COMPOSITION COMPRISING VLP AND AMYLOID-BETA PEPTIDE

The present invention relates to novel uses of a construct consisting of virus-like particle (VLP) structure chemically coupled to a fragment of the Aβ-1-42 peptide and its pharmaceutically acceptable salts (hereinafter CONSTRUCT), in particular to dosage regimens, modes of and dosage forms for the administration of a CONSTRUCT for the treatment of patients suffering from dementia, in particular dementia of the Alzheimer's type, especially mild to moderate or severe Alzheimer's Disease (AD), and vascular dementia with amyloid angiopathy to a method of isolating immune cells, especially antibody producing cells, and antibodies as well as there genes or fragments thereof generated by the immune system of a warm-blooded animal, especially a human, in response to the administration of the CONSTRUCT, the production of such antibodies and the pharmaceutical use of such antibodies.

The present invention relates to novel uses of a construct consisting of virus-like particle (VLP) structure chemically coupled to a fragment of the Aβ-1-42 peptide and its pharmaceutically acceptable salts (hereinafter CONSTRUCT), in particular to dosage regimens, modes of and dosage forms for the administration of a CONSTRUCT for the treatment of patients with increased Aβ-level , including but not limited to patients with dementia associated with Parkinson's disease, Lewy Body dementia.

The present invention also relates to novel uses of a construct consisting of virus-like particle (VLP) structure chemically coupled to a fragment of the Aβ-1-42 peptide and its pharmaceutically acceptable salts (hereinafter CONSTRUCT), in particular to dosage regimens, modes of and dosage forms for the administration of a CONSTRUCT for the prophylactic treatment of subjects at risk of developing AD, including but not limited to subjects with mild cognitive impairment , subjects with genotypes known to be associated with AD, such as ApoE4, subjects with Trisomy 21 and subjects with surrogate markers indicating risk for AD.

Considerable evidence has been accumulated suggesting that the β-amyloid peptide – the major component of senile amyloid plaques – plays a causal role in AD. Successful disease-modifying therapy for AD is likely to include products that affect the deposition of β-amyloid in the brain. Aβ-specific antibodies, actively generated by the immune system or passively administered, consistently reduce plaque burden in different transgenic mouse models for Aβ-amyloidosis. A first clinical attempt to stimulate the immune system of AD patients to

generate Aβ-antibody, however, had to be suspended due to unacceptable side effects (meningoencephalitis in 6 % of treated patients, Orgogozo JM, Gilman S, Dartigues JF, Laurent B, Puel M, Kirby LC, Jouanny P, Dubois B, Eisner L, Flitman S, Michel BF, Boada M, Frank F, Hock C (2003)] Subacute meningoencephalitis in a subset of patients with AD after Aβ42 immunization. Neurology; 61: 46-54.).

Surprisingly, lesser adverse immune reactions and a lesser incidence of microhemorrhages are observed with the CONSTRUCTS disclosed herein. In particular, no adverse immune reaction nor increased incidence of microhemorrhages, is observed with CONSTRUCTS consisting of a VLP chemically coupled to the Aβ-1-6 peptide.

In a first aspect of the present invention, it was surprisingly found that the CONSTRUCT advantageously can be applied subcutaneously to warm-blooded animals, especially humans, suffering from dementia.
In another aspect of the present invention, it was surprisingly found that the CONSTRUCT advantageously can be applied intramuscularly, intranasally and orally to warm-blooded animals, especially humans, suffering from dementia.

In a second aspect, the present invention provides a dosage form for subcutaneous administration of the CONSTRUCT. The preferred dosage form for subcutaneous administration of the CONSTRUCT is an aqueous solution containing Phosphate Buffer Saline (PBS), between 0.25 and 0.75 mg/mL CONSTRUCT, preferably between 0.4 and 0.6 mg/mL, e.g. 0.5 mg/mL CONSTRUCT, and no further excipients. The dosage form can be kept frozen until shortly before usage. The dosage form is administered preferably by subcutaneous injection with a syringe to the warm-blooded animal, especially into the abdomen. For thawing of the dosage form, the dosage form can be kept at ambient temperature for about between 15 minutes and 45 minutes, e.g. 30 minutes. Preferably, before withdrawing drug substance, the vials are gently inverted several times for dispersion of potential sub-visual particles.

The CONSTRUCTS as employed in the present invention are known as such. For example, WO 00/3227 to Cytos discloses a technology for providing a construct comprising a core-particle (such as a VLP), a linker and an antigen, all together forming an ordered and repetitive antigen array. WO 02/056907 to Cytos and Novartis describes constructs

comprising a VLP comprising recombinant proteins of a bacteriophage, such as Qβ, a linker and an antigen, e.g. Aβ1-42 or a fragment thereof, all together forming an ordered and repetitive antigen array. Preferably, a CONSTRUCT as used herein consists of capsid proteins of a RNA bacteriophage, more preferably of capsid proteins of the RNA bacteriophage Qβ, self-assembled into a highly ordered VLP structure chemically coupled with a bivalent linker to a fragment of the Aβ1-42 peptide, more preferably to Aβ-1-6. The CONSTRUCT can be prepared, purified and administered as disclosed in WO 00/3227, WO 02/056907 or WO2004/016282, especially in Example 13, which patent filings as well as the references cited therein are incorporated by reference into the present patent application, especially the end products of the Examples.

The term „treatment" as used herein relates in particular to a treatment aiming to halt pathogenic processes that lead to disease progression and/or has symptomatic effects.

The term „prophylactic treatment " as used herein relates in particular to a treatment aiming to halt pathogenic processes leading to disease.

The term „dementia of the Alzheimer's type" as used herein relates in particular to a disease as defined according to the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) criteria.

In a third aspect, the present invention relates to a method of treatment of dementia in human patients comprising administering 5 to 175 μg, preferably 15 to 125 μg, more preferably about 25 to 100 μg, e.g. 50 μg or 75 μg, of the CONSTRUCT to human patients in need thereof about every 4 to 8 weeks, preferably about every 5 to 7 weeks, in particular about every 6 weeks.

In a fourth aspect the present invention relates to a method of treatment of dementia in human patients comprising administering 5 to 1000 μg, preferably 5 to 300 μg, more preferably about 50 to 200, most preferably 50-150 μg, e.g. 50 μg or 75 μg,100μg, 125μg, 150μg of the CONSTRUCT to human patients in need thereof about every 4 to 8 weeks, preferably about every 5 to 7 weeks, in particular about every 6 weeks.
Frequency of injection can vary depending on the patient response.

For example the frequency of administration can vary if the injection has to be administered according to antibody titers.
The usefulness of the CONSTRUCTS in the treatment of the above-mentioned disorders can be confirmed in suitable clinical studies, e.g. those described in the Examples, e.g. applying a total daily dosage of 25 to 100 µg CONSTRUCT to patients every 4 to 8 weeks.

Suitable clinical studies are in particular randomized, double-blind, placebo-controlled, parallel studies in Alzheimer's patients or open label studies .

In a further aspect, the present invention pertains to a combination comprising at least one CONSTRUCT and at least one nootropic agent, preferably one cholinesterase-inhibitor, or memantine.

The term "nootropic agent" as used herein includes, but is not limited to nootropic plant extracts, calcium antagonists, cholinesterase inhibitors, dihydroergotoxin, nicergoline, piracetame, purine derivates, pyritinol, vincamine and vinpocetine.

The term "nootropic plant extracts" as used herein includes, but is not limited to extracts from Ginkgo leafs. The term "calcium antagonists" as used herein includes, but is not limited to cinnarizine and nimodipine. The term "cholinesterase inhibitors" as used herein includes, but is not limited to donepezil hydrochloride, rivastigmine and galantamine hydrobromide. The term "purine derivates" as used herein includes, but is not limited to pentifyllin.

Extracts from Ginkgo leafs can be administered, e.g., in the form as marketed, e.g. under the trademark Ginkodilat™ according to the information provided by the package insert. Cinnarizine can be administered, e.g., in the form as marketed, e.g. under the trademark Cinnarizin forte-ratiopharm™. Nimodipine can be administered, e.g., in the form as marketed, e.g. under the trademark Nimotop™. Donepezil hydrochloride can be administered, e.g., in the form as marketed, e.g. under the trademark Aricept™. Rivastigmine can be prepared as disclosed in US 5,602,176. It can be administered, e.g., in the form as marketed, e.g. under the trademark Exelon™. Galantamine hydrobromide can be administered, e.g., in the form as marketed, e.g. under the trademark Reminyl™. Dihydroergotoxin can be administered, e.g., in the form as marketed, e.g. under the trademark Hydergin™. Nicergoline can be administered, e.g., in the form as marketed, e.g.

under the trademark Sermion™. Piracetam can be administered, e.g., in the form as marketed, e.g. under the trademark Cerebroforte™. Pentifyllin can be administered, e.g., in the form as marketed, e.g. under the trademark Cosaldon™. Pyritinol can be administered, e.g., in the form as marketed, e.g. under the trademark Encephabol™. Vinpocetin can be administered, e.g., in the form as marketed, e.g. under the trademark Cavinton™. Memantine can be administered, e.g., in the form as marketed, e.g. under the trademarks Axura™ or Namenda™.

The structure of the active agents identified by code nos., generic or trade names may be taken from the actual edition of the standard compendium "The Merck Index" or from databases, e.g. Patents International (e.g. IMS World Publications). The corresponding content thereof is hereby incorporated by reference.

Hence, the present invention pertains also to a combination comprising a CONSTRUCT of the invention, and at least one nootropic agent selected from the group consisting of nootropic plant extracts, calcium antagonists, cholinesterase inhibitors, dihydroergotoxin, nicergoline, piracetame, purine derivates, pyritinol, vincamine and vinpocetine or memantine, in which the active ingredients are present in each case in free form or in the form of a pharmaceutically acceptable salt and optionally at least one pharmaceutically acceptable carrier, for simultaneous, separate or sequential use, especially for use in a method of treating dementia.

Such a combination is preferably a combined preparation.

Other agents can be used in combination with the CONSTRUCT, for example: antidepressants such as SSRIs, SNRIs, NRIs, antipsychotics such as risperidone, antidiabetic treatments such as insulin or metformin, antioxidative treatments such as selegiline, vitamin E, anti-inflammatory treatments such as NSAIDs, lipid-lowering agents such as statins, hormone substitution such as estrogens, amyloid lowering agents such as abeta secretase inhibitors, aggregation inhibitors such as beta-sheet blockers, chelators, immunomodulatory agents such as glatiramer acetate.

The term "a combined preparation", as used herein defines especially a "kit of parts" in the sense that the active ingredients as defined above can be dosed independently or by use of different fixed combinations with distinguished amounts of the ingredients, i.e., simultane-

ously or at different time points. The parts of the kit can then, e.g., be administered simultaneously or chronologically staggered, that is at different time points and with equal or different time intervals for any part of the kit of parts. Preferably, the time intervals are chosen such that the effect on the treated disease in the combined use of the parts is larger than the effect which would be obtained by use of only any one of the active ingredients.

Hence, the present invention also provides

- the use of a combination as disclosed herein for the preparation of a medicament for the treatment of dementia, in particular Alzheimer's disease; and
- a commercial package comprising a combination as disclosed herein together with instructions for simultaneous, separate or sequential use thereof in the treatment of dementia, in particular Alzheimer's disease.

In one preferred embodiment of the invention, the combination partner (b) is a cholinesterase inhibitor, especially rivastigmine, or memantine.

If the combination partners are administered as separate dosing forms, a dosage and mode of administration can be applied as provided in the package inserts. In particular, the following dosages of the combination partners (b) can be administered to the patient:

Cinnarizine may be administered to a patient in a total daily dosage of between about 75 to about 150 mg.

Nimodipine may be administered to a patient in a total daily dosage of between about 60 to about 120 mg.

Donepezil hydrochloride may be administered to a patient in a total daily dosage of between about 5 mg and 10 mg.

Rivastigmine may be administered to a patient in a total daily dosage of between about 6 and about 12 mg.

Galantamine may be administered to a patient in a total daily dosage of between about 12 and 24 mg, e.g. 12 mg twice daily.

Dihydroergotoxin may be administered in the form of its methansulfonate to a patient in a total daily dosage of between about 4 mg and 10 mg, e.g. about 8 mg.

Nicergoline may be administered in the form of its tartrate by intramuscular injection to a patient in a total daily dosage of between about 4 mg and 8 mg.

Piracetam may be administered to a patient in a total daily dosage of between about 1200 and 5000 mg, e.g. 4800 mg/day.

Pentifyllin may be administered to a patient in a total daily dosage of between about 400 and 800 mg.

Pyritinol may be administered in the form of its hydrochloride to a patient in a total daily dosage of about 600 mg.

Vinpocetin may be administered to a patient in a total daily dosage of between about 10 and 15 mg.

Memantine may be administered to a patient in the form of memantine hydrochloride in a total daily dosage of about 20 mg.

In a further aspect, the present invention provides human monoclonal antibodies against Aβ1-42 induced by the CONSTRUCT, preferably Aβ antibodies recognizing the N-terminus of Aβ1-42.

An efficient method to make human monoclonal antibodies from B cells isolated from the blood of a human patient is described by Elisabetta Traggiai, Stephan Becker, Kanta Subbarao, Larissa Kolesnikova, Yasushi Uematsu, Maria Rita Gismondo, Brian R Murphy, Rino Rappuoli & Antonio Lanzavecchia in *Nature Medicine* **10**, 871 - 875 (2004), which publication is included by reference into the present specification.

EXAMPLES

In the following Examples 1 to 4, male and female patients are included aged between 50 to 80 years (both inclusive), with mild to moderate AD as confirmed by a MMSE score of 16 to 26 (both inclusive), who are outpatients with caregivers (living together or, if living alone, with daily contact), who meet the DSM-IV criteria for dementia of the Alzheimer's type, and who satisfy the criteria for a clinical diagnosis of probable AD according to the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS-ADRDA). Each patient participates in a 4-week screening period (Day -28 to Day -1), a baseline period (pre-dose on Day 1 in week 0), three single dose treatments under ambulatory conditions in weeks 0, 6 and 18 (Days 1, 43, 127), ten additional ambulatory visits in bi- to four weekly intervals in weeks 2, 4, 8, 12, 16, 20, 22, 26, 30, and 34 (i.e. on Study Days 15, 29, 57, 85, 113, 141, 155, 183, 211 and 239), and two additional ambulatory visits in week 42 and 52 (i.e. on Study Days 295 and 365). Safety assessments include general physical examinations, neurological examinations, 12-lead electrocardiograms (ECGs), vital signs, standard clinical laboratory evaluations (hematology, blood chemistry, urinalysis), special immunological laboratory evaluations in blood and cerebrospinal fluid (CSF), cerebral magnetic resonance imagings (MRIs), as well as adverse event and serious adverse event monitoring. Further, patients and caregivers are instructed (verbally and in writing) to look for any unexpected deterioration in health status.

Aβ-antibody response is measured by determination of the Aβ-antibody titer (IgG and IgM) in serum and CSF using ELISA methods. The *ex vivo* Aβ-antibody binding properties in serum and CSF is explored by immunological methods on human and β-amyloid precursor protein (APP) transgenic mouse brain tissue. The VLP-antibody titer response in serum is measured to investigate the immune response to the carrier compound in relation to the immune response to Aβ.

Exploratory pharmacodynamic assessments include the following assessments: 1) determination of disease related markers in CSF (Aβ peptides and its isoforms, tau protein and its isoforms, phospho-tau) and plasma (Aβ peptides and isoforms); 2) volumetric MRIs, and 3) neuropsychological test battery, mini-mental state examination (MMSE), clinical dementia rating (CDR) and Alzheimer's Disease Cooperative Study - Activities of Daily Living (ADCS-ADL), 4) Positron emission tomography (PET) with ^{11}C-Pittsburgh Compound-B (^{11}C-PIB) which is a novel beta-amyloid selective tracer developed for *in vivo* detection of β-amyloid plaques in the brain and ^{18}F-fluorodeoxyglucose (^{18}FDG)

Responders are defined as those patients who show a significant increase of Aβ-specific antibody titers above baseline and who show an antibody isotype switch from IgM to IgG in serum at latest after the 3rd injection. Aβ-specific antibody titers are defined as titers above lower limit of quantification (LLOQ) in a validated enzyme-linked immunosorbent assay (ELISA) assay detecting specific antibodies relative to a standard serum as calibrator.

Example 1: A single or multi center, randomized, double-blind, placebo-controlled study in patients with mild to moderate Alzheimer's Disease (AD) with three subcutaneous injections of 25 µg of CONSTRUCT

A total of 30 patients is randomized to receive three s.c. injections of the CONSTRUCT or placebo. 24 patients receive the active drug CONSTRUCT and 6 patients receive placebo under double-blind conditions. Three s.c. injections of 25 µg CONSTRUCT or placebo are administered to each patient in weeks 0, 6 and 18.

Example 2: A single or multicenter, randomized, double-blind, placebo-controlled study in patients with mild to moderate Alzheimer's Disease (AD) with three subcutaneous injections of 50 µg of CONSTRUCT

A total of 30 patients is randomized to receive three s.c. injections of the CONSTRUCT or placebo. 24 patients receive the active drug CONSTRUCT and 6 patients receive placebo under double-blind conditions. Three s.c. injections of 50 µg CONSTRUCT or placebo are administered to each patient in weeks 0, 6 and 18.

Example 3: A single or multicenter, randomized, double-blind, placebo-controlled study in patients with mild to moderate Alzheimer's Disease (AD) with three subcutaneous injections of 100 µg of CONSTRUCT

A total of 30 patients is randomized to receive three s.c. injections of the CONSTRUCT or placebo. 24 patients receive the active drug CONSTRUCT and 6 patients receive placebo under double-blind conditions. Three s.c. injections of 100 µg CONSTRUCT or placebo are administered to each patient in weeks 0, 6 and 18.

Example 4: Determination of Antibody Titers in Serum

Blood samples are taken by direct venipuncture. A total of 10 mL venous blood is collected in plain barrier tubes. The sample are allowed to clot during 45 minutes at room temperature and then centrifuged for 10 minutes at approximately 2500 g. Serum tubes are frozen within 60 min after venipuncture and kept at <-70°C pending analysis.

Example 5: A single or multicenter, randomized, double-blind, placebo-controlled study in patients with mild to moderate Alzheimer's Disease (AD) with three subcutaneous injections of 150 µg of CONSTRUCT

A total of 30 patients is randomized to receive three s.c. injections of the CONSTRUCT or placebo. 24 patients receive the active drug CONSTRUCT and 6 patients receive placebo under double-blind conditions. Three s.c. injections of 150 µg CONSTRUCT or placebo are administered to each patient in weeks 0, 6 and 18.

Example 6: A single or multicenter, randomized, double-blind, placebo-controlled study in patients with mild to moderate Alzheimer's Disease (AD) with three subcutaneous injections of 300 µg of CONSTRUCT

A total of 30 patients is randomized to receive three s.c. injections of the CONSTRUCT or placebo. 24 patients receive the active drug CONSTRUCT and 6 patients receive placebo under double-blind conditions. Three s.c. injections of 300 µg CONSTRUCT or placebo are administered to each patient in weeks 0, 6 and 18.

What is claimed is:

1. The use of a construct consisting of a virus-like particle (VLP) structure chemically coupled to a fragment of the Aβ-1-42 peptide or its pharmaceutically acceptable salts for the manufacture of a pharmaceutical composition for the treatment of dementia in humans, wherein the pharmaceutical composition is administered subcutaneously or intramuscularly, and wherein 5 to 1000μg of the construct is administered about every 4 to 8 weeks.

2. The use according to claim 1 wherein the construct is administered at a total dose between 5 to 300μg every 4 to 8 weeks.

3. The use according to claim 1 wherein the construct is administered at a total dose between about 5 to 175 μg every 4 to 8 weeks.

4. The use according to any one of claims 1 to 3 wherein the construct is administered every 5 to 7 weeks.

5. The use according to any one of claims 1 to 4 wherein the construct is administered every 6 weeks.

6. The use according to any one of claims 1 to 5 wherein the dementia is dementia of the Alzheimer's type or vascular dementia with amyloid angipathy.

7. The use according to any one of claims 1 to 6 for the manufacture of a pharmaceutical composition for the treatment of patients with increased Aβ-level, including but not limited to patients with dementia associated with Parkinson's disease, Lewy Body dementia.

8. The use according to any one of claims 1 to 5 for the manufacture of a pharmaceutical composition for the prophylactic treatment of subjects at risk of developing Alzheimer's Disease, subjects with genotypes known to be associated with Alzheimer's Disease and subjects with Trisomy 21.

9. The use according to claim 8 wherein the subjects at risk of developing Alzheimer's Disease include those with mild cognitive impairment.

10. The use according to claim 8 wherein the subjects with genotypes known to be associated with Alzheimer's Disease include those with ApoE4.

11. The use according to any one of claim 1 to 10 wherein the construct consists of capsid proteins of a RNA bacteriophage self-assembled into a highly ordered VLP structure chemically coupled with a bivalent linker to a fragment of the Aβ-1-42 peptide.

12. The use according to claim 11 wherein the capsid proteins are taken from the RNA bacteriophage Qβ.

13. The use according to claim 11 or 12 wherein the fragment of the Aβ-1-42 peptide is Aβ-1-6.

14. A combination comprising at least one construct consisting of a VLP structure chemically coupled to a fragment of the Aβ-1-42 peptide or its pharmaceutically acceptable salts and at least one agent selected from the list of nootropic agents, antidepressants, antipsychotics, antidiabetic treatments, antioxydative treatments, vitamin E, anti-inflammatory treatments, lipid-lowering agents, hormone substitution, amyloid lowering agents, aggregation inhibitors, chelators, immunomodlatory agents, for the preparation of a medicament for the treatment of dementia in humans, wherein the construct consisting of a VLP structure chemically coupled to a fragment of the Aβ-1-42 peptide or its pharmaceutically acceptable salts is administered subcutaneously, at a total dose between 5 to 1000μg about every 4 to 8 weeks.

15. A combination according to claim 14 wherein:

(i) the nootropic agent is selected from nootropic plant extracts, calcium antagonists, cholinesterase inhibitors, dihydroergotoxin, nicergoline, piracetame, purine derivatives, pyritinol, vincamine, vinpocentine and memantine;

(ii) the antidepressant is selected from SSRIs, SNRIs, NRIs;

(iii) the antipsychotic is risperidone;

(iv) the antioxidative treatment is selegiline;

(v) the lipid-lowering agent is a statins;

(vi) the hormone substituent is estrogen;

(vii) the amyloid lowering agent is an abeta secretase inhibitors;

(viii) the aggregation inhibitor is a beta-sheet blocker; or

(ix) the immunomodulatory agent is glatiramer acetate.

16. A commercial package comprising a combination according to claim 14 or claim 15 together with instructions for simultaneous, separate or sequential use thereof in the treatment of dementia.

17. A combination according to claim 14 or claim 15 or a commercial package according to claim 16, wherein the construct consists of capsid proteins of a RNA bacteriophage self-assembled into a highly ordered VLP structure chemically coupled with a bivalent linker to a fragment of the Aβ-1-42 peptide.

18. The combination according to claim 14 or claim 15 or a commercial package according to claim 16 wherein the capsid proteins are taken from the RNA bacteriophage Qβ.

19. The combination according to claim 14 or claim 15 or a commercial package according to claim 16 wherein the fragment of the Aβ-1-42 peptide is Aβ-1-6.