleviating symptoms of an a-syn associated disease or disorder (synucleopathy).

Alpha-synuclein associated diseases or disorders include: Lewy Body Disorders (LBDs), especially Parkinson's Disease (PD), Parkinson's Disease with Dementia (PDD) and Dementia with Lewy Bodies (DLB), as well as Multiple System Atrophy (MSA) or Neurodegeneration with Brain Iron Accumulation type I (NBIA Type I).

The liposomal vaccine compositions of the invention optionally comprise at least one pharmaceutically acceptable carrier, which term explicitly includes diluents and excipients. Such agents are well known in the pharmaceutical art and are described, for example, in Remington's Pharmaceutical Sciences, 15th or 18th Ed. (Alfonso R. Gennaro, ed.; Mack Publishing Company, Easton, PA, 1990); Remington: the Science and Practice of Pharmacy 19th Ed. (Lippincott, Williams & Wilkins, 1995); Handbook of Pharmaceutical Excipients, 3rd Ed. (Arthur H. Kibbe, ed.; Amer. Pharmaceutical Assoc, 1999); Pharmaceutical Codex: Principles and Practice of Pharmaceutics 12th Ed. (Walter Lund ed.; Pharmaceutical Press, London, 1994); The United States Pharmacopeia: The National Formulary (United States Pharmacopeial Convention); Fiedler's "Lexikon der Hilfstoffe" 5th Ed., Edition Cantor Verlag Aulendorf 2002; "The Handbook of Pharmaceutical Excipients", 4th Ed., American Pharmaceuticals Association, 2003; and Goodman and Gilman's: the Pharmacological Basis of Therapeutics (Louis S. Goodman and Lee E. Limbird, eds.; McGraw Hill, 1992), the disclosures of which are hereby incorporated by reference. The carriers, diluents and excipients can be selected with regard to the intended route of administration and standard pharmaceutical practice. These compounds must be acceptable in the sense of being not

deleterious to the recipient thereof. A preferred example of a carrier that may be employed is a buffer.

In another aspect the invention pertains to a method of producing, or manufacturing, a liposomal construct of the invention. The method may comprise a step of providing a peptide containing at least one T-cell epitope which comprises, consists essentially of, or consists of the amino acid sequence of SEQ ID NO: 4 or an analogue thereof that retains alanine at position 2 and does not contain any methionine residues. The method may be adapted to produce any liposomal construct described herein. Thus, for example, this step of the method may comprise providing a peptide containing at least three universal T-cell epitopes. The at least three universal T-cell epitopes may comprise the amino acid sequences of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 4 or analogues thereof as defined herein. Alternatively, the at least three universal T-cell epitopes may consist of or may consist essentially of the amino acid sequences SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 4 or analogues thereof as defined herein. The method may further comprise a step of providing a liposome comprising at least one adjuvant.

The method comprises the step of combining the peptide containing at least one T-cell epitope with the liposome to generate the liposomal construct. The combining step preferably results in at least partial encapsulation of the peptide containing at least one T-cell epitope in the liposome. The peptide containing at least one T-cell epitope preferably comprises, consists essentially of, or consists of the amino acid sequence of SEQ ID NO: 6 or an analogue thereof. This is, in effect, the only essential step of the method given the other steps relate to providing the products for combining. Accordingly, the provision steps of the method of producing, or manufacturing, a liposomal construct may be performed in any order to allow the essential step of combining the two components.

In a further aspect the invention provides a method of producing, or manufacturing, a liposomal vaccine composition, comprising, following manufacturing the liposomal construct (as outlined below), a further step of incorporating at least one peptide antigen (as described herein), optionally a self-antigen peptide into the liposome. The antigen peptide is integrated to the liposome in a manner that the at least one antigen is displayed, at least partly, on the surface of the liposome. Thus, the essential step here is incorporating at least one peptide antigen into a liposomal construct of the invention to form a liposomal vaccine composition of the invention.

According to these methods, the at least one adjuvant may comprise MPLA and may further comprise a CpG adjuvant.

In methods comprising the step of incorporating at least one peptide antigen, the peptide antigen may be palmitoylated. In such embodiments, the palmitic acid residues are integrated in the outer layer of the liposome.

Suitable peptide antigens are described herein. Thus, in some embodiments, the peptide antigen is an amyloid-beta derived peptide. In a preferred embodiment, the peptide antigen is an amyloid-beta derived peptide antigen comprising, consisting of or consisting essentially of amino acids 1-15 of Abeta.

In other embodiments, the peptide antigen is an α -synuclein derived peptide. In a preferred embodiment, the peptide antigen is an α -synuclein derived antigenic peptide displayed on the surface of the liposome comprising, consisting essentially of or consisting of the amino acid sequence of SEQ ID NO: 9 or SEQ ID NO: 10.

Brief description of the drawings

Non-limiting embodiments of the invention are now described by way of example only, with reference to the accompanying drawings, in which:

Figure 1 is a graphical representation of oxidation stability tests with vaccine compositions ACI-24.043 (with SAT47) and ACI-24.060 (with SAT58 peptide).

Figure 2 is a graphical representation of the percentage of SAT peptide recovery using the liposomal construct (ACI-24.060) at different stages of the same production process. The recovery percentage of ACI-24.060 is compared to the peptide recovery of a different liposomal product (ACI-24.043) using the same production process.

Figure 3 is a graphical representation of the kinetics and amplitude of the anti-Abeta1-42 IgG profile (AU/mL) in the plasma of C57BL/6 mice over three immunizations with ACI-24.043 (with SAT47) or ACI-24.060 (with SAT58). Arrows indicate the days of immunization. Data shown as geometric mean $\pm$ 95% CI, with n=10 per group.

Non-limiting embodiments of the invention are now described by way of example only, with reference to the accompanying drawings.

Example 1: Design of T-cell epitope

The design of the universal T-cell epitope of the present invention is similar to the design of T-cell epitopes as described in WO2019/197414.

The ability of a universal T-cell epitope to activate T cells (immunogenicity score) is the result of two complementary properties: i) affinity to HLA and ii) capacity to bind different HLA haplotypes in a promiscuous manner. An in-silico evaluation (Epivax) of several T-cell epitopes from different origins was performed with the objective of selecting the peptides with the highest immunogenicity score. In a preliminary phase, 10 different peptides from different origins (Keyhole limpet hemocyanin-KLH, Diphtheria toxin, Influenza virus, Epstein Barr virus and Herpes virus) were evaluated.

Peptides with the best immunogenicity score (higher than 10) were selected due to their chance to be highly immunogenic in humans based on their predicted HLA affinity and HLA haplotype coverage (selected peptide sequences are shown in Table 1).

Table 1: Selected peptides sequences for T-cell epitope design.

Name	Sequence	Peptide origin
SEQ ID NO: 1	IVAQSIALSS	Diphtheria Toxin
SEQ ID NO: 2 Comparative Example	SMGVYQILAIYST	Influenza hemagglutinin
SEQ ID NO: 3	LYNLRRGTAL	Epstein Barr Virus
SEQ ID NO: 4	SAGVYQILAIYST	Influenza hemagglutinin

Following the screening results of the individual peptides, the promiscuous peptide SAT58 was designed (Table 2). The peptide SAT47 is described in WO2019/197414 and was reproduced in the below examples as a head-to-head comparator.

The goal was to increase the immunogenicity score without increasing the size of the final promiscuous peptide, due to peptide synthesis and vaccine encapsulation process constraints. In brief, the peptide synthesis yield and success rate reduces the more the length of the peptide increases. This effect is noted especially in peptides of over 30 amino acids in length, and in those composed mainly of hydrophobic residues.

In addition, the peptide encapsulation rate is lowered with the increasing length of the peptide, as the chances to accommodate it in the lumen of the liposomes are decreased as peptide length increases. The in-silico immunogenicity score of these 2 promiscuous T-cell epitopes was very high and, importantly, higher than that of the individual component peptides, therefore confirming that combining peptides from different origins can improve HLA affinity and HLA haplotype coverage (promiscuous T cell epitope sequence is shown in Table 2).

Table 2: Sequences of T-cell epitope containing peptides.

Name	Sequence	Peptide design	Peptide origin
SEQ ID NO: 5 (SAT47) Comparative Example	SMGVYQILAIYSTVVRIVAQS IALSSVVR YIKANSKFIGVVRL LYNLRRGTAL	SEQ ID NO: 2 + VVR + SEQ ID NO: 1 + VVR + Tetanus + VVR + SEQ ID NO: 3	Influenza hemagglutinin + Diphtheria + Tetanus + Epstein Barr
SEQ ID NO: 6 (SAT58)	SAGVYQILAIYSTVVRIVAQSI ALSSVVR YIKANSKFIGVVRL YNLRRGTAL	SEQ ID NO: 4+ VVR + SEQ ID NO: 1 + VVR + Tetanus + VVR + SEQ ID NO: 3	Influenza hemagglutinin + Diphtheria + Tetanus + Epstein Barr

Example 2: Preparation of the liposomal construct and liposomal vaccine composition ACI-24.060.

The manufacturing process comprises two main parts:

- i) the preparation of the SAT58 liposome construct containing the adjuvant, here 3D-(6-acyl) PHAD®, and the SAT58 peptide, and
- ii) the insertion of the palmitoylated antigenic peptide, here Abeta1-15 (Pal1-15), into the pre-formed SAT58 liposomes. Detailed steps are described below:

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 Ac₂O (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/ TBMP) 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.

Step 2: Preparation of the ACI-24.060 vaccine containing SAT58 (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 Na₂HPO₄ 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.

Example 3: Peptide oxidation analysis

ACI-24.060 (SAT58) was prepared as described above (Example 2).

ACI-24.043 (SAT47) was manufactured following the process as described in WO2019/197414.

Samples were stored for 12 months at 2-8°C. Oxidation stability over time was measured by means of reversed phase HPLC, results are shown in Table 3 and Figure 1.

SAT47 and RRT (relative retention time) 0.98 content (oxidised SAT47) were determined by reverse-phase HPLC with UV detection (207 nm) using an HPLC Ultimate 3000 system (Dionex / Thermo Fisher). Samples were prepared by dilution in isopropanol and water to a final composition of 70% isopropanol and 30% aqueous. Samples were stored at 8°C until injection in the HPLC system (injection volume = 4 µL). A C18 column (Acquity UPLC BEH C18 1,7µm, 2,1 x 100 mm (Waters, Art. Nr. 186002352), column temperature set at 80°C) was used to separate SAT47 and RRT 0.98 from other components using a gradient of water and acetonitrile (both mobile phases with 0.1% TFA) going from 10% acetonitrile to 90% acetonitrile over 6.2 minutes. The column was subsequently washed with isopropanol with 0.1% TFA during 2.1 minutes, and re-equilibrated at the starting conditions for 2.5 minutes. All chromatography steps were performed at a flow of 0.5 mL/min. The concentration of SAT47 and RRT 0.98 were calculated from a generated standard curve of SAT47.

SAT58 content was determined by reverse-phase HPLC with UV detection (207 nm) using an HPLC Ultimate 3000 system (Dionex / Thermo Fisher). Samples were prepared by dilution in 10mM histidine, 250mM sucrose, with 20% ethanol. Samples were stored at 8°C until injection in the HPLC system (injection volume = 4 µL). A C18 column (Acquity UPLC BEH C18 1,7 µm, 2,1 x 100 mm (Waters, Art. Nr. 186002352), column temperature set at 80°C) was used to separate SAT58 from other components using a gradient of water and acetonitrile (both mobile phases with 0.1% TFA) going from 10% acetonitrile to 90% acetonitrile over 6.2 minutes. The column was subsequently washed with isopropanol with 0.1% TFA during 2.1 minutes, and re-equilibrated at the starting conditions for 2.5 minutes. All chromatography steps were performed at a flow of 0.5 mL/min. No peak equivalent to RRT 0.98 was observed in the SAT58 chromatogram. The concentration of SAT58 was calculated from a generated standard curve of SAT58.

Table 3: Stability testing of ACI-24.043 (SAT47) and ACI-24.060 (SAT58)

Oxidation stability was analysed in vaccine compositions: ACI-24.043 containing the SAT47 peptide and ACI-24.060 containing the SAT58 peptide

Vaccine composition	ACI-24.043		ACI-24.043		ACI-24.060	ACI-24.060
Stability time-point (2-8°C)	SAT47 µg/mL	RRT 0.98 (SAT47-oxidised) µg/mL*	SAT47 µg/mL	RRT 0.98 (SAT47-oxidised) µg/mL	SAT58 µg/mL	SAT58 µg/mL
T0	142	7	121	13	117	141
3 Months	105	16	93	35	132	135
6 Months	99	22	43	75	120	141
9 Months	88	42	<	83	118	130
12 Months	89	49	<	76	107	127

The loss of SAT47 and increasing RRT 0.98 values confirm the ongoing production of oxidised SAT47 over time. No equivalent production was observed in the case of SAT58.

As can be seen from Figure 1 and Table 3 SAT58 concentration remained stable over time, whereas SAT47 concentration reduced substantially over the 12-month storage period.

Advantageously, it was observed that ACI-24.060 containing SAT58 displays improved oxidation stability properties compared to SAT47, in equivalent liposomal vaccine compositions. Without wishing to be bound by theory, it is believed that the improved oxidation profile may be due to the changing of the methionine residue in the amino acid sequence of the T-cell epitope comprising peptide SAT47 for an alanine residue in the T-cell epitope comprising peptide SAT58.

Example 4: Calculated recoveries of SAT peptide at different manufacturing steps

Peptide recovery of SAT58 throughout manufacturing was compared to the SAT47 recovery at same stage of manufacturing. The measures were taken at three points during the

manufacturing process and were analysed by means of HPLC. HPLC was performed as in Example 3.

Peptide recovery:

The recovery (or mass balance) for a process step is defined as the total peptide amount in solution or product at this specific step compared to its initial peptide input amount. SAT58 and SAT47 recovery was tested in the liposome preparation, the bulk product preparation and in the final product. The liposome preparation corresponds to the preparation before integration of the antigen molecule. The bulk product corresponds to the preparation after the integration of the antigen molecule. The final product corresponds to the preparation obtained after all steps of the process have been performed, i.e. ACI-24.060.

The recovery is determined by the SAT content (i.e. concentration) measured by HPLC, multiplied by the solution or product volume at this step. It is typically expressed as a percentage of the initial input SAT amount and is a measure of process loss.

Surprisingly, it was observed that SAT58 had a higher peptide recovery in the final product compared to the peptide recovery of SAT47.

As seen in Figure 2, in the SAT47 Liposomal preparation about 70% of the initial amount of SAT47 was recovered compared to about 78% recovery for SAT58.

In the bulk product preparation, about 55% of SAT47 was recovered compared to about 68% of SAT58.

In the final product only about 45% of SAT47 was recovered in the SAT47 final drug product compared to about 65% of SAT58 in the SAT58 final product.

Thus, a higher recovery of SAT58 is observed compared to SAT47 during the whole manufacturing process, including before the addition of the antigen peptide Pal1-15.

Example 5: Liposomal construct and liposomal vaccine composition with an α -synuclein derived antigenic peptide.

The vaccine was produced as follows in a three-step approach, i.e., preparation of intermediate SAT58 liposome followed by integration of CpG-Chol to generate the fully adjuvanted liposomes and finally insertion of a palmitoylated α -synuclein (α -syn) derived antigenic peptide comprising the amino acid SEQ ID NO: 9 (GG-KESMPVDPDNEA), described in WO2022/029181.

Intermediate liposomes: first, lipids (DMPG, DMPC, cholesterol and monophosphoryl Hexa-acyl Lipid A (3D-(6-acyl) PHAD™ (Avanti Polar Lipids, USA)), the first adjuvant) were dissolved in ethanol at 60°C. After complete dissolution, the lipid/ethanol solution is filtered through a 0.2 µm pore size filter into an injection system preheated at 60°C. In a separate vessel, the peptide comprising T-cell epitope SAT58 was dispersed in ethanol at room temperature by the aid of sonication and solubilized by dilution with 10 mM Histidine, 250 mM Sucrose. The SAT58 solution was filtered through a 0.2 µm pore size filter and heated up to 40°C. The lipid/ethanol solution and the SAT58 solution were then mixed using a crossflow injection module to form the intermediate liposomes, which were subsequently subjected to active cooling followed by size reduction using repeated extrusion cycles. Finally ultra-/diafiltration (UDF) was performed to remove ethanol. The intermediate SAT58 liposomes were filtered through a 0.2 µm pore size filter and stored at 4°C until used.

CpG-Chol integration: The intermediate SAT58 liposomes were diluted to a concentration of 1 mg/mL of lipid in 20 mM Histidine, 145 mM NaCl and warmed up to 60°C. The second adjuvant, CpG-Cholesterol, was added to the liposomes dropwise. The liposomal dispersion was incubated at 60°C for an additional 30 minutes whilst stirring. The fully adjuvanted liposomes were purified by UDF and then filtered through a 0.45 µm followed by a 0.2 µm pore size filter and stored at 4°C.

α-synuclein (a-syn) derived antigenic peptide insertion: the fully adjuvanted liposomes were diluted to a concentration of 1 mg/mL of total lipid content in 20 mM Histidine, 145 mM NaCl. Concomitantly, N-terminal di-palmitoylated α-synuclein derived antigenic peptide SEQ ID NO: 9 was dissolved to a final concentration of 1 mg/mL in 20 mM Histidine, 145 mM NaCl at 60°C and the solution was filtered through a 0.2 µm pore size filter. The liposomes and the peptide solution were finally mixed using a crossflow module and the liposomal dispersion was incubated at temperature of 60°C under stirring for 30 minutes. A UDF step was performed to exchange the buffer to the final formulation system i.e. with 10 mM Histidine, 250 mM Sucrose buffer. The product was concentrated down to its final volume, filtered through a 0.45 µm pore size filter followed by a 0.2 µm pore size filter. The product was stored at 4°C.

T-cell epitope peptide stability analysis

Samples of the liposomal vaccine with the α-synuclein derived antigenic peptide derived antigenic peptide comprising the amino acid SEQ ID NO: 9 (GG-KESMPVDPDNEA), prepared as described above, were stored for 6 months at 2-8°C. T-cell epitope peptide SAT58 stability over time was measured by means of reversed phase HPLC, as described in Example 3 above. Results are shown in Table 4.

Table 4: Stability testing of T-cell epitope peptide SAT58

Stability time-point (2-8°C)	SAT58 µg/mL
T0	114
1 Month	109
2 Months	116
3 Months	117
6 Months	110

As can be seen from Table 4 SAT58 concentration remained stable over time, and no degradation products were observed.

Example 6: Immunogenicity of the liposomal constructs containing SAT58

An *in vivo* study in mice was performed to assess immunogenicity of liposomal vaccine composition ACI-24.043 (containing T-cell epitope peptide SAT47) and liposomal vaccine composition ACI-24.060 (containing T-cell epitope peptide SAT58). Two groups of C57BL/6 mice (n=10) received a total of three s.c. immunizations, of either ACI-24.043 or ACI-24.060, at a target dose of 80 µg of Pa1-15 (SEQ ID NO:7) on days 1, 15 and 29. Plasma samples were obtained at predose (day -7) and one week after each immunization (days 8, 22 and 36) for analysis of the antibody response against Abeta1-42 by ELISA.

Plates were coated with 10 µg/ml of human Aβ1-42 peptide film (Bachem, Switzerland) overnight at 4°C. After washing with 0.05% Tween 20/PBS and blocking with 1%BSA/0.05%Tween/PBS, serial dilutions of plasma were added to the plates and incubated at 37°C for 2 hours. After washing, plates were incubated with alkaline phosphatase (AP) conjugated anti-mouse IgG antibody (Jackson ImmunoResearch, USA) for 2 hours at 37°C. After final washing, plates were incubated for 2.5 hours with AP substrate (pNPP) and read at 405 nm using an ELISA plate reader. The anti-Aβ1-42 antibody concentrations were back-calculated against a standard curve established using serial dilutions of the commercially available antibody 6E10 (Biolegend, UK, Cat. 803002).

The results in Figure 3 show that animals immunized with ACI-24.043 and ACI-24.060 developed similar anti-Abeta1-42 antibody profiles, demonstrating that liposomal vaccine composition ACI-24.060 (with SAT58) displays an excellent immunogenicity profile, and one which is comparable to the profile obtained with the liposomal vaccine composition ACI-24.043 (with SAT47).

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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 construct comprising:
 - a liposome,
 - at least one adjuvant; and
 - a peptide containing at least one T-cell epitope which comprises, consists essentially of, or consists of the amino acid sequence of SEQ ID NO: 4 or an analogue thereof that retains alanine at position 2 and does not contain any methionine residues.
2. A liposomal construct according to claim 1, wherein the peptide containing at least one T-cell epitope comprises at least one additional T-cell epitope, wherein the at least one additional T-cell epitope comprises, consists of, or consists essentially of an amino acid sequence selected from SEQ ID NO: 1 and/or SEQ ID NO: 3, or an analogue thereof.
3. A liposomal construct according to claim 1 or 2, wherein the peptide contains at least three T-cell epitopes which comprise, consist essentially of, or consist of the amino acid sequences SEQ ID NO:1, SEQ ID NO:3, and SEQ ID NO:4, or analogues thereof.
4. The liposomal construct according to claim 2 or 3, wherein the peptide further comprises at least one linker between at least two T-cell epitopes.
5. The liposomal construct according to claim 4, wherein the at least one linker comprises, consists essentially of, or consists of the amino acid sequence: VVR; TVGLR; KSVR; PMGAP or PMGLP.
6. The liposomal construct according to claim 4 or 5, wherein the at least one linker comprises, consists of or consists essentially of the amino acid sequence VVR.
7. The liposomal construct according to any of claims 1 to 6, wherein the peptide containing at least one T-cell epitope comprises, consists essentially of, or consists of the amino acid sequence of SEQ ID NO: 6, or an analogue thereof.
8. The liposomal construct according to any of claims 1 to 7, wherein the peptide containing at least one T-cell epitope is at least partially encapsulated within the liposome.
9. The liposomal construct according to any of claims 1 to 8, wherein the at least one adjuvant is at least in part displayed on the surface of the liposome.

10. The liposomal construct according to any of claims 1 to 9, wherein the at least one adjuvant is at least in part integrated within a lipid bilayer of the liposome.
11. The liposomal construct according to any of claims 1 to 10, wherein the at least one adjuvant is selected from the group consisting of: monophosphoryl lipid A (MPLA); diphosphoryl lipid A (DPLA); alum; Pam2CSK4; Pam3CSK4; Pam3CAG; saponins; CpG; lipidated CpG, preferably CpG-Cholesterol; cationic lipids; phosphorothioated PS-CpG-ODNs; CpG oligodeoxynucleotides (CpG-ODN); CpG-A; CpG-B or CpG-C.
12. The liposomal construct of any of claims 1 to 11, wherein the at least one adjuvant is a TLR4 agonist, preferably monophosphoryl lipid A (MPLA).
13. The liposomal construct according to any of claims 1 to 11, wherein the at least one adjuvant is a TLR9 agonist, preferably a CpG oligonucleotide.
14. The liposomal construct according to any claim 1 to 13 for use in the preparation of a liposomal vaccine composition.
15. A liposomal vaccine composition comprising:
- a liposomal construct according to any of claims 1 to 13; and
 - at least one antigenic peptide, and optionally two different antigenic peptides, displayed on the surface of the liposome, optionally wherein the composition additionally comprises at least one pharmaceutically acceptable carrier.
16. The liposomal vaccine composition according to claim 15, wherein the at least one antigenic peptide is modified with one or more lipophilic or hydrophobic moieties, optionally wherein the one or more lipophilic or hydrophobic moieties is selected from: a fatty acid, a triglyceride, diglyceride, steroid, sphingolipid, glycolipid, or a phospholipid.
17. The liposomal vaccine composition according to claim 16, wherein the one or more lipophilic or hydrophobic moieties comprises a palmitoyl residue (palmitic acid).
18. The liposomal vaccine composition according to claim 17, wherein the at least one antigenic peptide is tetrapalmitoylated.
19. The liposomal vaccine composition according to any of claims 15 to 18, wherein the at least one antigenic peptide is a self-antigen.

20. The liposomal vaccine composition according to claim 19, wherein the self-antigen is derived from IL-17, IL-27, Abeta, Tau, α -synuclein, huntingtin, prion, or amylin protein.

21. The liposomal vaccine composition according to claim 19 or 20, wherein the at least one antigenic peptide is an Abeta peptide or fragment thereof, preferably comprising, consisting essentially of or consisting of amino acids 1-15 of Abeta.

22. A liposomal vaccine composition comprising:

a liposome

an MPLA adjuvant

a peptide which comprises, consists essentially of, or consists of the amino acid sequence of SEQ ID NO: 6; and

an amyloid-beta derived peptide antigen displayed on the surface of the liposome comprising, consisting of or consisting essentially of amino acids 1-15 of Abeta.

23. A liposomal vaccine composition comprising:

a liposome

an MPLA adjuvant integrated in the liposome

a peptide which consists essentially of, or consists of the amino acid sequence of SEQ ID NO: 6; and

an amyloid-beta derived peptide antigen consisting of or consisting essentially of amino acids 1-15 of Abeta, wherein the antigen is palmitoylated and the palmitic acid residues integrate in the outer layer of the liposome.

24. A liposomal vaccine composition comprising:

a liposome

an MPLA adjuvant integrated in the liposome

a peptide which consists of the amino acid sequence of SEQ ID NO: 6; and

an amyloid-beta derived peptide antigen consisting of amino acids 1-15 of Abeta, wherein the antigen is tetrapalmitoylated via N and C terminal lysine residues (2 at each end) added to the peptide and the palmitic acid residues integrate in the outer layer of the liposome.

25. A liposomal vaccine composition comprising:

a liposome

an MPLA adjuvant

CpG-Cholesterol

a peptide which comprises, consists essentially of, or consists of the amino acid sequence of SEQ ID NO: 4 or SEQ ID NO: 6; and

an α -synuclein derived antigenic peptide displayed on the surface of the liposome comprising, consisting essentially of or consisting of the amino acid sequence of SEQ ID NO: 9 or SEQ ID NO: 10.

26 The liposomal vaccine composition of any of claims 15 to 25 wherein the peptide which comprises, consists essentially of, or consists of the amino acid sequence of SEQ ID NO: 6 is, at least partially, encapsulated within the liposome.

27. The liposomal vaccine composition of any of claims 15 to 26 wherein the liposome comprises phospholipids and cholesterol.

28. The liposomal vaccine composition of claim 27 wherein the phospholipids comprise dimyristoylphosphatidyl-choline (DMPC) and dimyristoylphosphatidyl-glycerol (DMPG), optionally wherein the molar ratio of DPMC:DMPG:cholesterol is 9:1:7.

29. The liposomal vaccine composition of claim 28 wherein the molar ratio of DPMC:DMPG:cholesterol:MPLA in the liposome is 9:1:7:0.05.

30. The liposomal vaccine composition according to any of claims 15 to 18, wherein the at least one antigenic peptide is a foreign antigen.

31. The liposomal vaccine composition according to claim 30, wherein the foreign antigen is a viral antigen or an allergen.

32. The liposomal vaccine composition according to claim 31, wherein the viral antigen is derived from a virus selected from rhinoviruses, coronaviruses, enteroviruses, adenoviruses, parainfluenza viruses and respiratory syncytial viruses.

33. The liposomal vaccine composition according to any of claims 15 to 32 for use in therapy.

34. The liposomal vaccine composition according to any of claims 15 to 33 for use in a method of prevention or treatment, of symptoms of, or associated with, a neurodegenerative disease or disorder.

35. The liposomal vaccine composition according to claim 34, wherein the neurodegenerative disease is Alzheimer's disease, or Down's syndrome.

36. A method of manufacturing a liposomal construct as defined in any one of claims 1 to 13 comprising the steps of:

- a) providing a peptide containing at least one T-cell epitope which comprises, consists essentially of, or consists of the amino acid sequence of SEQ ID NO: 4 or an analogue thereof that retains alanine at position 2 and does not contain any methionine residues,
- b) providing a liposome comprising at least one adjuvant; and
- c) combining the peptide containing at least one T-cell epitope with the liposome to generate the liposomal construct.

37. The method according to claim 36, wherein the peptide containing at least one T-cell epitope comprises, consists of, or consists essentially of:

- a) the amino acid sequences SEQ ID NO: 1, SEQ ID NO: 3 and SEQ ID NO: 4 in combination; or
- b) the amino acid sequence of SEQ ID NO: 6, or an analogue thereof.

38. A method of manufacturing a liposomal vaccine composition as defined in any one of claims 15 to 32, wherein the method comprises the steps of:

- a) providing a peptide containing at least one T-cell epitope which comprises, consists essentially of, or consists of the amino acid sequence of SEQ ID NO: 4 or an analogue thereof that retains alanine at position 2 and does not contain any methionine residues,

- b) providing a liposome comprising at least one adjuvant,
- c) combining the peptide containing at least one T-cell epitope with the liposome; and
- d) incorporating at least one peptide antigen into the liposome such that the at least one peptide antigen is displayed on the surface of the liposome.

39. The method according to claim 38, wherein the peptide containing at least one T-cell epitope comprises, consists of, or consists essentially of:

- a) the amino acid sequences SEQ ID NO: 1, SEQ ID NO: 3 and SEQ ID NO: 4 in combination; or
- b) the amino acid sequence of SEQ ID NO: 6, or an analogue thereof.

40. The method according to claim 38 or 39 wherein the at least one adjuvant comprises MPLA.

41. The method according to claim 40 wherein the at least one adjuvant further comprises a CpG adjuvant.

42. The method according to any one of claims 38 to 41 wherein the peptide antigen is palmitoylated and the palmitic acid residues are integrated in the outer layer of the liposome.

43. The method according to any one of claims 38 to 42 wherein the peptide antigen is an amyloid-beta derived peptide, preferably an amyloid-beta derived peptide antigen comprising, consisting of or consisting essentially of amino acids 1-15 of Abeta.

44. The method according to any one of claims 38 to 42 wherein the peptide antigen is an α -synuclein derived peptide, preferably an α -synuclein derived antigenic peptide displayed on the surface of the liposome comprising, consisting essentially of or consisting of the amino acid sequence of SEQ ID NO: 9 or SEQ ID NO: 10.