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(54) **Title**: THERAPEUTIC ANTIBODIES TARGETING APP-C99

(57) **Abstract**: The present invention relates to an isolated and/or purified antibody, antibody fragment or derivative thereof able to block the gamma-secretase-dependent processing of the amyloid precursor protein (APP) and to pharmaceutical compositions containing said antibody/ies.

Therapeutic antibodies targeting APP-C99

FIELD OF THE INVENTION

The present invention relates to an isolated and/or purified antibody, antibody fragment or derivative thereof able to block the gamma-secretase-dependent processing of the amyloid precursor protein (APP) and to pharmaceutical compositions containing said antibody.

BACKGROUND OF THE INVENTION

Alzheimer's disease (AD) is presently affecting close to 30 million people worldwide. There is presently no efficient treatment available to cure or prevent this fatal disease considered by far as the most frequent age related neurological disorder (1). The neuropathological features of AD consist of extracellular deposition of amyloid-β (Aβ) peptides into senile plaques, and intracellular accumulation of hyperphosphorylated tau proteins leading to the formation of neurofibrillary tangles. The amyloid cascade hypothesis of AD pathogenesis proposes that polymerization of Aβ into soluble oligomeric and/or insoluble amyloid deposits is a critical and early event that triggers hyperphosphorylation of tau and formation of neurofibrillary lesions, neuroinflammation and neuronal death ultimately leading to dementia (2, 3).
Aβ peptides are derived from the amyloid precursor protein (APP) that undergoes a first shedding step by either alpha- or beta-secretase, leading to the formation of the membrane bound 83- or 99-amino-acid-long APP-carboxy-terminal fragments (APP-CTFs) designated APP-C83 and APP-C99, respectively. APP-C83 and APP-C99 are further processed by the intramembrane gamma-secretase to generate APP intracellular domains (AICDs), and extracellular nontoxic p3 fragments from APP-C83 or amyloidogenic Aβ peptides from APP-C99 (3-5). As enhanced production, and subsequent accumulation/aggregation of Aβ lead ultimately to neurodegeneration, a rational strategy to lower Aβ production consists in selectively inhibiting gamma-secretase-dependent APP-C99 processing without affecting the cleavage of other gamma-secretase substrates such as Notch, involved in cell proliferation and differentiation (6, 7) .

Indeed, preclinical studies with gamma-secretase inhibitors have shown gut toxicity and atrophy of spleen and thymus, and have been linked to impaired Notch processing (8, 9). Furthermore, intestinal adverse effects and skin toxicity, likely due to an alteration of Notch

signaling pathways, have been observed in patients treated with gamma-secretase inhibitors (10-12). Thus, it remains a priority to identify new Aβ lowering compounds that do not impair other signaling pathways of gamma-secretase.

This object has been achieved by providing an isolated and/or purified antibody, antibody fragment or derivative thereof able to block the gamma-secretase-dependent cleavage of the amyloid precursor protein (APP) without impairing the Notch processing.

SUMMARY OF THE INVENTION

The present invention provides an isolated and/or purified antibody, antibody fragment or derivative thereof able to block the gamma-secretase-dependent processing of the amyloid precursor protein (APP) but not the Notch processing, wherein said isolated and/or purified antibody, antibody fragment or derivative thereof i) specifically recognizes at least one sequence of said APP consisting essentially in the amino acid sequence set forth in SEQ ID No 1, a fragment thereof and/or a conservative variant thereof.

A further object of the present invention is to provide an isolated and/or purified antibody, antibody fragment or derivative thereof for the treatment and/or prevention of APP-associated diseases.

The present invention also relates to an isolated and/or purified nucleic acid sequence comprising

i) a nucleotide sequence encoding an isolated and/or purified antibody, an antibody fragment or derivative of said antibody,

ii) a nucleic acid sequence having substantial sequence identity or homology to a nucleic acid sequence encoding an isolated and/or purified antibody, an antibody fragment of or derivative of said antibody,

iii) a nucleic acid sequence complementary to i) or ii),

iv) a degenerated nucleic acid sequence of i), ii) or iii), or

v) a nucleic acid sequence capable of hybridizing under stringent conditions to i), ii), iii) or iv).

Another object concerns a pharmaceutical composition comprising a pharmaceutically effective amount of an isolated and/or purified antibody, antibody fragment or derivative thereof of the invention.

An expression vector comprising an isolated and/or purified nucleic acid sequence of the invention, a host cell comprising said expression vector and a hybridoma and/or clone secreting the monoclonal antibody of any one of the invention are also contemplated.

In a further aspect, there is provided a method for treating and/or preventing an APP-associated disease, in a patient in need thereof, comprising administering a pharmaceutically effective amount of an isolated and/or purified antibody, antibody fragment or derivative thereof of any one of the invention.

In another aspect, the invention relates to an immune composition for the treatment and/or prevention of an APP-associated disease comprising at least one or several isolated and/or purified antibody, antibody fragment or derivative thereof of any one of the invention.

In another aspect, the invention relates to a kit for treating and/or preventing an APP-associated disease, comprising a pharmaceutically effective amount of an isolated and/or purified antibody, antibody fragment or derivative thereof of any one of the invention, optionally with reagents and/or instructions for use.

In another aspect, the invention relates to a method for reducing Aβ production or delaying Aβ plaque formation in a patient in need thereof, comprising administering a pharmaceutically effective amount of an isolated and/or purified antibody, antibody fragment or derivative thereof of any one of the invention.

DESCRIPTION OF THE FIGURES

Fig. 1. Epitopes determination for the 4 groups of antibodies generated after mice immunization with recombinant and active human APP-C99 (APP-C99).

(A) Start, unbound, and eluted fraction of APP-C99 in presence of 0.1% phosphatidylcholine (PC) were migrated on 4-12% SDS-PAGE and stained with Coomassie blue. *: APP-C99 dimer. APP-C99 was cleaved in its lipidic environment when incubated with gamma-secretase microsomes (γ-Sec) and generated the AICD product (in fact AICD-His, indicated by an arrow) as probed by western blot using anti-APP A8717 antibody. In the absence of gamma-secretase microsomes, no AICD band was detected. **(B)** To determine the binding regions of the generated antibodies, several N-terminal and C-terminal fragments overlapping with the full APP-C99 sequence were cloned and purified with an anti-Histidine-tag resin. As the N-terminal fragments start with the Aβ sequence of APP-C99, they were named Aβ60, 70, 80 and 90. The C-terminal ones were designated C89, C83, C59 and C50. TMD: transmembrane domain. **(C)** These fragments were normalized and tested by enzyme-linked immunosorbent assay (ELISA) with the 12 clones obtained after immunization. As all fragments are carrying a Histidine-tag (His-tag), results are expressed in percentage of the binding observed with an anti-His-tag antibody. Four main groups of antibodies could be identified, namely mC99(1-7), mC99(70-80), mC99(80-90) and mC99(90-99) (mean ± standard deviation (s.d.), n=2). **(D)** Immunobloting of the APP-C99 fragments with each of the 12 antibodies demonstrated a comparable pattern of recognition. Here, the same anti-His-tag antibody was used as immuno-blotting control. **(E, top panel)** More precisely, the anti-N-terminal APP-C99 antibodies bind 3 synthetic peptides 1-7, 1-14 and 1-20 (ELISA). Therefore, the linear epitope of this group was designated 1-7. **(E, bottom panel)** The same group of antibodies recognized both C83 and C99 by Native-PAGE, thus revealing a structural epitope. For convenience, the linear epitope of this group was designated 1-7 and the antibodies designated mC99(1-7). **(F)** Overall, immunization with APP-C99 generated 4 groups of monoclonal antibodies: mC99(1-7) (comprising 5 clones) targeting the epitope 1-7, 5 clones designated mC99(70-80) for the epitope 70-80 , 1 clone mC99(80-90) for the epitope 80-90, and 1 clone mC99(90-99) for the epitope 90-99. The different classes of epitopes obtained, as well as the number of clones per group of epitope, relates the accessible versus non-accessible regions of APP-C99 in a membrane bilayer.

Fig. 2. Monoclonal antibodies targeting APP-C99 decrease its processing by gamma-secretase and reduce the production of secreted Aβ after their internalization in human cell lines.

(A) Immunoblot detection of APP-CTFs (including APP-C99 and APP-C83) accumulated in HEK and HeLa cells as a result of 250 nM of anti-APP-C99 antibodies treatment during 24 hours. DAPT (10 μM, a gamma-secretase inhibitor) was used as a control for APP-CTFs accumulation. Here, the 12 selected monoclonal antibodies targeting the epitopes 1-7 , 70-80, 80-90, and 90-99 were tested. **(B)** Five clones were selected to perform the following experiments. To confirm the inhibition of APP-C99 cleavage by gamma-secretase in presence of the antibodies, HEK APP-C99-Gal4-VP16 cells were incubated for 48 hours with increasing concentrations of the selected clones. Contrary to treatments with IgG A control antibody, the luminescence signal decreased accordingly to the treatment dose, confirming the impairment of APP-C99 processing in presence of anti-APP-C99 antibodies (mean ± s.d., t test with $*p<0.05$, $**p<0.01$, $***p<0.005$, $n=3$). **(C)** APP-CTFs accumulation, as well as secreted Aβ40 and 42 levels were assessed in HEK APPSwe cells incubated 24 hours with different concentrations of anti-APP-C99 antibodies. We observed a dose-dependent response to the treatments on both Aβ levels and APP-CTFs/APP-full length (APP-FL) accumulation. The decrease in Aβ40 and 42 productions reached respectively 27.6 ±4.2% and 25 ±6.6% at 500nM treatment when compared to IgG A control, for C-terminal antibodies. For the mC99(1-7) group, the 92 ±1% decrease in Aβ40 and 42 includes the direct depletion of the Aβ peptides in the medium (mean ± s.d., t test with $*p<0.05$, $**p<0.01$, $***p<0.005$, $n=3$). **(D)** Incubations of HEK NotchΔE cells with 500 nM of anti-APP-C99 antibodies during 24 hours showed no effect on Notch ΔE processing, whereas APP-CTFs were accumulated, thus emphasizing the specific effect of the antibodies on APP-C99/APP-full length (APP-FL) processing. **(E)** APP-C99 and APP-FL co-immunoprecipitated (co-IPed) with mC99(1-7) while both APP-C83/APP-C99 and APP-FL co-IPed with mC99(70-80) after lysis of HEK APPSwe cells pretreated with 250 nM of each antibody for 24 hours. The co-immunoprecipitation of the substrate and its specific antibody proves a substrate-antibody interaction in cells.

Fig. 3. Monoclonal antibodies targeting APP-C99 decrease its intracellular processing by gamma-secretase in a dose-dependent and non-toxic manner.

(A) Dose-dependent accumulation of APP-CTFs/APP-FL in HEK APPSwe cells treated with mC99(70-80) or mC99(1-7). Treatments of HEK APPSwe cells for 24 hours with increasing concentrations of these antibodies, show a dose-dependent accumulation of APP-CTFs/APP-FL

starting at a concentration as low as 25 nM. No effects on APP-CTFs/APP-FL were observed, following IgGA control antibody treatments. **(B)** Western blot detection of APP- CTFs/APP-FL in HEK APPSwe cells incubated with 250 nM of mC99(1-7) or mC99(70-80), or a monoclonal antibody targeting the N-terminus of APP-FL (22C11, against residues 66-81 of APP), or the gamma-secretase inhibitor DAPT (10μM). **(C)** Anti-APP-C99 antibodies do not cause significant toxicity in different human cell lines. Lactate Deshydrogenase (LDH) and crystal violet assays were performed to assess cell death in HEK, HeLa, HEK APP-C99-Gal4-VP16 (T-20), HEK APPSwe, and HEK NotchΔE cells, after treatments with 250 nM or 500 nM of the indicated antibodies (250 nM for HEK and HeLa cells, and 500 nM for NotchΔE and HEK APPSwe cells). Cell death and viability were expressed as percentage of LDH lysis control (for LDH assays) and MPP+ control (inhibitor of mitochondrial respiratory chain, for crystal violet assays) (mean ± s.d., t-test comparison with cell death control, ***$p<0.005$, n=3).

DETAILED DESCRIPTION OF THE INVENTION

Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. The publications and applications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. In addition, the materials, methods, and examples are illustrative only and are not intended to be limiting.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in art to which the subject matter herein belongs. As used herein, the following definitions are supplied in order to facilitate the understanding of the present invention.

The term “comprise” or “comprising” is generally used in the sense of include/including, that is to say permitting the presence of one or more features or components.

As used in the specification and claims, the singular form “a”, “an” and “the” include plural references unless the context clearly dictates otherwise.

As used herein, “at least one” means “one or more.”

As used herein the term “patient” is well-recognized in the art, and, is used herein to refer to a mammal, including dog, cat, rat, mouse, monkey, cow, horse, goat, sheep, pig, camel, and, most preferably, a human. The term does not denote a particular age or sex. Thus, adult and newborn subjects, whether male or female, are intended to be covered.

“Alzheimer’s disease” refers to a devastating neurodegenerative disorder for which the prevalence has been predicted to quadruple by 2050 (1). As the average lifespan increases, it has become a major socio-economical priority to identify new disease-modifying treatments to prevent or cure this disease. Although several underlying mechanisms that could explain the etiology of the disease are still unclear. The amyloid cascade remains so far the major and most documented hypothesis to explain the pathogenesis of AD.

The main histopathological hallmark of AD is the extracellular deposition of senile plaques principally containing misfolded and toxic Aβ peptides. Aβ peptides are the cleavage products of the 99 membrane-tethered APP-CTF (APP-C99) by gamma-secretase, an intramembrane enzymatic complex that can accomplish peptidic bond hydrolysis within the membrane bilayer (2-4, 13). Therefore, it is currently accepted that Aβ–lowering compounds might be therapeutically beneficial if they do not interfere with other gamma-secretase functions. Indeed, impairment of Notch processing and signaling leads to severe toxicity because of interference with cell renewal and differentiation (8, 14).

Surprisingly, the Applicants have shown that antibodies selected after immunization with an APP sequence, preferably a natively folded sequence of said APP consisting essentially in the amino acid sequence set forth in SEQ ID No 1, a fragment of said sequence and/or a conservative variant of said sequence, selectively impair the gamma-secretase-dependent processing of the amyloid precursor protein (APP) in cell-based *in vitro* assays without impairing the Notch processing. When incubated with cells, the same antibodies also impair the gamma-secretase-dependent processing of APP processing and reduce Aβ production. Taken together,

these results validate this new strategy as an immunotherapeutic approach to reduce Aβ production and therefore prevent or delay Aβ plaque formation.

Accordingly, the present invention relates to an isolated and/or purified antibody, antibody fragment or derivative thereof able to block the gamma-secretase-dependent processing of the amyloid precursor protein (APP) but not the Notch processing, wherein said isolated and/or purified antibody, antibody fragment or derivative thereof specifically recognizes at least one sequence of said APP consisting essentially in the amino acid sequence set forth in SEQ ID No 1, a fragment thereof and/or a conservative variant thereof. Preferably, said at least one APP sequence consisting essentially in the amino acid sequence set forth in SEQ ID No 1, a fragment thereof and/or a conservative variant thereof is a natively folded sequence.

Amyloid precursor protein (APP) is an integral membrane protein expressed in many tissues and concentrated in the synapses of neurons. Its primary function is not known, though it has been implicated as a regulator of synapse formation, neural plasticity and iron export. APP is best known and most commonly studied as the precursor molecule whose proteolysis generates beta amyloid (Aβ), a 39- to 42-amino acid peptide whose amyloid fibrillar form is the primary component of amyloid plaques found in the brains of Alzheimer's disease patients. Preferably this APP sequence of the invention consists essentially in the amino acid sequence set forth in **SEQ ID No 1** and containing 99 amino acids.

The phrase "consisting essentially in" when referring to a particular amino acid means a sequence having the properties of a given SEQ ID NO. For example, when used in reference to an amino acid sequence, the phrase includes the sequence *per se* and molecular modifications that would not affect the essential characteristics of the sequence. Preferably, the amino acid sequence will have substantial sequence identity for example at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, or 85% amino acid sequence identity; more preferably 90% amino acid sequence identity; and most preferably at least 95%, 96%, 97%, 98%, or 99% % amino acid sequence identity.

The present invention also refers to a fragment of the amyloid precursor protein (APP) **SEQ ID No 1**, preferably human APP. This refers to a biologically active sequence containing less amino acids in length than the corresponding sequence. This biologically active fragment

can be used as long as it exhibits the same properties as the sequence from which it derives. Preferably this sequence contains less than 90%, preferably less than 60%, in particular less than 30% amino acids in length than the respective sequence. Preferably also these sequences contain at least 5 to 20, most preferably 7 to 11 contiguous amino acids in length in common with the APP sequence of the invention.

Preferably, the fragment of said amino acid sequence set forth in SEQ ID No 1 is selected from the group of sequences comprising the N-terminal sequence 1-7 (SEQ ID No 2), the C-terminal sequence 70-80 (SEQ ID No 3), the C-terminal sequence 80-90 (SEQ ID No 4) and the C-terminal sequence 90-99 (SEQ ID No 5), and/or a combination of said sequences.

The present invention further refers to a conservative variant of the amyloid precursor protein (APP), or of a fragment thereof as disclosed above. This conservative variant refers to polypeptides having amino acid sequences that differ to some extent from the native sequence polypeptide, that is amino acid sequences that vary from the native 3D sequence whereby one or more amino acids are substituted by another one. The variants can occur naturally (e.g. polymorphism) or can be synthesized. Variants possess substitutions, deletions, and/or insertions at certain positions within the amino acid sequence of the native amino acid sequence. Amino acid substitutions are herein defined as exchanges within one of the following five groups:

I. Small aliphatic, nonpolar or slightly polar residues: Ala, Ser, Thr, Pro, Gly
II. Polar, positively charged residues: His, Arg, Lys
III. Polar, negatively charged residues: and their amides: Asp, Asn, Glu, Gln
IV. Large, aromatic residues: Phe, Tyr, Trp
V. Large, aliphatic, nonpolar residues: Met, Leu, Ile, Val, Cys.

Preferably the amino acid substitutions are conservative, i.e. occur within one of the above-identified group and do not affect the biological activity/ies of said APP sequence, i.e. its immunogenicity property.

Surprisingly, the Applicants have shown that an isolated and/or purified antibody, antibody fragment or derivative thereof of the present invention is/are able to block the gamma-secretase-dependent processing of the amyloid precursor protein (APP) but not the Notch processing. This refers to that the fact that the antibodies of the invention are selective and can neutralize the APP/gamma-secretase (in particular the human APP C99/ gamma-secretase) binding site, and/or

block the entrance of the substrate into the internal chamber of the complex, and/or inhibit its cleavage to subsequently inhibit the release of Aβ.

As used herein, an "antibody" is a protein molecule that reacts with a specific antigenic determinant or epitope and belongs to one or five distinct classes based on structural properties: IgA, IgD, IgE, IgG and IgM. The antibody may be a polyclonal (e.g. a polyclonal serum) or a monoclonal antibody, including but not limited to fully assembled antibody, single chain antibody, antibody fragment, and chimeric antibody, humanized antibody as long as these molecules are still biologically active and still bind to at least one peptide of the invention. Preferably the antibody is a monoclonal antibody. Preferably also the monoclonal antibody will be selected from the group comprising the IgG1, IgG2, IgG2a, IgG2b, IgG3 and IgG4 or a combination thereof. Most preferably, the monoclonal antibody is selected from the group comprising the IgG1, IgG2, IgG2a, and IgG2b, or a combination thereof. Non-limiting examples of antibodies are those listed in table 4.

A typical antibody is composed of two immunoglobulin (Ig) heavy chains and two Ig light chains. Several different types of heavy chain exist that define the class or isotype of an antibody. These heavy chain types vary between different animals. All heavy chains contain a series of immunoglobulin domains, usually with one variable (VH) domain that is important for binding antigen and several constant (CH) domains. Each light chain is composed of two tandem immunoglobulin domains: one constant (CL) domain and one variable domain (VL) that is important for antigen binding.

The term "isolated", when used as a modifier of an antibody of the invention means that the antibody is made by the hand of man or is separated, completely or at least in part, from their naturally occurring in vivo environment. Generally, isolated antibodies are substantially free of one or more materials with which they normally associate with in nature, for example, one or more protein. The term "isolated" does not exclude alternative physical forms of the antibodies, such as multimers/oligomers, modifications (e g , phosphorylation, glycosylation, lipidation) or derivatized forms, or forms expressed in host cells produced by the hand of man

An "isolated" antibody can also be "substantially pure" or "purified" when free of most or all of the materials with which it typically associates with in nature. Thus, an isolated antibody

that also is substantially pure or purified does not include polypeptides or polynucleotides present among millions of other sequences, such as antibodies of an antibody library or nucleic acids in a genomic or cDNA library.

The inventors have generated and selected 12 monoclonal antibodies after BALB/c mice immunization with active human APP-C99 (processed *in vitro* by gamma-secretase) purified in a lipidic environment to stabilize its native structure. Based on immunogenicity predictions (15-18) as well as on APP-C99 structural studies (19, 20), the different groups of epitopes (1-7, 70-80, 80-90, and 90-99) were interpreted as being the most accessible, hydrophilic, and flexible regions of APP-C99 embedded in a lipid-bilayer. Further immunohistochemical characterization confirmed these binding regions on cerebral amyloid deposits in Alzheimer brain slices (data not shown). After, their incubation with human cell lines, the different antibodies triggered intracellular and endogenous APP-CTFs accumulation. Moreover, selected clones for each group of epitopes (mC99(1-7), mC99(70-80), mC99(80-90), and mC99(90-99)) triggered a specific dose-dependent effect on APP/APP-CTFs processing as demonstrated by (i) their internalization in cells and binding to their APP-based targets (ii) the accumulation of non-processed APP-CTFs (iii) the decrease in AICD production as witnessed by the luciferase reporter assay concomitantly with (iii) the reduction in Aβ40 and 42 peptides production. Finally, intracerebroventricular (ICV) delivery of the mC99(1-7) and mC99(70-80) antibodies in young AD transgenic mice led to decreased brain Aβ levels (manuscript submitted for publication).

Additionally, experimental results show that monoclonal antibodies targeting the N- or C-terminal regions of APP-C99 (i) are internalized by cells and bind to their APP-based target, (ii) lower specifically the processing of APP-C99 by gamma-secretase, and (iii) decrease Aβ production, presumably by preventing the substrate binding and/or cleavage by gamma-secretase, due to steric hindrance.

Antibodies used in the present invention are not limited to whole antibody molecules and may be antibody fragments or derivatives as long as they are able to block the gamma-secretase dependent processing of the amyloid precursor protein (APP) and that they specifically recognizes at least one sequence of said APP, a fragment thereof and/or a conservative variant thereof without affecting the Notch processing.

Examples of isolated and/or purified antibody fragment or derivative thereof are selected

amongst the group comprising a Fab-fragment, a F(ab2)'-fragment, a single-chain antibody, a chimeric antibody, a CDR-grafted antibody, a bivalent antibody-construct, a humanized antibody, a synthetic antibody, a chemically modified derivative thereof, a multispecific antibody, a diabody, a scFv-fragment; a dsFv-fragment, a labeled antibody, or another type of recombinant antibody. Specifically, an antibody fragment is synthesized by treating the antibody with an enzyme such as papain or pepsin, or genes encoding these antibody fragments are constructed, and expressed by appropriate host cells as known to the skilled artisan.

Preferably, the isolated and/or purified antibody of the present invention consists essentially in an amino acid sequence selected from the group comprising
i) SEQ ID No 26 and SEQ ID No 27, a biologically active fragment thereof and/or a variant thereof,
ii) SEQ ID No 28 and SEQ ID No 29, a biologically active fragment thereof and/or a variant thereof,
iii) SEQ ID No 30 and SEQ ID No 31, a biologically active fragment thereof and/or a variant thereof,
iv) SEQ ID No 32 and SEQ ID No 33, a biologically active fragment thereof and/or a variant thereof ,or
v) SEQ ID No 34 and SEQ ID No 35, a biologically active fragment thereof and/or a variant thereof,
and/or a combination of said sequences. Examples of combination may comprise a gamma VH chain of a first clone, a biologically active fragment thereof and/or a variant thereof, associated with a kappa VL chain of another clone, a biologically active fragment thereof and/or a variant thereof.

A “Biologically active fragment” of the amino acid sequence of an antibody of the invention refers to a sequence containing less amino acids in length than the corresponding sequence. This biologically active fragment can be used as long as it exhibits the same properties as the sequence from which it derives, i.e. able to block the gamma-secretase-dependent processing of the amyloid precursor protein (APP) but not the Notch processing. The biologically active fragment of the invention is selective and can neutralize the APP/gamma-secretase (in particular the human APP C99/gamma-secretase) binding site, and/or block the entrance of the substrate into the internal chamber of the complex, and/or inhibit its cleavage to subsequently inhibit the release of Aβ.

Preferably this biologically active fragment contains less than 90%, preferably less than 60%, in particular less than 30% amino acids in length than the respective sequence.

Alternatively, this biologically active fragment represents the complementary determining region. The term “complementary determining region” or “CDR” is well-defined in the art (see, for example ref 27) and refers to the stretches of amino acids within the variable region of an antibody that primarily makes contact with the antigen.

Usually, the isolated and/or purified antibody, antibody fragment or derivative thereof of the invention comprises:

i) at least one complementary determining region 1 (CDR1) of the VH region, wherein the amino acid sequence determining said CDR1 consists essentially in an amino acid sequence selected from the group comprising SEQ ID No 6, SEQ ID No 8, SEQ ID No 10, SEQ ID No 12, SEQ ID No 14, a biologically active fragment thereof and/or a variant thereof, and/or a combination of said sequences, and/or

ii) at least one complementary determining region 2 (CDR2) of the VH region, wherein the amino acid sequence determining said CDR2 consists essentially in an amino acid sequence selected from the group comprising SEQ ID No 7, SEQ ID No 9, SEQ ID No 11, SEQ ID No 13, SEQ ID No 15, a biologically active fragment thereof and/or a variant thereof, and/or a combination of said sequences, and/or

iii) at least one complementary determining region 3 (CDR3) of the VH region, wherein the amino acid sequence determining said CDR3 consists essentially in an amino acid sequence selected from the group comprising SEQ ID No 56, SEQ ID No 57, SEQ ID No 58, SEQ ID No 59, SEQ ID No 60, a biologically active fragment thereof and/or a variant thereof, and/or a combination of said sequences

Additionally, the isolated and/or purified antibody, antibody fragment or derivative thereof of the invention may also comprise:

i) at least one complementary determining region 1 (CDR1) of the VL region, wherein the amino acid sequence determining said CDR1 consists essentially in an amino acid sequence selected from the group comprising SEQ ID No 16, SEQ ID No 18, SEQ ID No 20, SEQ ID No 22, SEQ ID No 24, a biologically active fragment thereof and/or a variant thereof, and/or a combination of said sequences, and/or

ii) at least one complementary determining region 2 (CDR2) of the VL region, wherein the amino acid sequence determining said CDR2 consists essentially in an amino acid sequence selected from the group comprising SEQ ID No 61, SEQ ID No 62, SEQ ID No 63, SEQ ID No 64, SEQ ID No 65, a biologically active fragment thereof and/or a variant thereof, and/or a combination of said sequences, and/or

iii) at least one complementary determining region 3 (CDR3) of the VL region, wherein the amino acid sequence determining said CDR3 consists essentially in an amino acid sequence selected from the group comprising SEQ ID No 17, SEQ ID No 19, SEQ ID No 21, SEQ ID No 23, SEQ ID No 25, a biologically active fragment thereof and/or a variant thereof, and/or a combination of said sequences.

Preferably, the biologically active fragment contains less than 90%, preferably less than 60%, in particular less than 30% amino acids in length than the respective sequence(s) of CDR of the VH and/or CDR of the VL regions.

Fragments or derivatives of the above antibodies which are able to block the gamma-secretase-dependent processing of the amyloid precursor protein (APP) can be obtained by using methods which are described, e.g., in (27). When derivatives of said antibodies are obtained by the phage display technique, surface plasmon resonance as employed in the BIAcore system can be used to increase the efficiency of phage antibodies which bind to an epitope of EAG1 (28)".

The term "CDR-grafted antibody" refers to an antibody in which the CDR from one antibody, or a biologically active fragment of said CDR, is inserted into the framework of another antibody. In certain embodiments, the antibody from which the CDR is derived and the antibody from which the framework is derived are of different species. In certain embodiments, the antibody from which the CDR is derived and the antibody from which the framework is derived are of different isotypes.

The term "humanized antibody" refers to an antibody in which all or part of an antibody framework region is derived from a human, but all or part of one or more CDR regions is derived from another species, for example, including, but not limited to, a mouse. The term "humanized

antibody" refers to antibodies in which the framework or "complementarity determining regions" (CDR) have been modified to comprise the CDR of an immunoglobulin of different specificity as compared to that of the parent immunoglobulin. Humanized antibodies can be produced using synthetic or recombinant DNA technology using standard methods or other suitable techniques. Nucleic acid (e.g. , cDNA) sequences coding for humanized variable regions can also be constructed using PCR mutagenesis methods to alter DNA sequences encoding a human or humanized chain, such as a DNA template from a previously humanized variable region (see e.g. (29), (30), (31) and (32)). Using these or other suitable methods, variants can also be readily produced. In one embodiment, cloned variable regions (e.g., dAbs) can be mutated, and sequences encoding variants with the desired specificity can be selected (e.g., from a phage library; see e.g., Krebber et al. , U.S. 5,514,548; Hoogenboom et al. , WO 93/06213, published April 1, 1993). Methods for the production of humanized antibodies are also described in, e.g., EP-A1 0 239 400 and W090/07861.

The term "bivalent or bispecific antibody" as used herein refers to an antibody in which each of the two pairs of heavy chain and light chain (HC/LC) is specifically binding to a different antigen, i.e. the first heavy and the first light chain (originating from an antibody against a first antigen) are specifically binding together to a first antigen, and , the second heavy and the second light chain (originating from an antibody against a second antigen) are specifically binding together to a second antigen; such bivalent, bispecific antibodies are capable of specifically binding to two different antigens at the same time, and not to more than two antigens, in contrary to, on the one hand a monospecific antibody capable of binding only to one antigen, and on the other hand e.g. a tetravalent, tetraspecific antibody which can bind to four antigen molecules at the same time.

Among the antibody fragments of the present invention, Fab, F(ab')2 and the like can be obtained by treating an antibody of the invention with a proteolytic enzyme such as papain or pepsin, or alternatively, can be prepared by constructing a gene encoding the resulting antibody fragment and introducing this construct into an expression vector, followed by expression in an appropriate host cell.

The term "recombinant antibody", as used herein, is intended to include all antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies

isolated from a host cell (such as a NSO or CHO cell) or from an animal (e.g. a mouse) that is transgenic for, for example, human immunoglobulin genes or antibodies expressed using a recombinant expression vector transfected into a host cell. Such recombinant antibodies have variable and constant regions in a rearranged form.

The “diabody” refers to an antibody produced in accordance with the technology described in (33). These authors have provided an alternative mechanism for making bispecific antibody fragments. The fragments comprise a heavy-chain variable domain (VH) connected to a light-chain variable domain (VL) by a linker which is too short to allow pairing between the two domains on the same chain. Accordingly, the VH and VL domains of one fragment are forced to pair with the complementary VL and VH domains of another fragment, thereby forming two antigen-binding sites. Another strategy for making bispecific antibody fragments by the use of single-chain Fv (sFv) dimers has also been reported. See ref. 34.

Antibodies with more than two valencies are also contemplated. For example, multispecific antibodies such as trispecific antibodies can be prepared as known to the skilled artisan.

Among the antibody fragments of the present invention, single chain Fv (scFv) can be prepared by linking together an H chain V region and an L chain V region from an antibody of the present invention by using an appropriate peptide linker or the like. Alternatively, scFv can be prepared by constructing a DNA segment encoding the entire sequences or desired amino acid sequences of a gene encoding an H chain or H chain V region from the above antibody and a gene encoding an L chain or L chain V region from the antibody, and introducing this construct into an expression vector, followed by expression in an appropriate host cell.

Among the antibody fragments of the present invention, disulfide-stabilized antibody (dsFv) is an antibody fragment in which polypeptides modified to replace one amino acid residue by a cysteine residue in both H and L chain V regions from an antibody of the present invention are linked together between these cysteine residues via a disulfide linkage. An amino acid residue to be replaced by a cysteine residue can be selected by stereostructural estimation of the antibody. dsFv can be prepared by constructing a DNA segment encoding the entire sequence or a desired amino acid sequence of a gene encoding the antibody fragment, and introducing this construct into an expression vector, followed by expression in an appropriate host cell.

Among the antibody fragments of the present invention, a CDR-containing peptide comprises at least one or more CDR regions selected from CDR regions in H or L chains of an antibody of the invention. Also, multiple CDR regions may be linked together by techniques using an appropriate peptide linker or the like. The CDR-containing peptide may also be prepared by constructing a DNA segment encoding the entire sequence or a desired amino acid sequence of a gene encoding the peptide, and introducing this construct into an expression vector, followed by expression in an appropriate host cell. Alternatively, the CDR-containing peptide can also be prepared by chemical synthesis such as Fmoc or tBoc method.

The production of chimeric antibodies is described, for example, in W089/09622. A further source of antibodies to be utilized in accordance with the present invention are so-called xenogenic antibodies. The general principle for the production of xenogenic antibodies such as human antibodies in mice is described in, e.g., WO 91/10741, WO 94/02602, WO 96/34096 and WO 96/33735. As discussed above, the antibody of the invention may exist in a variety of forms besides complete antibodies; including, for example, Fv, Fab and F(ab)2, as well as in single chains; see e.g. W088/09344.

To further demonstrate the specificity of the antibodies for APP-C99, the Applicants have investigated their effects on Notch intracellular domain (NICD) production in HEK cells stably expressing human Notch ΔE (see example 5). In contrast to DAPT, a well-known gamma-secretase inhibitor, incubation with these antibodies did not affect Notch processing (Fig. 2D). Importantly, detailed toxicity assays revealed that antibodies treatments did not show significant cell death (Fig. 3C). Accordingly, adverse effects due to an alteration of the cleavage of other gamma-secretase substrates, such as for example in the Notch signaling pathways, in patients treated with so far known gamma-secretase inhibitors (10-12) are not expected with the present antibodies.

The Notch receptor, in particular the Notch-I receptor, is a known substrate of gamma -secretase and crucial signaling molecule involved in the differentiation and proliferation of a wide variety of cell types (6, 21). Indeed, significant adverse effects of gamma-secretase inhibitors have been described in preclinical and clinical studies, including gastrointestinal, haematopoetic, and skin toxicity, all of which have been attributed to impaired Notch processing (8, 9, 11, 12). Thus, it was important for the Applicants to develop drugs that lower Aβ production without adversely affecting other gamma-secretase substrates and associated functions.

As discussed above, the 12 monoclonal antibodies have been obtained after mice immunization with a natively folded sequence of human APP-C99. ELISA and immunobloting experiments thus confirmed the existence of structural epitopes on APP-C99. For example, a structural epitope in the N-terminal region has been determined in which the asparate 7 (D7) is in close vicinity with lysin16 (K16) and its adjacent residues (arrow in Fig. 1F). That observation was further supported by Native-PAGE immunoblotting revealing that both APP-C83 and -C99 were immunoreactive for mC99(1-7), while the 6E10 antibody (epitope 1-16 of Aβ) only bound APP-C99, and A8717 (targeting the last 19 residues of human APP) recognized both APP-C83 and -C99 (Fig. 1E bottom panel).

The present invention further contemplates an isolated and/or purified nucleic acid sequence comprising

i) a nucleotide sequence encoding an isolated and/or purified antibody, an antibody fragment or derivative of said antibody of the invention,

ii) a nucleic acid sequence having substantial sequence identity or homology to a nucleic acid sequence encoding an isolated and/or purified antibody, an antibody fragment of or derivative of said antibody of the invention,

iii) a nucleic acid sequence complementary to i) or ii),

iv) a degenerated nucleic acid sequence of i), ii) or iii), or

v) a nucleic acid sequence capable of hybridizing under stringent conditions to i), ii), iii) or iv).

"An isolated and/or purified nucleic acid sequence" refers to nucleic acid free or substantially free of material with which it is naturally associated such as other polypeptides or nucleic acids with which it is found in its natural environment, or the environment in which it is prepared (e. g. cell culture) when such preparation is by recombinant nucleic acid technology practiced *in vitro* or *in vivo*.

The term "nucleic acid" is intended to refer either to DNA or to RNA.

In case the nucleic acid is DNA, then DNA which can be used herein is any polydeoxynuclotide sequence, including, e.g. double-stranded DNA, single-stranded DNA, double-stranded DNA wherein one or both strands are composed of two or more fragments, double-stranded DNA wherein one or both strands have an uninterrupted phosphodiester

backbone, DNA containing one or more single-stranded portion(s) and one or more double-stranded portion(s), double-stranded DNA wherein the DNA strands are fully complementary, double-stranded DNA wherein the DNA strands are only partially complementary, circular DNA, covalently-closed DNA, linear DNA, covalently cross-linked DNA, cDNA, chemically-synthesized DNA, semi-synthetic DNA, biosynthetic DNA, naturally-isolated DNA, enzyme-digested DNA, sheared DNA, labeled DNA, such as radiolabeled DNA and fluorochrome-labeled DNA, DNA containing one or more non-naturally occurring species of nucleic acid.

DNA sequences that encode the isolated and/or purified antibody, an antibody fragment of or derivative of said antibody of the invention, or a biologically active fragment thereof, can be synthesized by standard chemical techniques, for example, the phosphotriester method or via automated synthesis methods and PCR methods.

The purified and/or isolated DNA sequence encoding an isolated and/or purified antibody, an antibody fragment of or derivative of said antibody of the invention according to the invention may also be produced by enzymatic techniques. Thus, restriction enzymes, which cleave nucleic acid molecules at predefined recognition sequences can be used to isolate nucleic acid sequences from larger nucleic acid molecules containing the nucleic acid sequence, such as DNA (or RNA) that codes for the isolated and/or purified antibody, an antibody fragment of or derivative of said antibody of the invention or for a fragment thereof.

Encompassed by the present invention is also a nucleic acid in the form of a polyribonucleotide (RNA), including, e.g., single-stranded RNA, double-stranded RNA, double-stranded RNA wherein one or both strands are composed of two or more fragments, double-stranded RNA wherein one or both strands have an uninterrupted phosphodiester backbone, RNA containing one or more single-stranded portion(s) and one or more double-stranded portion(s), double-stranded RNA wherein the RNA strands are fully complementary, double-stranded RNA wherein the RNA strands are only partially complementary, covalently crosslinked RNA, enzyme-digested RNA, sheared RNA, mRNA, chemically-synthesized RNA, semi-synthetic RNA, biosynthetic RNA, naturally-isolated RNA, labeled RNA, such as radiolabeled RNA and fluorochrome-labeled RNA, RNA containing one or more non-naturally-occurring species of nucleic acid.

The isolated and purified nucleic acid sequence, DNA or RNA, also comprises an isolated and/or purified nucleic acid sequence having substantial sequence identity or homology to a nucleic acid sequence encoding an isolated and/or purified antibody, an antibody fragment

of or derivative of said antibody of the invention. Preferably, the nucleic acid will have substantial sequence identity for example at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, or 85% nucleic acid identity; more preferably 90% nucleic acid identity; and most preferably at least 95%, 96%, 97%, 98%, or 99% sequence identity.

Identity as known in the art and used herein, is a relationship between two or more amino acid sequences or two or more nucleic acid sequences, as determined by comparing the sequences. It also refers to the degree of sequence relatedness between amino acid or nucleic acid sequences, as the case may be, as determined by the match between strings of such sequences. Identity and similarity are well known terms to skilled artisans and they can be calculated by conventional methods (for example see 35-41).

Methods which are designed to give the largest match between the sequences are generally preferred. Methods to determine identity and similarity are codified in publicly available computer programs including the GCG program package (42); BLASTP, BLASTN, and FASTA (43). The BLAST X program is publicly available from NCBI and other sources (43).

Also encompassed by the present invention is a nucleic acid sequence complementary to the isolated and purified nucleic acid sequence encoding an isolated and/or purified antibody, an antibody fragment of or derivative of said antibody/ies of the invention.

Also within the scope of the invention is a degenerated nucleic acid sequence having a sequence which differs from a nucleic acid sequence encoding the isolated and/or purified antibody, an antibody fragment or derivative of said antibody of the invention, or a derivative or complementary sequence thereof, due to degeneracy in the genetic code. Such nucleic acid encodes functionally equivalent isolated and/or purified antibody, antibody fragment or derivative of said antibody of the invention but differs in sequence from the sequence due to degeneracy in the genetic code. This may result in silent mutations which do not affect the amino acid sequence. Any and all such nucleic acid variations are within the scope of the invention.

In addition, also considered is a nucleic acid sequence capable of hybridizing under stringent conditions, preferably high stringency conditions, to a nucleic acid sequence encoding an isolated and/or purified antibody, an antibody fragment of or derivative of said antibody of the invention, a nucleic acid sequence complementary thereof or a degenerated nucleic acid

sequence thereof. Appropriate stringency conditions which promote DNA hybridization are known to those skilled in the art, or can be found in ref. 44. For example, 6.0X sodium chloride/sodium citrate (SSC) at about 45°C, followed by a wash of 2.0XSSC at 50°C may be employed. The stringency may be selected based on the conditions used in the wash step. By way of example, the salt concentration in the wash step can be selected from a high stringency of about 0.2XSSC at 50°C. In addition, the temperature in the wash step can be at high stringency conditions, at about 65° C.

The present invention also includes an isolated and/or purified nucleic acid encoding an antibody, an antibody fragment or derivative of said antibody of the invention comprising a nucleic acid sequence encoding a truncation or an analog of the antibody, antibody fragment or derivative of said antibody of the invention. The term “truncation” refers to a sequence encoding a peptide containing less amino acid than the native but exhibiting the same properties.

The invention also encompasses allelic variants of the disclosed isolated and/or purified nucleic sequence; that is, naturally-occurring alternative forms of the isolated and/or purified nucleic acid that also encode peptides that are identical, homologous or related to that encoded by the isolated and/or purified nucleic sequences. Alternatively, non-naturally occurring variants may be produced by mutagenesis techniques or by direct synthesis.

A biologically active fragment of the disclosed isolated and/or purified nucleic sequence is also considered and refers to a sequence containing less nucleotides in length than the nucleic acid sequence encoding an isolated and/or purified antibody, an antibody fragment of or derivative of said antibody of the invention, a nucleic acid sequence complementary thereof or a degenerated nucleic acid sequence thereof. This sequence can be used as long as it exhibits the same properties as the native sequence from which it derives. Preferably this sequence contains less than 90%, preferably less than 60%, in particular less than 30% amino acids in length than the respective isolated and/or purified nucleic sequence of the antibody, antibody fragment or derivative of said antibody of the invention.

Yet another concern of the present invention is to provide an expression vector comprising the isolated and/or purified nucleic acid sequence encoding an isolated and/or purified antibody, an antibody fragment or derivative of said antibody of the invention. The choice of an expression vector depends directly, as it is well known in the art, on the functional

properties desired, e.g., an isolated and/or purified antibody, an antibody fragment of or derivative of said antibody of the invention expression and the host cell to be transformed or transfected.

A further concern of the present invention is to provide a host cell comprising the expression vector of the invention. Generally, the host cell is a bacterium, a fungal, a plant, or an animal cell. Preferably, the animal cell is a mammalian cell and most preferably a human cell or a human cell line.

The present invention also provides one or more hybridoma and/or clones secreting the monoclonal antibody of the invention. Non limiting examples of clones include the followings: 2C5, 1D8, 2F9, 1H9, 1H10 which produce antibodies that bind to human and murine APP and/or a fragment of said APP and block the gamma-secretase-dependent processing of the amyloid precursor protein (APP).

The term “monoclonal antibody” refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced. Methods for producing and screening for specific antibodies using hybridoma technology are routine and well known in the art.

Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. For example, monoclonal antibodies can be produced using hybridoma techniques including those known in the art and taught, for example, in (27), (45) (said references incorporated by reference in their entireties). The term "monoclonal antibody" as used herein is not limited to antibodies produced through hybridoma technology.

One additional aspect of the present invention is to provide a pharmaceutical composition comprising a pharmaceutically effective amount of an isolated and/or purified antibody, antibody fragment or derivative thereof as disclosed herein. The pharmaceutical agent can be in a variety of well known formulations and administered using any of a variety of well known methods of administration such as intra-nasal, oral, subcutaneous, intravenous, intraarterial, intraperitoneal and/or intramuscular are also contemplated or the like.

Pharmaceutical compositions adapted for nasal administration wherein the pharmaceutically acceptable carrier is a solid include a coarse powder having a particle size for example in the range 20 to 500 microns which is administered in the manner in which snuff is taken, e.g, by rapid inhalation through the nasal passage from a container of the powder held close up to the nose. Suitable compositions wherein the carrier is a liquid, for administration as a nasal spray or as nasal drops, include aqueous or oil solutions of the active ingredient.

Pharmaceutical compositions adapted for administration by inhalation include fine particle dusts or mists which may be generated by means of various types of metered dose pressurized aerosols, nebulizers or insufflators.

In cases where the agent of the invention is included in a suspension, the formulation may contain suspending agents, as for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, or mixtures of these substances, among others.

Useful intranasal formulations may contain at least one stabilizer and surfactant. Among the pharmaceutically acceptable surfactants are polyoxyethylene castor oil derivatives, such as polyoxyethylene-glycerol-triricinoleate, also known as polyoxyl 35 caster oil (CREMOPHOR EL), or poloxyl 40 hydrogenated castor oil (CREMOPHOR RH40) both available from BASF Corp.; mono-fatty acid esters of polyoxyethylene (20) sorbitan, such as polyoxyethylene (20) sorbitan monolaurate (TWEEN 80), polyoxyethylene monostearate (TWEEN 60), polyoxyethylene (20) sorbitan monopalmitate (TWEEN 40), or polyoxyethylene 20 sorbitan monolaurate (TWEEN 20) (all available from ICI Surfactants of Wilmington, Del.); polyglyceryl esters, such as polyglyceryl oleate; and polyoxyethylated kernel oil (LABRAFIL, available from Gattefosse Corp.). Preferably, the surfactant will be between about 0.01% and 10% by weight of the pharmaceutical composition. Among the pharmaceutically useful stabilizers are antioxidants such as sodium sulfite, sodium metabisulfite, sodium thiosulfate, sodium formaldehyde sulfoxylate, sulfur dioxide, ascorbic acid, isoascorbic acid, thioglycerol, thioglycolic acid, cysteine hydrochloride, acetyl cysteine, ascorbyl palmitate, hydroquinone, propyl gallate, nordihydroguaiaretic acid, butylated hydroxytoluene, butylated hydroxyanisole, alpha-tocopherol and lecithin. Preferably, the stabilizer will be between about 0.01% and 5% by weight of the pharmaceutical composition.

Suspensions may also include chelating agents such as ethylene diamine tetraacetic acid, its derivatives and salts thereof, dihydroxyethyl glycine, citric acid and tartaric acid among

others. Additionally, proper fluidity of a suspension can be maintained, for example, by the use of coating materials such as lecithin, by the maintenance of the required particle size in the case of dispersions and by the use of surfactants, such as those previously mentioned.

Solid dosage forms for oral administration include capsules, tablets, pills, powders and granules. In such solid dosage forms, the pharmaceutically effective amount of an agent of the invention may be mixed with at least one inert, pharmaceutically acceptable excipient or carrier, such as sodium citrate or dicalcium phosphate and/or (a) fillers or extenders such as starches, lactose, sucrose, glucose, mannitol and silicic acid; (b) binders such as carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidone, sucrose and acacia; (c) humectants such as glycerol; (d) disintegrating agents such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates and sodium carbonate; (e) solution retarding agents such as paraffin; (f) absorption accelerators such as quaternary ammonium compounds; (g) wetting agents such as cetyl alcohol and glycerol monostearate;(h) absorbents such as kaolin and bentonite clay; and (i) lubricants such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate and mixtures thereof. In the case of capsules, tablets and pills, the dosage form may also comprise buffering agents.

Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like.

The solid dosage forms of tablets, capsules, pills and granules can be prepared with coatings and shells such as enteric coating and other coatings well-known in the pharmaceutical formulating art. They may optionally contain opacifying agents and may also be of a composition such that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes.

Liquid dosage forms for oral administration or for spray formulation include pharmaceutically acceptable emulsions, solutions, suspensions, syrups and elixirs. In addition to the pharmaceutically effective amount of an agent of the invention, the liquid dosage forms may contain inert diluents commonly used in the art such as, for example, water or other solvents, solubilizing agents and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethyl formamide, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor and sesame oils),

glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan and mixtures thereof.

This invention also envisages the use of an isolated and/or purified antibody, antibody fragment or derivative thereof of the invention in a pharmaceutically acceptable salt form. Examples of such salts may include sodium, potassium, calcium, aluminum, gold and silver salts. Also contemplated are salts formed with pharmaceutically acceptable amines such as ammonia, alkyl amines, hydroxyalkylamines, N-methylglucamine and the like. Certain basic compounds also form pharmaceutically acceptable salts, e.g., acid addition salts. For example, pyrido-nitrogen atoms may form salts with strong acid, while compounds having basic substituents such as amino groups also form salts with weaker acids. Examples of suitable acids for salt formation are hydrochloric, sulfuric, phosphoric, acetic, citric, oxalic, malonic, salicylic, malic, fumaric, succinic, ascorbic, maleic, pamoic, methanesulfonic and other mineral and carboxylic acids well known to those skilled in the art.

The salts are prepared by contacting the free base form with a sufficient amount of the desired acid to produce a salt in the conventional manner. The free base forms may be regenerated by treating the salt with a suitable dilute aqueous base solution such as dilute aqueous NaOH, potassium carbonate, ammonia and sodium bicarbonate. The free base forms differ from their respective salt forms somewhat in certain physical properties, such as solubility in polar solvents, but the acid and base salts are otherwise equivalent to their respective free base forms for purposes of the invention.

All such acid and base salts are intended to be pharmaceutically acceptable salts within the scope of the invention and all acid and base salts are considered equivalent to the free forms of the corresponding compounds for purposes of the invention.

The pharmaceutical composition comprising an isolated and/or purified antibody, antibody fragment or derivative thereof of the invention, as described herein, as an active ingredient may also be incorporated or impregnated into a bioabsorbable matrix, with the matrix being administered in the form of a suspension of matrix, a gel or a solid support. In addition the matrix may be comprised of a biopolymer.
Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semi permeable matrices of solid hydrophobic polymers containing a

pharmaceutically effective amount of an agent of the invention, which matrices are in the form of shaped articles, e.g. films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and [gamma] ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT(TM) (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid. The formulations to be used for in vivo administration must be sterile. This is readily accomplished for example by filtration through sterile filtration membranes.

It is understood that the suitable dosage of a pharmaceutically effective amount of an isolated and/or purified antibody, antibody fragment or derivative thereof of the present invention will be dependent upon the age, sex, health, and weight of the recipient, kind of concurrent treatment, if any and the nature of the effect desired.

The appropriate dosage form will depend on the disease, the isolated and/or purified antibody, antibody fragment or derivative thereof of the invention, and the mode of administration.

Usually, the pharmaceutically effective amount of an isolated and/or purified antibody, antibody fragment or derivative thereof of the invention is present in an amount between about 0.001% and 20% by weight of the pharmaceutical composition.

While a preferred pharmaceutical composition of the present invention comprises a pharmaceutically effective amount of an isolated and/or purified antibody, antibody fragment or derivative thereof as an active ingredient, an alternative pharmaceutical composition may contain an isolated and/or purified nucleic acid sequence encoding said isolated and/or purified antibody, antibody fragment or derivative thereof, as described herein, as an active ingredient (alone or in combination with an isolated and/or purified antibody, antibody fragment or derivative thereof) as an active ingredient. This pharmaceutical composition may include either the sole isolated and/or purified DNA sequence, an expression vector comprising said isolated and/or purified DNA sequence or a host cell previously transfected or transformed with an expression vector described herein.

In this latter example, host cell will preferably be isolated from the patient to be treated in order to avoid any antigenicity problem. These gene and cell therapy approaches are especially

well suited for patients requiring repeated administration of the pharmaceutical composition, since the said purified and/or isolated DNA sequence, expression vector or host cell previously transfected or transformed with an expression vector can be incorporated into the patient's cell which will then produce the protein endogenously.

The pharmaceutical compositions of the invention are preferably for the treatment and/or prevention of diseases of aging based on or associated with amyloid-like proteins and characterized, in part, by the buildup of extracellular deposits of amyloid or amyloid-like material that contribute to the pathogenesis, as well as the progression of the disease. These diseases include, but are not limited to, neurological disorders such as Alzheimer's Disease (AD), Lewy body dementia (LBD), Down's syndrome, hereditary cerebral hemorrhage with amyloidosis (Dutch type); the Guam Parkinson- Dementia complex. Other diseases which are based on or associated with amyloid- like proteins are progressive supranuclear palsy, multiple sclerosis; Creutzfeld Jacob disease, Parkinson's disease, HIV-related dementia, ALS (amyotropic lateral sclerosis), Adult Onset Diabetes, senile cardiac amyloidosis, endocrine tumors, and others, including ocular diseases associated with pathological abnormalities/changes in the tissues of the visual system, particularly associated with amyloid-beta-related pathological abnormalities/changes in the tissues of the visual system, such as neuronal degradation. Said pathological abnormalities may occur, for example, in different tissues of the eye, such as the visual cortex leading to cortical visual deficits; the anterior chamber and the optic nerve leading to glaucoma; the lens leading to cataract due to beta-amyloid deposition; the vitreous leading to ocular amyloidosis; the retina leading to primary retinal degeneration, and macular degeneration, for example age-related macular degeneration; the optic nerve leading to optic nerve drusen, optic neuropathy and optic neuritis; and the cornea leading to lattice dystrophy.

Preferably, the diseases is selected among the group comprising Alzheimer's disease, Down syndrom, diseases caused by an extra copy of Chromosome 21 or extra copy(ies) of the gene APP, cerebral amyloid angiopathy (CAA), hereditary cerebral hemorrhage with amyloidosis Dutch type (HCHWAD), amyloid microangiopathy in the vascular variant of Alzheimer's disease, Abeta related angiitis (ABRA), fronto temporal dementias (FTD), Parkinson's disease and amyotrophic lateral sclerosis .

Also encompassed in the present invention is a method for treating and/or preventing a disease disclosed herein, in a patient in need thereof, comprising administering a

pharmaceutically effective amount of an isolated and/or purified antibody, antibody fragment or derivative thereof of the invention.

The present invention is also directed to an immune composition for the treatment and/or prevention of an APP-associated disease comprising at least one or several antibodies or antibody fragments of the invention, e.g. two or three antibodies or antibody fragments of the invention. In a further aspect, the present invention is also directed to an acid nucleic vaccine composition for the treatment and/or prevention of an APP-associated disease comprising at least one expression vector comprising at least one copy of the purified and isolated nucleic acid sequence of the invention, fragments thereof, molecular chimeras thereof, combinations thereof and/or variants thereof.

Further encompassed in the present invention is a kit for treating and/or preventing a disease disclosed supra, comprising a pharmaceutically effective amount of an isolated and/or purified antibody, antibody fragment or derivative thereof of the invention, optionally with reagents and/or instructions for use. Alternatively, or additionally, the kit may further include other materials desirable from a commercial and user standpoint, including buffers, diluents, filters, needles, and syringes.

Various references are cited throughout this Specification, each of which is incorporated herein by reference in its entirety.

The foregoing description will be more fully understood with reference to the following Examples. Such Examples, are, however, exemplary of methods of practising the present invention and are not intended to limit the scope of the invention.

EXAMPLES

Example 1

Plasmids. The coding regions of human APP-C99, APP-C83, APP-C59, APP-C50 (C-terminal numbering), Aβ1-60, Aβ1-70, Aβ1-80, Aβ1-90 (Aβ numbering) were amplified by PCR and cloned in the peT21b vector. One part of these fragments was generated with a methionine followed by a His-tag on their N-terminal extremities, giving the resulting constructs: Met-His-Aβ1-60, Met-His-Aβ1-70, Met-His-Aβ1-80, Met-His-Aβ1-90 (Aβ numbering). The other part of the fragments was generated with a methionine at their N-termini and a His-tag at their C-termini, giving the following constructs: Met-C89-His, Met-C59-His, Met-C50-His (Aβ numbering). All sequences were verified by DNA sequencing.

Expression and purification of recombinant proteins. The obtained vectors were transformed in BL21(DE3) *Escherichia Coli* bacteria. The transformed bacteria were then cultured in Luria Bertani medium supplied with ampicillin (100 µg/ml), with shaking at 37°C, until OD_{600nm} reached 0.8. Expression of recombinant proteins was then induced during 4 hours, by addition of 1mM isopropyl thiogalactoside (IPTG, Applichem). After 4 hours induction, bacteria were harvested during 1 hour by centrifugation at 5000 g. After lysis of bacterial pellets, and 3 centrifugation steps, recombinant proteins were purified using Ni-NTA agarose affinity resin (Invitrogen). All fragments were analyzed by SDS-PAGE followed by Coomassie blue staining (Invitrogen).

***In vitro* gamma-secretase activity assays.** *In vitro* gamma-secretase activity assays using the recombinant APP-C99 substrate and purified gamma-secretase were performed as previously reported (22). After 4 hours incubation at 37°C, the reaction was stopped by adding Laemmli buffer and the samples were analyzed by western blot for AICD-His.

Mice injection with purified and active human APP-C99. To generate the anti-APP-C99 monoclonal antibodies, 100 µg of recombinant and active APP-C99 supplied with 200 µg of poly I/C adjuvant were injected intraperitoneally in 8-week-old female BALB/c mice. Three injections were performed at 2 week-intervals. Four days prior hybridoma fusion, mice with positively reacting sera were reinjected. Spleen cells were fused with Sp2/0 Agl4 myeloma cells as described previously (23). Hybridoma culture supernatants were tested 10 days later by ELISA for cross-reaction with APP-C99 recombinant protein. Positive supernatants were then tested by Western blot. Specific cultures were cloned twice on soft agar. Twelve specific hybridomas were established. Subclass determination was performed using an isotyping kit

(Roche). For ascites fluid production, 2 x 10^6 cells of hybridomas with the highest antibody titers were injected into pristane primed BALB/c mice.

ELISA tests for epitopes determination. Plates were coated with 100 µl per well of the diluted antigens (2 µg/ml in PBS) and incubated at 4°C overnight, following what they were washed twice with PBS-tween (0.05% of tween 20). Non-specific binding was blocked by adding 200 µl of 1% gelatin/PBS. After 30 minutes incubation at room temperature, plates were washed twice with PBS-tween, and 100 µl of hybridomas supernatants were supplied to appropriate wells for 1 hour at room temperature. Washing steps were repeated and 100 µl of secondary antibody conjugated with Alkaline Phosphatase were subsequently incubated for 1 hour. After 2 washing steps, 100 µl of substrate (pNPP 1 mg/ml in 1M diethanolamine pH 9.8, 0.5 mM $MgCl_2$) was added and absorbances were read at 405-410 nm with an ELISA plate reader.

Luminescence T20-cell-based assay. T20 cells, a luciferase-based reporter system (24) (a gift from Dr. M. S. Wolfe) was used for measuring the processing of APP-C99 by gamma-secretase. T20 cells are stably overexpressing APP-C99 fused with Gal4-VP16 on its C-terminus and luciferase placed under the control of a Gal4 promoter. In presence of tetracycline (1 µg/ml), APP-C99 fused with Gal4-VP16 is expressed and cleaved by gamma-secretase. The AICD-Gal4-VP16 product is then translocated to the nucleus to activate the expression of luciferase, which generates the luminescence signal reporting APP-C99 processing by gamma-secretase. T20-cells were plated in 96-wells plates (Corning), incubated during 24 hours with 350 nM, 500 nM, and 750 nM of each purified monoclonal antibody. Their luminescence signal was subsequently measured with a Tecan Infinite M200 plate reader, using the Bright-Glo luciferase kit (Promega).

Cell lines culture. HEK cells stably expressing APPSwe (a gift from Dr. D. J. Selkoe), T20, HEK and HeLa cells were cultured in DMEM containing 10% FBS and Penicillin-Streptomycin (Invitrogen). For HEK APPSwe, medium was supplied with 150 µg/ml G418, for T20 cells with 50 µg/ml blasticidin, 200 µg/ml zeocin, and 500 µg/ml hygromycin.

Cells treatments and proteins extraction. Cells were treated for 24 hours at 37°C with 10 µM DAPT or different concentrations of each monoclonal antibody. Cells were then lysed with Hepes buffer pH7, containing NP40 1% and protease inhibitors (Roche). Protein extracts were quantified by BCA (Pierce) and analyzed by western-blot.

Immunoblotting and antibodies. Proteins extracts obtained from cells treatments were separated by SDS-PAGE or Native-SDS-PAGE and analyzed by western-blot with the following primary antibodies: rabbit monoclonal A8717 antibody (targeting the last 19 amino acids of human APP, Sigma), anti-β-actin antibody (Sigma), Val1744 anti-Notch cleavage site antibody (Cell signaling). Secondary goat anti-mouse and goat anti-rabbit 680 antibodies (Alexa fluor)

were used to probe the membrane and the fluorescence signal was detected with a Li-Cor Odyssey scanner.

Aβ peptides ELISA. Cultures media from HEKAPPSwe were collected and secreted Aβ peptides were quantified using human Aβ40 and Aβ42 ELISA kits from Invitrogen (KHB3482, KHB3544).

Immunoprecipitation. HEK APPSwe cells were lysed with CHAPSO 1% and proteins were extracted. Proteins extracts were then immunoprecipitated with proteins G beads (Invitrogen) and analyzed by immunoblotting.

Cell death assays. Cell death in HEK, HeLa, HEK APPSwe, N7 and T20 cells was assessed with the LDH CytoTox-one kit (Promega) as well as the crystal violet test. Briefly, cells were incubated 10 min at RT with 0.2% crystal violet solution. After 2 washing-steps, 1% SDS was added to solubilize the dye, and the absorbance was read at 570 nm.

Example 2

RESULTS

Mice immunization with active APP-C99 generates monoclonal antibodies targeting 2 major and 2 minor groups of epitopes.

To generate monoclonal antibodies directed against natively folded, active gamma-secretase substrate APP-C99, we first purified recombinant APP-C99 (Met-APP-C99-His) in the presence of phosphatidylcholine (PC) as a lipidic environment (Fig. 1A, top panel). This allowed the substrate to be processed in an activity assay using semi-purified gamma-secretase microsomes (22)(Fig. 1A, bottom panel). Next, this active APP-C99 was supplied with poly I/C adjuvant and intraperitoneally injected in BALB/c mice. Six weeks after several antigen injections, spleen cells from mice with positively reacting sera were fused with Sp2/0 Agl4 myeloma cells. After 10 days, ~400 hybridoma culture supernatants were tested by Enzyme-linked immunosorbent assay (ELISA), followed by western-blotting for cross-reaction with recombinant APP-C99. Selected cultures were cloned twice and 12 specific hybridomas were established. Purified monoclonal antibodies from hybridomas supernatants were further probed against recombinant APP-C99 and N- and C-terminal truncated APP-C99 fragments (Fig. 1B), by ELISA (Fig. 1C) or by immuno-blotting (Fig. 1D) to determine the binding region of each of them. All fragments including APP-C99 were protein normalized by silver stained SDS-PAGE and by western blot using an anti Histidine-tag antibody (Fig. 1D, Anti His-Tag). Among the selected monoclonal antibodies, 5 were directed against the N-terminal region of APP-C99. The 7 others antibodies were directed against the C-terminal region of APP-C99, with 5 of them targeting the region 70-

80 (mC99(70-80)), 1 binding the region 80-90 (mC99(80-90)) and 1 recognizing the region 90-99 (mC99(90-99), see Fig. 1C and 1D). When tested by ELISA against short synthetic peptides (1-7, 1-14, and 1-20, see Fig. 1E), the group of anti-N-terminal antibodies was found to bind all the aforementioned fragments. Thus, we concluded that the linear epitope of this group was 1-7. However, for C83 binding, this specific group showed almost no signal by western blot whereas it was positive by ELISA (compare mC99(1-7) for C83 on Fig. 1C and mC99(1-7) Fig. 1D). This difference can be attributed to ELISA conditions which are intrinsically more native than western blot conditions. Based on this observation, and to obtain such an ELISA profile, a putative hypothesis would be that aspartate residue 7 (D7) is in close vicinity with lysin 16 (K16) to form a structural epitope recognized by this group of antibodies (see arrow in Fig. 1F). For convenience, we attributed based on ELISA and western blot data, the epitope 1-7 to these 5 anti-N-terminal antibodies. Based on recent structural studies performed in experimental LMPG micelles (19) or simulated membrane environment (20), we next represented the epitopes for each group of antibodies on a hypothetical topology model of APP-C99 (Fig. 1F). The model comprises a long transmembrane helix (residues 30-52), and 2 small helical domains interacting with the membrane bilayer (the first one being either 13-22 or 19-22 depending on the hydrophobic environment of the protein (19, 20), and the second one being 90-99). As immunization with a protein generates monoclonal antibodies against its most immunogenic regions, likely to be the most accessible, hydrophilic and flexible (15-18), our data strongly support a model in which the most exposed regions of active APP-C99 embedded into the lipid bilayer contain the N- and C-terminal epitopes 1-7 and 70-80, respectively. Apparently, the epitopes 80-90 and 90-99 are less exposed and accessible regions as only one antibody has been identified per group. No antibodies have been found to target the putative natively folded, non-exposed 30-69 region that includes the transmembrane domain (TMD) of APP-C99.

Anti-APP-C99 monoclonal antibodies reduce gamma-secretase-dependent APP-C99 processing and alter Aβ production following internalization in human cell lines.

To investigate whether anti-APP-C99 monoclonal antibodies can modulate APP-C99 processing, the accumulation of APP-C-terminal fragments (APP-CTFs including APP-C99 and APP-C83) was probed in response to inhibited gamma-secretase processing (common characteristics among known γ-secretase substrates), in HEK and HeLa cells previously treated with 250 nM of each of the 12 antibodies during 24 hours. As shown in Fig. 2A, anti-APP-C99 antibodies treatments caused intracellular accumulation of APP-CTFs, when compared to non-treated, PBS-treated or cells treated with both IgG A or commercially available IgG mix control antibodies. For further

investigation of the clones, we selected 5 of them based on their epitope, their affinity, and the number of clones obtained per epitope: in the N-terminal group, one antibody was chosen (mC99(1-7)#4), 2 different clones were selected (mC99(70-80) #9 and mC99(70-80)#10, see Fig.2A) for the most frequent C-terminal epitope, and 1 antibody was kept for each of the remaining epitope (mC99(80-90)#11 and mC99(90-99)#12). Impairment of APP-C99 processing was dose-dependent as observed after 48 hours incubation with 350, 500, or 750 nM of each antibody in a previously described gamma-secretase-based luciferase reporter assay using HEK cells stably expressing APP-C99 fused to Gal4-VP16 (T20 cells, (24) see Fig. 2B). However, as the luminescence signal depends on AICD-Gal4-VP16 activating the expression of luciferase, one cannot exclude that reduced luminescence can be partly attributed to the binding of the C-terminal antibodies to AICD thus preventing it from reaching the luciferase promoter. mC99(1-7) antibody showed a significant luminescence reduction (40 ±2% at 750nM when compared to IgG A control), and recognizes only the N-terminal part of APP-C99. Next, the effect of anti-APP-C99 antibodies on APP-C99 processing by gamma-secretase was further validated by measuring Aβ secretion in HEK293T cells stably overexpressing human APP carrying the Swedish mutation (HEK APPSwe). As shown in Fig. 2C, all antibodies triggered a dose-dependent decrease of both Aβ40 and Aβ42 production in these cells. Except for mC99(70-80)#10 treatment, the C-terminal antibodies induced a significant decrease at 500 nM treatment (27.6 ±4.2% and 25 ±6.6% for Aβ40 and Aβ42, respectively) when compared to IgG A control (Fig. 2C bottom panel), without affecting the Aβ40/42 ratio. As mC99(1-7) targets the Aβ sequence of APP-C99, it also probably depletes the Aβ peptides contained in the medium and generates a 92 ±1% drop of Aβ40 and 42 levels when compared to control antibodies, at 500 nM treatment. Consistent with impaired gamma-secretase-dependent APP-C99 processing, dose-dependent accumulations of APP-CTFs were detected in these cells treated for 24 hours with anti-APP-C99 antibodies concentrations as low as 25–75 nM (Fig. 2C top panel and Fig. 3A), but not in cells incubated with IgGA, IgGmix, or a monoclonal antibody targeting the N-terminus of APP full-length (APP-FL) (22C11 against aa 66–81 of APP) (Fig. 3A and 3B). Interestingly, treatments with antibodies also triggered a dose-dependent accumulation of APP full-length (APP-FL, Fig. 2C, top panel). This was in contrast to DAPT, a well-known gamma-secretase inhibitor, supporting APP-FL/APP-CTFs as specific intracellular targets. To further demonstrate the specificity of the antibodies for APP-C99, we next investigated their effects on Notch intracellular domain (NICD) production in HEK cells stably expressing human Notch ΔE (N7 cells,(24)). In contrast to DAPT, incubation with these antibodies did not affect Notch

processing (Fig. 2D). Importantly, detailed toxicity assays revealed that antibodies treatments did not trigger significant cell death (Fig. 3B).
In an effort to demonstrate a direct interaction between the antibodies and their cellular APP-based target(s), protein extracts from HEK APPSwe incubated for 24 hours with 250 nM of anti-N-terminal (epitope 1-7), anti-C-terminal (epitope 70-80) antibodies or IgG A control or PBS, were immunoprecipated with protein G beads (with high affinity for IgG antibodies) and probed on western blot with an anti-APP-FL commercially available C-terminal antibody (A8717). Results revealed that both anti-N-terminal and anti-C-terminal APP-C99 antibodies bound and immunoprecipitated both APP-CTFs and APP-FL, in contrast to the IgG A control (Fig. 2E).
To get new insights into the mechanisms by which anti-APP-C99 monoclonal antibodies affect both APP-C99 processing and Aβ production, and in particular those targeting its intra-cytoplasmic C-terminal part, we assessed whether mC99(1-7) and mC99(70-80) antibodies were internalized by cells to recognize the N or C-terminal part of their target. Immunofluorescence and electron microscopy analyses demonstrated (i) that both types of antibodies were internalized in cells, (ii) where they partially colocalized with their APP-based targets, and (iii) that they were present within vesicular structures of the late endosomal compartement (data not shown)

Taken together, these results show that monoclonal antibodies targeting the N- and C-terminal regions of APP-C99 (i) are internalized by cells and bind to their APP-based target, (ii) lower specifically the processing of APP-C99 by gamma-secretase, and (iii) decrease Aβ production, presumably by preventing the substrate binding and/or cleavage by gamma-secretase, due to steric hindrance.

Example 3

Confirmation of a structural epitope targeted by the mC99(1-7) antibodies.

For epitope determination, the monoclonal antibodies were probed by ELISA and by immunoblotting against recombinant N- and C-terminal truncated APP-C99 fragments (Fig. 1B). Interestingly, and although ELISA unequivocally revealed C83 binding (Fig. 1C), the mC99(1-7) antibodies barely detected the same protein under denaturing conditions by Western blot (Fig. 1D). This suggests the existence of a structural epitope on APP-C99, in which the asparate 7 (D7) is in close vicinity with lysin 16 (K16) and its adjacent residues (arrow in Fig. 1F). That observation was further supported by Native-PAGE immunoblotting revealing that both APP-

C83 and -C99 were immunoreactive for mC99(1-7), while the 6E10 antibody (epitope 1-16 of Aβ) only bound APP-C99, and A8717 (targeting the last 19 residues of human APP) recognized both APP-C83 and -C99 (Fig. 1E bottom panel).

Example 4

mC99(1-7) and mC99(70-80) antibodies bind to their corresponding epitopes in Alzheimer brain tissue sections.

mC99(1-7) and mC99(70-80) antibodies were tested for detection of their corresponding epitopes in brain sections from Aβ-containing Alzheimer brain slices. Strong immunolabeling of Aβ plaques was achieved with mC99(1-7)). In contrast, no staining of the congophilic Aβ plaques could be detected with mC99(70-80), which instead decorated the periphery of Congo Red-positive deposits as dense granular puncta or more elongated filaments likely corresponding to dystrophic neurites (data not shown). Therefore, these antibodies could potentially disrupt Aβ-amyloid deposits.

Example 5

Anti-APP-C99 monoclonal antibodies affect specifically the gamma-secretase cleavage.

Consistent with impaired gamma-secretase-dependent APP-C99 processing, dose-dependent accumulations of APP-CTFs were detected in these cells treated for 24 hours with anti-APP-C99 antibody concentrations as low as 25-75 nM (Fig. 2C and 3A), but not in cells incubated with IgGA, IgGmix, or a mAb targeting the N-terminus of APP-FL (22C11 against aa 66-81 of APP) (Fig. 3B). Importantly, the processing of Notch was not impaired by anti-APP-C99 antibodies, thus underlying their specificity of action towards the APP substrates (Fig. 2D).

SEQUENCES

The different chains and CDRs of the antibodies were determined with NCBI Ig blast (IMGT®, the international ImMunoGeneTics information system).

SEQ ID No	**Table 1**	
1	DAEFRHDSG YEVHHQKLVF FAEDVGSNKG AIIGLMVGGV VIATVIVITL VMLKKKQYTS IHHGVVEVDA AVTPEERHLS KMQQNGYENP TYKFFEQMQN	**APP-C99**
2	DAEFRHD	Linear Epitope 1-7
3	AVTPEERHLSK	Epitope 70-80
4	KMQQNGYENPT	Epitope 80-90
5	TYKFFEQMQN	Epitope 90-99
	Table 2	**VH chain (CDR)**
6	GFDFRRYW	Clone 2C5 CDR1-VH
7	INPDSSTI	Clone 2C5 CDR2-VH
56	A	Clone 2C5 CDR3-VH
8	GFSLSTSGMN	Clone 1D8 CDR1-VH
9	IWWDDDK	Clone 1D8 CDR2-VH
57	AR	Clone 1D8 CDR3-VH
10	GFAFSRYA	Clone 2F9 CDR1-VH
11	ISNGGSYT	Clone 2F9 CDR2-VH
58	AR	Clone 2F9 CDR3-VH

12	GYPFSSYW	Clone 1H9 CDR1-VH
13	ILPGSGST	Clone 1H9 CDR2-VH
59	AS	Clone 1H9 CDR3-VH
14	GFTFSRYA	Clone 1H10 CDR1-VH
15	ISSGGSYT	Clone 1H10 CDR2-VH
60	AR	Clone 1H10 CDR3-VH

	Table 3	**VL chain (CDR)**
16	QSLLDSDGKTY	Clone 2C5 CDR1-VL
61	LVS	Clone 2C5 CDR2-VL
17	WQGTHIP	Clone 2C5 CDR3-VL
18	QSIVHSNGNTY	Clone 1D8 CDR1-VL
62	KVS	Clone 1D8 CDR2-VL
19	FQGSRVP	Clone 1D8 CDR3-VL
20	QSVDYDGNSY	Clone 2F9 CDR1-VL
63	AAS	Clone 2F9 CDR2-VL
21	QQSNENP	Clone 2F9 CDR3-VL
22	QSLLDSGGKTY	Clone 1H9 CDR1-VL
64	LVS	Clone 1H9 CDR2-VL
23	WQGTHFP	Clone 1H9 CDR3-VL

24	QSVDFDGVSY	Clone 1H10 CDR1-VL
65	AAS	Clone 1H10 CDR2-VL
25	QQTNENP	Clone 1H10 CDR3-VL

	Table 4	
26	MDFGLIFFIVALLKGVQCEVKLLESGGGLVQPGGSLKLSCAASGFDFRRYWM SWVRQAPG KGLEWIGEINPDSSTINFTPSLKDKFIISRDNAKNTLYLQMSKVRSEDTALY YCATYYPY WGQGTSVTVSS	2C5 gamma-VH PROT
27	MMSPAQFLFLLVLWIRDTNGDVVMTQTPFTLSVTIGQPASISCKSSQSLLDS DGKTYLKW LLQRPGQSPKRLIYLVSKLDSGVPDRFTGSGSGTDFTLKISRVEAEDLGVYY CWQGTHIP YTFGGGTKLEIK	2C5 kappa-VL PROT
28	MDRLTSSFLLLIVPAYVLSQVTLKESGPGILKPSQTLSLTCSFSGFSLSTSG MNVGWIRQ PSGKGLEWLAHIWWDDDKFYNPSLKSQLTISKDTSRNQVFLKITNVDSSDTA TYYCARRT SGNSDFFDYWGQGTTLTVSS	1D8 gamma-VH PROT
29	MKLPVRLLVLMFWIPVSRSDVLMTQSPLSLPVSLGDQASISCRSSQSIVHSN GNTYLEWY LQKSGQSPKGLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYC FQGSRVPL TFGAGTKLELK	1D8 kappa-VL PROT
30	MNFVLSLIFLALILKGVQCEVQLVESGGGLVKPGGSLKLSCAASGFAFSRYA MSWVRQTP EKRLEWVATISNGGSYTYYPDRVKGRFTISRDNAKNTLYLQMSNLRSEDTAK YYCARRDY GSRDYVMDYWGQGTSVTVSS	2F9 gamma-VH PROT
31	METDTILLWVLLLWVPGSTGDIVLTQSPASLAVSLGQRATISCKASQSVDYD GNSYMNWY QQKPGQPLKLLIYAASNLESGIPARFSGSGSGTDFTLNIHPVEEEDAATYYC QQSNENPT FGGGTKLELK	2F9 kappa-VL PROT
32	MEWTWVFLFLLSVTAGVHSQVQVQQSGAELMKPGASVKMSCKASGYPFSSYW IDWVKQRP GHGLEWIGEILPGSGSTNYNEKFKGKATFTADTSSNTAYMQLSSLTSEDSAV YFCASGPY WGQGTTLTVSS	1H9-gamma-VH PROT
33	MMSPAQFLFLLVLWIRETNGDVVMTQTPLTLSVTIGQPASISCKSSQSLLDS GGKTYLIW LLQRPGQSPKRLIYLVSKLDSGVPDRFTGSGSGTDFTLKISRVEAEDLGAYY CWQGTHFP	1H9-kappa-VL PROT

	YTFGGGTKLEIK	
34	MNFVLSLIFLALILKGVQCEVQLVESGGGLVKPGGSLKLSCAASGFTFSRYA MSWVRQTP EKRLEWVATISSGGSYTYYPDSVKGRFTISRDNAKNTLYLRMSSLRSEDTAM YYCARRDY GSRDYVMDYWGHGTSVTVSS	1H10gamma-VH PROT
35	METDTILLWVLLLWVPGSTGDIVLTQSPASLAVSLGQRATISCKASQSVDFD GVSYMNWY QQKPGQPPKLLIYAASNLESGIPARFSGIGSGTDFTLNIHPVEEEDAATYYC QQTNENPT FGGGTKLEIK	1H10-kappa-VL PROT
36	MDFGLIFFIVALLKGVQCEVKLLESGGGLVQPGGSLKLSCAASGFDFRRYWM SWVRQAPG KGLEWIGEINPDSSTINFTPSLKDKFIISRDNAKNTLYLQMSKVRSEDTALY YCATYYPY WGQGTSVTVSSAKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVTW NSGSLSSG VHTFPAVLQSDLYTLSSSVTVPSSTWPSETVTCNVAHPASSTKVDKKIVPRD CGCKPCIC TVPEVSSVFIFPPKPKDVLTITLTPKVTCVVVDISKDDPEVQFSWFVDDVEV HTAQTQPR EEQFNSTFRSVSELPIMHQDWLNGKEFKCRVNSAAFPAPIEKTISKTKGRPK APQVYTIP PPKEQMAKDKVSLTCMITDFFPEDITVEWQWNGQPAENYKNTQPIMDTDGSY FVYSKLNV QKSNWEAGNTFTCSVLHEGLHNHHTEKSLSHSPGK	2C5 PROT gamma FULL
37	ATGGATTTTGGGCTGATTTTTTTTATTGTTGCTCTTTTAAAAGGGGTCCAGT GTGAGGTGAAGCTTCTCGAGTCTGGAGGTGGCCTGGTGCAGCCTGGAGGATC CCTGAAACTCTCCTGTGCAGCCTCAGGATTCGACTTTAGAAGATACTGGATG AGTTGGGTCCGGCAGGCTCCAGGGAAAGGGCTAGAATGGATTGGAGAAATTA ATCCAGATAGCAGTACGATAAACTTTACGCCATCTCTAAAGGATAAATTCAT CATCTCCAGAGACAACGCCAAAAATACGCTGTACCTGCAAATGAGCAAAGTG AGATCTGAGGACACAGCCCTTTATTACTGTGCAACTTACTACCCTTACTGGG GTCAAGGAACCTCAGTCACCGTCTCCTCAGCCAAAACGACACCCCCATCTGT CTATCCACTGGCCCCTGGATCTGCTGCCCAAACTAACTCCATGGTGACCCTG GGATGCCTGGTCAAGGGCTATTTCCCTGAGCCAGTGACAGTGACCTGGAACT CTGGATCCCTGTCCAGCGGTGTGCACACCTTCCCAGCTGTCCTGCAGTCTGA CCTCTACACTCTGAGCAGCTCAGTGACTGTCCCCTCCAGCACCTGGCCCAGC GAGACCGTCACCTGCAACGTTGCCCACCCGGCCAGCAGCACCAAGGTGGACA AGAAAATTGTGCCCAGGGATTGTGGTTGTAAGCCTTGCATATGTACAGTCCC AGAAGTATCATCTGTCTTCATCTTCCCCCCAAAGCCCAAGGATGTGCTCACC ATTACTCTGACTCCTAAGGTCACGTGTGTTGTGGTAGACATCAGCAAGGACG ATCCCGAGGTCCAGTTCAGCTGGTTTGTAGATGATGTGGAGGTGCACACAGC TCAGACGCAACCCCGGGAGGAGCAGTTCAACAGCACTTTCCGCTCAGTCAGT GAACTTCCCATCATGCACCAGGACTGGCTCAATGGCAAGGAGTTCAAATGCA GGGTCAACAGTGCAGCTTTCCCTGCCCCCATCGAGAAAACCATCTCCAAAAC CAAAGGCAGACCGAAGGCTCCACAGGTGTACACCATTCCACCTCCCAAGGAG CAGATGGCCAAGGATAAAGTCAGTCTGACCTGCATGATAACAGACTTCTTCC CTGAAGACATTACTGTGGAGTGGCAGTGGAATGGGCAGCCAGCGGAGAACTA CAAGAACACTCAGCCCATCATGGACACAGATGGCTCTTACTTCGTCTACAGC AAGCTCAATGTGCAGAAGAGCAACTGGGAGGCAGGAAATACTTTCACCTGCT CTGTGTTACATGAGGGCCTGCACAACCACCATACTGAGAAGAGCCTCTCCCA CTCTCCTGGTAAATGA	2C5 DNA gamma FULL
38	MMSPAQFLFLLVLWIRDTNGDVVMTQTPFTLSVTIGQPASISCKSSQSLLDS DGKTYLKW LLQRPGQSPKRLIYLVSKLDSGVPDRFTGSGSGTDFTLKISRVEAEDLGVYY CWQGTHIP YTFGGGTKLEIKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVK	2C5 PROT kappa FULL

	WKIDGSER QNGVLNSWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEATHKTSTSPIVK SFNRNEC	
39	ATGATGAGTCCTGCCCAGTTCCTGTTTCTGTTAGTGCTCTGGATTCGGGACA CCAACGGT GATGTTGTGATGACCCAGACTCCATTCACTTTGTCGGTTACCATTGGACAAC CAGCCTCC ATCTCTTGCAAGTCAAGTCAGAGCCTCTTAGATAGTGATGGAAAGACATATT TGAAATGG TTGTTACAGAGGCCAGGCCAGTCTCCAAAGCGCCTAATCTATCTGGTGTCTA AACTGGAC TCTGGAGTCCCTGACAGGTTCACTGGCAGTGGATCAGGGACAGATTTCACAC TGAAAATC AGCAGAGTGGAGGCTGAGGATTTGGGAGTTTATTATTGCTGGCAAGGTACAC ATATTCCG TACACGTTCGGAGGGGGGACCAAGCTGGAAATAAAACGGGCTGATGCTGCAC CAACTGTA TCCATCTTCCCACCATCCAGTGAGCAGTTAACATCTGGAGGTGCCTCAGTCG TGTGCTTC TTGAACAACTTCTACCCCAAAGACATCAATGTCAAGTGGAAGATTGATGGCA GTGAACGA CAAAATGGCGTCCTGAACAGTTGGACTGATCAGGACAGCAAAGACAGCACCT ACAGCATG AGCAGCACCCTCACGTTGACCAAGGACGAGTATGAACGACATAACAGCTATA CCTGTGAG GCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATG AGTGTTAG	2C5 DNA kappa FULL
40	MDRLTSSFLLLIVPAYVLSQVTLKESGPGILKPSQTLSLTCSFSGFSLSTSG MNVGWIRQ PSGKGLEWLAHIWWDDDKFYNPSLKSQLTISKDTSRNQVFLKITNVDSSDTA TYYCARRT SGNSDFFDYWGQGTTLTVSSAKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGY FPEPVTVT WNSGSLSSGVHTFPAVLQSDLYTLSSSVTVPSSTWPSETVTCNVAHPASSTK VDKKIVPR DCGCKPCICTVPEVSSVFIFPPKPKDVLTITLTPKVTCVVVDISKDDPEVQF SWFVDDVE VHTAQTQPREEQFNSTFRSVSELPIMHQDWLNGKEFKCRVNSAAFPAPIEKT ISKTKGRP KAPQVYTIPPPKEQMAKDKVSLTCMITDFFPEDITVEWQWNGQPAENYKNTQ PIMDTDGS YFVYSKLNVQKSNWEAGNTFTCSVLHEGLHNHHTEKSLSHSPGK	1D8 PROT gamma FULL
41	ATGGACAGGCTTACTTCTTCATTCCTGCTGCTGATTGTCCCTGCATATGTCT TGTCCCAAGTTACTCTAAAAGAGTCTGGCCCTGGGATATTGAAGCCCTCACA GACCCTCAGTCTGACTTGTTCTTTCTCTGGGTTTTCACTGAGCACTTCTGGT ATGAATGTAGGCTGGATTCGACAGCCTTCAGGGAAGGGTCTGGAGTGGCTGG CACACATTTGGTGGGATGATGATAAGTTCTATAACCCATCCCTGAAGAGCCA GCTCACAATCTCCAAGGATACCTCCAGAAACCAGGTATTCCTCAAGATCACC AATGTAGACTCTTCAGATACTGCCACTTACTACTGTGCTCGAAGAACTTCCG GTAACTCCGACTTCTTTGACTACTGGGGCCAAGGCACCACTCTCACAGTCTC CTCAGCCAAAACGACACCCCCATCTGTCTATCCACTGGCCCCTGGATCTGCT GCCCAAACTAACTCCATGGTGACCCTGGGATGCCTGGTCAAGGGCTATTTCC CTGAGCCAGTGACAGTGACCTGGAACTCTGGATCCCTGTCCAGCGGTGTGCA CACCTTCCCAGCTGTCCTGCAGTCTGACCTCTACACTCTGAGCAGCTCAGTG ACTGTCCCCTCCAGCACCTGGCCCAGCGAGACCGTCACCTGCAACGTTGCCC ACCCGGCCAGCAGCACCAAGGTGGACAAGAAAATTGTGCCCAGGGATTGTGG CTGTAAGCCTTGCATATGTACAGTCCCAGAAGTATCATCTGTCTTCATCTTC CCCCCAAAGCCCAAGGATGTGCTCACCATTACTCTGACTCCTAAGGTCACGT GTGTTGTGGTAGACATCAGCAAGGATGATCCCGAGGTCCAGTTCAGCTGGTT	1D8 DNA gamma FULL

	TGTAGATGATGTGGAGGTGCACACAGCTCAGACGCAACCCCGGGAGGAGCAG TTCAACAGCACTTTCCGCTCAGTCAGTGAACTTCCCATCATGCACCAGGACT GGCTCAATGGCAAGGAGTTCAAATGCAGGGTCAACAGTGCAGCTTTCCCTGC CCCCATCGAGAAAACCATCTCCAAAACCAAAGGCAGACCGAAGGCTCCACAG GTGTACACCATTCCACCTCCCAAGGAGCAGATGGCCAAGGATAAAGTCAGTC TGACCTGCATGATAACAGACTTCTTCCCTGAAGACATTACTGTGGAGTGGCA GTGGAATGGGCAGCCAGCGGAGAACTACAAGAACACTCAGCCCATCATGGAC ACAGATGGCTCTTACTTCGTCTACAGCAAGCTCAATGTGCAGAAGAGCAACT GGGAGGCAGGAAATACTTTCACCTGCTCTGTGTTACATGAGGGCCTGCACAA CCACCATACTGAGAAGAGCCTCTCCCACTCTCCTGGTAAATGA	
42	MKLPVRLLVLMFWIPVSRSDVLMTQSPLSLPVSLGDQASISCRSSQSIVHSN GNTYLEWY LQKSGQSPKGLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYC FQGSRVPL TFGAGTKLELKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKW KIDGSERQ NGVLNSWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEATHKTSTSPIVKS FNRNEC	1D8 PROT kappa FULL
43	ATGAAGTTGCCTGTTAGGCTGTTGGTGCTGATGTTCTGGATTCCTGTTTCCA GAAGTGATGTTTTGATGACCCAAAGTCCACTCTCCCTGCCTGTCAGTCTTGG AGATCAAGCCTCCATCTCTTGCAGATCAAGTCAGAGCATTGTACATAGTAAT GGAAACACCTATTTAGAATGGTACCTGCAGAAATCAGGCCAGTCTCCAAAGG GCCTGATCTACAAAGTTTCCAACCGATTTTCCGGGGTCCCAGACAGGTTCAG TGGCAGTGGATCAGGGACAGATTTCACACTCAAGATCAGCAGAGTGGAGGCT GAGGATCTGGGAGTTTATTACTGCTTTCAAGGTTCACGTGTTCCGCTCACGT TCGGTGCTGGGACCAAGCTGGAGCTGAAACGGGCTGATGCTGCACCAACTGT ATCCATCTTCCCACCATCCAGTGAGCAGTTAACATCTGGAGGTGCCTCAGTC GTGTGCTTCTTGAACAACTTCTACCCCAAAGACATCAATGTCAAGTGGAAGA TTGATGGCAGTGAACGACAAAATGGCGTCCTGAACAGTTGGACTGATCAGGA CAGCAAAGACAGCACCTACAGCATGAGCAGCACCCTCACGTTGACCAAGGAC GAGTATGAACGACATAACAGCTATACCTGTGAGGCCACTCACAAGACATCAA CTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTGTTAG	1D8 DNA kappa FULL
44	MNFVLSLIFLALILKGVQCEVQLVESGGGLVKPGGSLKLSCAASGFAFSRYA MSWVRQTP EKRLEWVATISNGGSYTYYPDRVKGRFTISRDNAKNTLYLQMSNLRSEDTAK YYCARRDY GSRDYVMDYWGQGTSVTVSSAKTTPPSVYPLAPGCGDTTGSSVTLGCLVKGY FPESVTVT WNSGSLSSSVHTFPALLQSGLYTMSSSVTVPSSTWPSQTVTCSVAHPASSTT VDKKLEPS GPISTINPCPPCKECHKCPAPNLEGGPSVFIFPPNIKDVLMISLTPKVTCVV VDVSEDDP DVQISWFVNNVEVHTAQTQTHREDYNSTIRVVSTLPIQHQDWMSGKEFKCKV NNKDLPSP IERTISKIKGLVRAPQVYILPPPAEQLSRKDVSLTCLVVGFNPGDISVEWTS NGHTEENY KDTAPVLDSDGSYFIYSKLNMKTSKWEKTDSFSCNVRHEGLKNYYLKKTISR SPGK	2F9 PROT gamma FULL
45	ATGAACTTTGTGCTCAGCTTGATTTTCCTTGCCCTCATTTTAAAAGGTGTCC AGTGTGAAGTGCAGCTGGTGGAGTCTGGGGGAGGCTTAGTGAAGCCTGGAGG GTCCCTGAAACTCTCCTGTGCAGCCTCTGGATTCGCTTTCAGTAGATATGCC ATGTCTTGGGTTCGCCAGACTCCGGAGAAGAGGCTGGAGTGGGTCGCAACCA TTAGTAATGGTGGTAGTTACACTTATTATCCAGACCGTGTGAAGGGTCGATT CACCATCTCCAGAGACAATGCCAAGAACACCCTATACCTGCAAATGAGCAAT CTGAGGTCTGAGGACACGGCCAAGTATTACTGTGCAAGACGGGACTACGGTA GTAGAGACTATGTTATGGACTACTGGGGTCAAGGAACCTCAGTCACCGTCTC CTCAGCCAAAACAACACCCCCATCAGTCTATCCACTGGCCCCTGGGTGTGGA GATACAACTGGTTCCTCCGTGACTCTGGGATGCCTGGTCAAGGGCTACTTCC CTGAGTCAGTGACTGTGACTTGGAACTCTGGATCCCTGTCCAGCAGTGTGCA	2F9 DNA gamma FULL

	CACCTTCCCAGCTCTCCTGCAGTCTGGACTCTACACTATGAGCAGCTCAGTG ACTGTCCCCTCCAGCACCTGGCCAAGTCAGACCGTCACCTGCAGCGTTGCTC ACCCAGCCAGCAGCACCACGGTGGACAAAAAACTTGAGCCCAGCGGGCCCAT TTCAACAATCAACCCCTGTCCTCCATGCAAGGAGTGTCACAAATGCCCAGCT CCTAACCTCGAGGGTGGACCATCCGTCTTCATCTTCCCTCCAAATATCAAGG ATGTACTCATGATCTCCCTGACACCCAAGGTCACGTGTGTGGTGGTGGATGT GAGCGAGGATGACCCAGACGTCCAGATCAGCTGGTTTGTGAACAACGTGGAA GTACACACAGCTCAGACACAAACCCATAGAGAGGATTACAACAGTACTATCC GGGTGGTCAGCACCCTCCCCATCCAGCACCAGGACTGGATGAGTGGCAAGGA GTTCAAATGCAAGGTCAACAACAAAGACCTCCCATCACCCATCGAGAGAACC ATCTCAAAAATTAAAGGGCTAGTCAGAGCTCCACAAGTATACATCTTGCCGC CACCAGCAGAGCAGTTGTCCAGGAAAGATGTCAGTCTCACTTGCCTGGTCGT GGGCTTCAACCCTGGAGACATCAGTGTGGAGTGGACCAGCAATGGGCATACA GAGGAGAACTACAAGGACACCGCACCAGTCCTGGACTCTGACGGTTCTTACT TCATATATAGCAAGCTCAATATGAAAACAAGCAAGTGGGAGAAAACAGATTC CTTCTCATGCAACGTGAGACACGAGGGTCTGAAAAATTACTACCTGAAGAAG ACCATCTCCCGGTCTCCGGGTAAATGA	
46	METDTILLWVLLLWVPGSTGDIVLTQSPASLAVSLGQRATISCKASQSVDYD GNSYMNWY QQKPGQPLKLLIYAASNLESGIPARFSGSGSGTDFTLNIHPVEEEDAATYYC QQSNENPT FGGGTKLELKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWK IDGSERQN GVLNSWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEATHKTSTSPIVKSF NRNEC	2F9 PROT kappa FULL
47	ATGGAGACAGACACAATCCTGCTATGGGTGCTGCTGCTCTGGGTTCCAGGCT CCACTGGTGACATTGTGCTGACCCAATCTCCAGCTTCTTTGGCTGTGTCTCT AGGGCAGAGGGCCACCATCTCCTGCAAGGCCAGCCAAAGTGTTGATTATGAT GGTAATAGTTATATGAACTGGTACCAACAGAAACCAGGACAGCCACTCAAAC TCCTCATCTATGCTGCATCCAATCTAGAATCTGGAATCCCAGCCAGGTTTAG TGGCAGTGGGTCTGGGACAGACTTCACCCTCAACATCCATCCTGTGGAGGAG GAGGATGCTGCAACCTATTACTGTCAGCAAAGTAATGAAAATCCCACGTTCG GAGGGGGGACCAAGCTGGAATTAAAACGGGCTGATGCTGCACCAACTGTATC CATCTTCCCACCATCCAGTGAGCAGTTAACATCTGGAGGTGCCTCAGTCGTG TGCTTCTTGAACAACTTCTACCCCAAAGACATCAATGTCAAGTGGAAGATTG ATGGCAGTGAACGACAAAATGGCGTCCTGAACAGTTGGACTGATCAGGACAG CAAAGACAGCACCTACAGCATGAGCAGCACCCTCACGTTGACCAAGGACGAG TATGAACGACATAACAGCTATACCTGTGAGGCCACTCACAAGACATCAACTT CACCCATTGTCAAGAGCTTCAACAGGAATGAGTGTTAG	2F9 DNA kappa FULL
48	MEWTWVFLFLLSVTAGVHSQVQVQQSGAELMKPGASVKMSCKASGYPFSSYW IDWVKQRP GHGLEWIGEILPGSGSTNYNEKFKGKATFTADTSSNTAYMQLSSLTSEDSAV YFCASGPY WGQGTTLTVSSAKTTAPSVYPLAPVCGDTTGSSVTLGCLVKGYFPEPVTLTW NSGSLSSG VHTFPAVLQSDLYTLSSSVTVTSSTWPSQSITCNVAHPASSTKVDKKIEPRG PTIKPCPP CKCPAPNLLGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPDVQISWF VNNVEVHT AQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFKCKVNNKDLPAPIERTISK PKGSVRAP QVYVLPPPEEEMTKKQVTLTCMVTDFMPEDIYVEWTNNGKTELNYKNTEPVL DSDGSYFM YSKLRVEKKNWVERNSYSCSVVHEGLHNHHTTKSFSRTPGLDLDDVCAEAQD GELDGLWT TITIFISLFLLSVCYSASVTLFKVKWIFSSVVELKQTISPDYRNMIGQGA	1H9 PROT gamma FULL
49	ATGGAATGGACCTGGGTCTTTCTCTTCCTCCTGTCAGTAACTGCAGGTGTCC ACTCCCAGGTTCAGGTGCAGCAGTCTGGAGCTGAGCTGATGAAACCTGGGGC	1H9 DNA gamma FULL

	CTCAGTGAAGATGTCCTGCAAGGCTAGTGGCTACCCATTCAGTAGTTACTGG ATAGACTGGGTAAAGCAGAGGCCTGGCCATGGCCTTGAGTGGATTGGAGAGA TTTTACCTGGAAGTGGTAGTACTAACTACAATGAGAAGTTCAAGGGCAAGGC CACATTCACTGCAGATACATCCTCCAACACAGCCTACATGCAACTCAGCAGC CTGACATCTGAGGACTCTGCCGTCTATTTCTGTGCAAGCGGCCCCTACTGGG GCCAAGGCACCACTCTCACAGTCTCCTCAGCCAAAACAACAGCCCCATCGGT CTATCCACTGGCCCCTGTGTGTGGAGATACAACTGGCTCCTCGGTGACTCTA GGATGCCTGGTCAAGGGTTATTTCCCTGAGCCAGTGACCTTGACCTGGAACT CTGGATCCCTGTCCAGTGGTGTGCACACCTTCCCAGCTGTCCTGCAGTCTGA CCTCTACACCCTCAGCAGCTCAGTGACTGTAACCTCGAGCACCTGGCCCAGC CAGTCCATCACCTGCAATGTGGCCCACCCGGCAAGCAGCACCAAGGTGGACA AGAAAATTGAGCCCAGAGGGCCCACAATCAAGCCCTGTCCTCCATGCAAATG CCCAGCACCTAACCTCTTGGGTGGACCATCCGTCTTCATCTTCCCTCCAAAG ATCAAGGATGTACTCATGATCTCCCTGAGCCCCATAGTCACATGTGTGGTGG TGGATGTGAGCGAGGATGACCCAGATGTCCAGATCAGCTGGTTTGTGAACAA CGTGGAAGTACACACAGCTCAGACACAAACCCATAGAGAGGATTACAACAGT ACTCTCCGGGTGGTCAGTGCCCTCCCCATCCAGCACCAGGACTGGATGAGTG GCAAGGAGTTCAAATGCAAGGTCAACAACAAAGACCTCCCAGCGCCCATCGA GAGAACCATCTCAAAACCCAAAGGGTCAGTAAGAGCTCCACAGGTATATGTC TTGCCTCCACCAGAAGAAGAGATGACTAAGAAACAGGTCACTCTGACCTGCA TGGTCACAGACTTCATGCCTGAAGACATTTACGTGGAGTGGACCAACAACGG GAAAACAGAGCTAAACTACAAGAACACTGAACCAGTCCTGGACTCTGATGGT TCTTACTTCATGTACAGCAAGCTGAGAGTGGAAAAGAAGAACTGGGTGGAAA GAAATAGCTACTCCTGTTCAGTGGTCCACGAGGGTCTGCACAATCACCACAC GACTAAGAGCTTCTCCCGGACTCCGGGGCTAGACCTGGATGATGTCTGTGCT GAGGCCCAGGACGGGGAGCTGGACGGCCTCTGGACGACCATCACCATCTTCA TCAGCCTCTTCCTGCTCAGCGTGTGCTACAGCGCCTCTGTCACACTCTTCAA GGTAAAGTGGATCTTCTCCTCTGTGGTGGAGCTGAAGCAGACGATCTCCCCT GACTACAGAAACATGATTGGGCAGGGAGCCTAG	
50	MMSPAQFLFLLVLWIRETNGDVVMTQTPLTLSVTIGQPASISCKSSQSLLDS GGKTYLIW LLQRPGQSPKRLIYLVSKLDSGVPDRFTGSGSGTDFTLKISRVEAEDLGAYY CWQGTHFP YTFGGGTKLEIKRADAAPAVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVK WKIDGSER QNGVLNSWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEAAHKTSTSPIVK SFNRNEC	1H9 PROT kappa FULL
51	ATGATGAGTCCTGCCCAGTTCCTGTTTCTGTTAGTGCTCTGGATTCGGGAAA CCAACGGTGATGTTGTGATGACCCAGACTCCACTCACTTTGTCGGTTACCAT TGGACAACCAGCCTCCATCTCTTGCAAGTCAAGTCAGAGTCTCTTAGATAGT GGTGGAAAGACATATTTGATTTGGTTGTTACAGAGGCCAGGCCAGTCTCCAA AGCGCCTAATCTATCTGGTGTCTAAACTGGACTCTGGAGTCCCTGACAGGTT CACTGGCAGTGGATCAGGGACAGATTTCACACTGAAAATCAGCAGAGTGGAG GCTGAGGATTTGGGAGCTTATTATTGCTGGCAAGGTACACATTTTCCGTACA CGTTCGGAGGGGGGACCAAGCTGGAAATAAAACGGGCTGATGCTGCACCAGC TGTATCCATCTTCCCACCATCCAGTGAGCAGTTAACATCTGGAGGTGCCTCA GTCGTGTGCTTCTTGAACAACTTCTACCCCAAAGACATCAATGTCAAGTGGA AGATTGATGGCAGTGAACGACAAAATGGCGTCCTGAACAGTTGGACTGATCA GGACAGCAAAGACAGCACCTACAGCATGAGCAGCACCCTCACGTTGACCAAG GACGAGTATGAACGACATAACAGCTATACCTGTGAGGCCGCTCACAAGACAT CAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTGTTAG	1H9 DNA kappa FULL
52	MNFVLSLIFLALILKGVQCEVQLVESGGGLVKPGGSLKLSCAASGFTFSRYA MSWVRQTP EKRLEWVATISSGGSYTYYPDSVKGRFTISRDNAKNTLYLRMSSLRSEDTAM YYCARRDY GSRDYVMDYWGHGTSVTVSSAKTTAPSVYPLAPVCGDTTGSSVTLGCLVKGY FPEPVTLT WNSGSLSSGVHTFPAVLQSDLYTLSSSVTVTSSTWPSQSITCNVAHPASSTK	1H10 PROT gamma FULL

	VDKKIEPR GPTIKPCPPCKCPAPNLLGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSED DPDVQISW FVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFKCKVNNKDLP APIERTIS KPKGSVRAPQVYVLPPPEEEMTKKQVTLTCMVTDFMPEDIYVEWTNNGKTEL NYKNTEPV LDSDGSYFMYSKLRVEKKNWVERNSYSCSVVHEGLHNHHTTKSFSRTPGLDL DDVCAEAQ DGELDGLWTTITIFISLFLLSVCYSASVTLFKVKWIFSSVVELKQTISPDYR NMIGQGA	
53	ATGAACTTTGTGCTCAGCTTGATTTTCCTTGCCCTCATTTTAAAAGGTGTCC AGTGTGAAGTGCAGCTGGTGGAGTCTGGGGGAGGCTTAGTGAAGCCTGGAGG GTCCCTGAAACTCTCCTGTGCAGCCTCTGGATTCACTTTCAGTAGGTATGCC ATGTCTTGGGTTCGCCAGACTCCGGAGAAGAGGCTGGAGTGGGTCGCAACCA TTAGTAGTGGTGGTAGTTACACTTATTATCCAGACAGTGTGAAGGGTCGATT CACCATCTCCAGAGACAATGCCAAGAACACCCTGTACCTCCGAATGAGCAGT CTGAGGTCTGAGGACACGGCCATGTATTACTGTGCAAGACGGGACTACGGTA GTAGAGACTATGTTATGGACTACTGGGGTCACGGAACCTCAGTCACCGTCTC CTCAGCCAAAACAACAGCCCCATCGGTCTATCCACTGGCCCCTGTGTGTGGA GATACAACTGGCTCCTCGGTGACTCTAGGATGCCTGGTCAAGGGTTATTTCC CTGAGCCAGTGACCTTGACCTGGAACTCTGGATCCCTGTCCAGTGGTGTGCA CACCTTCCCAGCTGTCCTGCAGTCTGACCTCTACACCCTCAGCAGCTCAGTG ACTGTAACCTCGAGCACCTGGCCCAGCCAGTCCATCACCTGCAATGTGGCCC ACCCGGCAAGCAGCACCAAGGTGGACAAGAAAATTGAGCCCAGAGGGCCCAC AATCAAGCCCTGTCCTCCATGCAAATGCCCAGCACCTAACCTCTTGGGTGGA CCATCCGTCTTCATCTTCCCTCCAAAGATCAAGGATGTACTCATGATCTCCC TGAGCCCCATAGTCACATGTGTGGTGGTGGATGTGAGCGAGGATGACCCAGA TGTCCAGATCAGCTGGTTTGTGAACAACGTGGAAGTACACACAGCTCAGACA CAAACCCATAGAGAGGATTACAACAGTACTCTCCGGGTGGTCAGTGCCCTCC CCATCCAGCACCAGGACTGGATGAGTGGCAAGGAGTTCAAATGCAAGGTCAA CAACAAAGACCTCCCAGCGCCCATCGAGAGAACCATCTCAAAACCCAAAGGG TCAGTAAGAGCTCCACAGGTATATGTCTTGCCTCCACCAGAAGAAGAGATGA CTAAGAAACAGGTCACTCTGACCTGCATGGTCACAGACTTCATGCCTGAAGA CATTTACGTGGAGTGGACCAACAACGGGAAAACAGAGCTAAACTACAAGAAC ACTGAACCAGTCCTGGACTCTGATGGTTCTTACTTCATGTACAGCAAGCTGA GAGTGGAAAAGAAGAACTGGGTGGAAAGAAATAGCTACTCCTGTTCAGTGGT CCACGAGGGTCTGCACAATCACCACACGACTAAGAGCTTCTCCCGGACTCCG GGGCTAGACCTGGATGATGTCTGTGCTGAGGCCCAGGACGGGGAGCTGGACG GCCTCTGGACGACCATCACCATCTTCATCAGCCTCTTCCTGCTCAGCGTGTG CTACAGCGCCTCTGTCACACTCTTCAAGGTAAAGTGGATCTTCTCCTCTGTG GTGGAGCTGAAGCAGACGATCTCCCCTGACTACAGAAACATGATTGGGCAGG GAGCCTAG	1H10 DNA gamma FULL
54	METDTILLWVLLLWVPGSTGDIVLTQSPASLAVSLGQRATISCKASQSVDFD GVSYMNWY QQKPGQPPKLLIYAASNLESGIPARFSGIGSGTDFTLNIHPVEEEDAATYYC QQTNENPT FGGGTKLEIKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWR IDGSERQN GVLNSWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEAAHKTSTSPIVKSF NRNEC	1H10 PROT kappa FULL
55	ATGGAGACAGACACAATCCTGCTATGGGTGCTGCTGCTCTGGGTTCCAGGCT CCACTGGTGACATTGTGCTGACCCAATCTCCAGCTTCTTTGGCTGTGTCTCT AGGGCAGAGGGCCACCATCTCCTGCAAGGCCAGCCAAAGTGTTGATTTTGAT GGTGTTAGTTATATGAACTGGTACCAACAGAAACCAGGACAGCCACCCAAAC TCCTCATCTATGCTGCATCCAATCTAGAATCTGGGATCCCAGCCAGGTTTAG TGGCATTGGGTCTGGGACAGACTTCACCCTCAACATCCATCCTGTGGAGGAG GAGGATGCTGCAACCTATTACTGTCAGCAAACTAATGAAAATCCCACATTCG GAGGGGGGACCAAGCTGGAAATAAAACGGGCTGATGCTGCACCAACTGTATC CATCTTCCCACCATCCAGTGAGCAGTTAACATCTGGAGGTGCCTCAGTCGTG	1H10 DNA kappa FULL

	TGCTTCTTGAACAACTTCTACCCCAAAGACATCAATGTCAAGTGGAGGATTG ATGGCAGTGAACGACAAAATGGCGTCCTGAACAGTTGGACTGATCAGGACAG CAAAGACAGCACCTACAGCATGAGCAGCACCCTCACGTTGACCAAGGACGAG TATGAACGACATAACAGCTATACCTGTGAGGCCGCTCACAAGACATCAACTT CACCCATTGTCAAGAGCTTCAACAGGAATGAGTGTTAG	

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CLAIMS

1. An isolated and/or purified antibody, antibody fragment or derivative thereof able to block the gamma-secretase-dependent processing of the amyloid precursor protein (APP) but not the Notch processing, wherein said isolated and/or purified antibody, antibody fragment or derivative thereof specifically recognizes at least one sequence of said APP consisting essentially in the amino acid sequence set forth in SEQ ID No 1, a fragment thereof and/or a conservative variant thereof.

2. The isolated and/or purified antibody, antibody fragment or derivative thereof of claim 1 characterized in that said APP sequence consisting essentially in the amino acid sequence set forth in SEQ ID No 1, a fragment thereof and/or a conservative variant thereof is a natively folded sequence.

3. The isolated and/or purified antibody, antibody fragment or derivative thereof of claim 1 or 2, characterized in that the fragment of said amino acid sequence set forth in SEQ ID No 1 and/or the conservative variant thereof, is selected from the group of sequences comprising the N-terminal sequence 1-7 (SEQ ID No 2), the C-terminal sequence 70-80 (SEQ ID No 3), the C-terminal sequence 80-90 (SEQ ID No 4) and the C-terminal sequence 90-99 (SEQ ID No 5), and/or a combination of said sequences.

4. The isolated and/or purified antibody, antibody fragment or derivative thereof of any one of the preceding claims, characterized in that said isolated and/or purified antibody, antibody fragment or derivative thereof comprises

i) at least one complementary determining region 1 (CDR1) of the VH region, wherein the amino acid sequence determining said CDR1 consists essentially in an amino acid sequence selected from the group comprising SEQ ID No 6, SEQ ID No 8, SEQ ID No 10, SEQ ID No 12, SEQ ID No 14, a biologically active fragment thereof and/or a variant thereof, and/or a combination of said sequences, and/or

ii) at least one complementary determining region 2 (CDR2) of the VH region, wherein the amino acid sequence determining said CDR2 consists essentially in an amino acid sequence selected from the group comprising SEQ ID No 7, SEQ ID No 9, SEQ ID No 11, SEQ ID No 13, SEQ ID No 15, a biologically active fragment thereof and/or a variant thereof, and/or a combination of said sequences, and/or

iii) at least one complementary determining region 3 (CDR3) of the VH region, wherein the amino acid sequence determining said CDR3 consists essentially in an amino acid sequence selected from the group comprising SEQ ID No 56, SEQ ID No 57, SEQ ID No 58, SEQ ID No 59, SEQ ID No 60, a biologically active fragment thereof and/or a variant thereof, and/or a combination of said sequences.

5. The isolated and/or purified antibody, antibody fragment or derivative thereof of any one of the preceding claims, characterized in that said isolated and/or purified antibody, antibody fragment or derivative thereof comprising

i) at least one complementary determining region 1 (CDR1) of the VL region, wherein the amino acid sequence determining said CDR1 consists essentially in an amino acid sequence selected from the group comprising SEQ ID No 16, SEQ ID No 18, SEQ ID No 20, SEQ ID No 22, SEQ ID No 24, a biologically active fragment thereof and/or a variant thereof, and/or a combination of said sequences, and/or

ii) at least one complementary determining region 2 (CDR2) of the VL region, wherein the amino acid sequence determining said CDR2 consists essentially in an amino acid sequence selected from the group comprising SEQ ID No 61, SEQ ID No 62, SEQ ID No 63, SEQ ID No 64, SEQ ID No 65, a biologically active fragment thereof and/or a variant thereof, and/or a combination of said sequences, and/or

iii) at least one complementary determining region 3 (CDR3) of the VL region, wherein the amino acid sequence determining said CDR3 consists essentially in an amino acid sequence selected from the group comprising SEQ ID No 17, SEQ ID No 19, SEQ ID No 21, SEQ ID No 23, SEQ ID No 25, a biologically active fragment thereof and/or a variant thereof, and/or a combination of said sequences.

6. The isolated and/or purified antibody, antibody fragment or derivative thereof of any one of the preceding claims, characterized in that the biologically active fragment contains less than 90%, preferably less than 60%, in particular less than 30% amino acids in length than the respective sequence of CDR of the VH and/or CDR of the VL regions.

7. The isolated and/or purified antibody, antibody fragment or derivative thereof of any one of the preceding claims, characterized in that said isolated and/or purified antibody consists essentially in an amino acid sequence selected from the group

i) SEQ ID No 26 and SEQ ID No 27, a biologically active fragment thereof and/or a variant thereof,
ii) SEQ ID No 28 and SEQ ID No 29, a biologically active fragment thereof and/or a variant thereof
iii) SEQ ID No 30 and SEQ ID No 31, a biologically active fragment thereof and/or a variant thereof
iv) SEQ ID No 32 and SEQ ID No 33, a biologically active fragment thereof and/or a variant thereof or
v) SEQ ID No 34 and SEQ ID No 35, a biologically active fragment thereof and/or a variant thereof,
and/or a combination of said sequences.

8. The isolated and/or purified antibody, antibody fragment or derivative thereof of any one of the preceding claims, characterized in that said isolated and/or purified antibody fragment or derivative thereof is a Fab-fragment, a F(ab2)'-fragment, a single-chain antibody, a chimeric antibody, a CDR-grafted antibody, a CDR-containing peptide, a bivalent antibody-construct, a humanized antibody, a synthetic antibody, a chemically modified derivative thereof, a multispecific antibody, a diabody, a Fv-fragment, a labeled antibody or another type of recombinant antibody.

9. The isolated and/or purified antibody, antibody fragment or derivative thereof of any one of the preceding claims, characterized in that it is a monoclonal antibody.

10. The isolated and/or purified antibody, antibody fragment or derivative thereof of claim 9, characterized in that the monoclonal antibody is selected from the group comprising IgG1, IgG2, IgG2a, IgG2b, IgG3 and IgG4, or a combination thereof.

11. An isolated and/or purified antibody, antibody fragment or derivative thereof of any one of the preceding claims for the treatment and/or prevention of an APP-associated disease selected from the group comprising Alzheimer’s disease, Down syndrom, diseases caused by an extra copy of Chromosome 21 or extra copy(ies) of the gene APP, cerebral amyloid angiopathy (CAA), hereditary cerebral hemorrhage with amyloidosis Dutch type (HCHWAD), amyloid microangiopathy in the vascular variant of Alzheimer’s disease, Abeta related angiitis (ABRA), fronto temporal dementias (FTD), Parkinson’s disease, amyotrophic lateral sclerosis .

12. An isolated and/or purified nucleic acid sequence comprising
i) a nucleotide sequence encoding an isolated and/or purified antibody, an antibody fragment or derivative of any one of claims 1 to 10,
ii) a nucleic acid sequence having substantial sequence identity or homology to a nucleic acid sequence encoding an isolated and/or purified antibody, an antibody fragment of or derivative of said antibody of any one of claims 1 to 10,
iii) a nucleic acid sequence complementary to i) or ii),
iv) a degenerated nucleic acid sequence of i), ii) or iii), or
v) a nucleic acid sequence capable of hybridizing under stringent conditions to i), ii), iii) or iv).

13. A pharmaceutical composition comprising a pharmaceutically effective amount of one or more isolated and/or purified antibody, antibody fragment or derivative thereof of any of claims 1 to 10 and/or one or more isolated and/or purified nucleic acid sequence encoding said isolated and/or purified antibody, antibody fragment or derivative thereof of claim 12.

14. An expression vector comprising an isolated and/or purified nucleic acid sequence of claim 12.

15. A host cell comprising an expression vector of claim 14.

16. A hybridoma and/or clone secreting the monoclonal antibody of any one of claims 1 to 10.

17. A method for treating and/or preventing an APP-associated disease, in a patient in need thereof, comprising administering a pharmaceutically effective amount of an isolated and/or purified antibody, antibody fragment or derivative thereof of any one of claims 1 to 10.

18. An immune composition for the treatment and/or prevention of an APP-associated disease comprising at least one or several isolated and/or purified antibody, antibody fragment or derivative thereof of any one of claims 1 to 10.

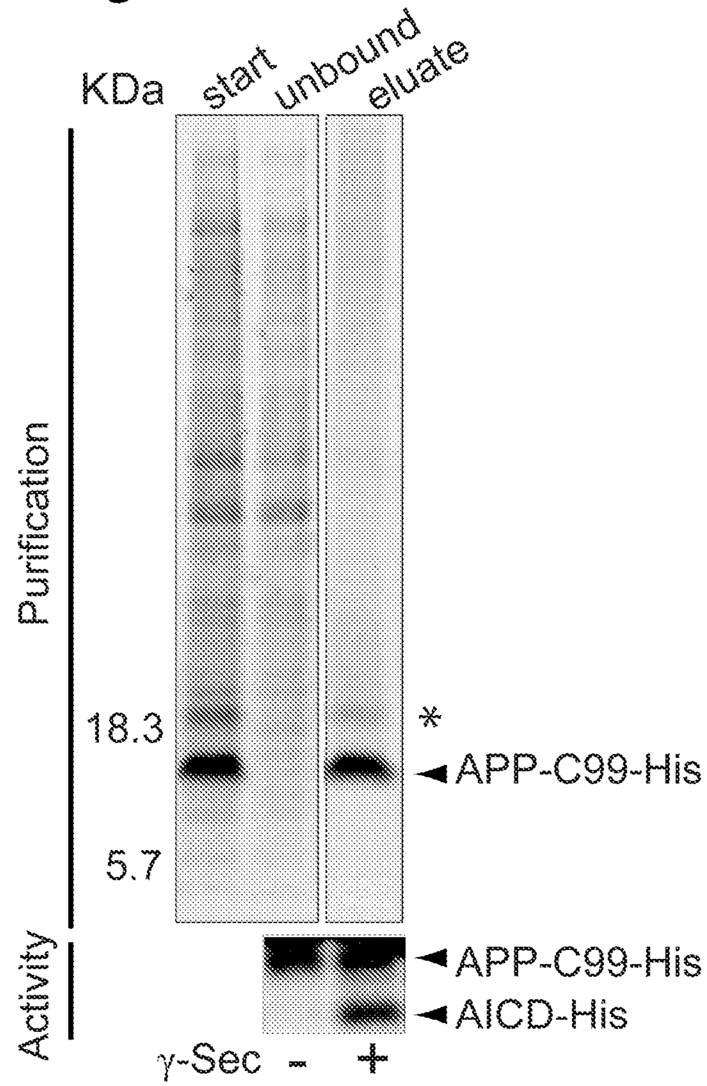
19. A kit for treating and/or preventing an APP-associated disease, comprising a pharmaceutically effective amount of an isolated and/or purified antibody, antibody fragment or

derivative thereof of any one of claims 1 to 10, optionally with reagents and/or instructions for use.

20. A method for reducing Aβ production in a patient in need thereof, comprising administering a pharmaceutically effective amount of an isolated and/or purified antibody, antibody fragment or derivative thereof of any one of claims 1 to 10.

21. A method for delaying Aβ plaque formation in a patient in need thereof, comprising administering a pharmaceutically effective amount of an isolated and/or purified antibody, antibody fragment or derivative thereof of any one of claims 1 to 10.

Fig. 1A



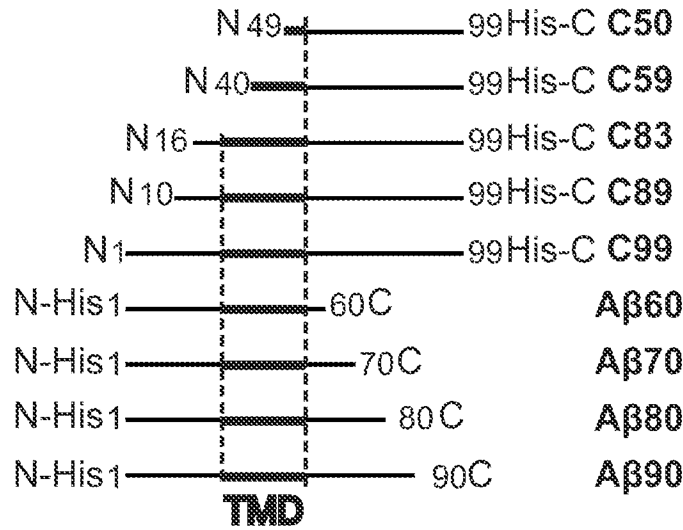


Fig. 1B
N 49 99 His-C C50
N 40 99 His-C C59
N 16 99 His-C C83
N 10 99 His-C C89
N 1 99 His-C C99
N-His 1 60 C Aβ60
N-His 1 70 C Aβ70
N-His 1 80 C Aβ80
N-His 1 90 C Aβ90
TMD

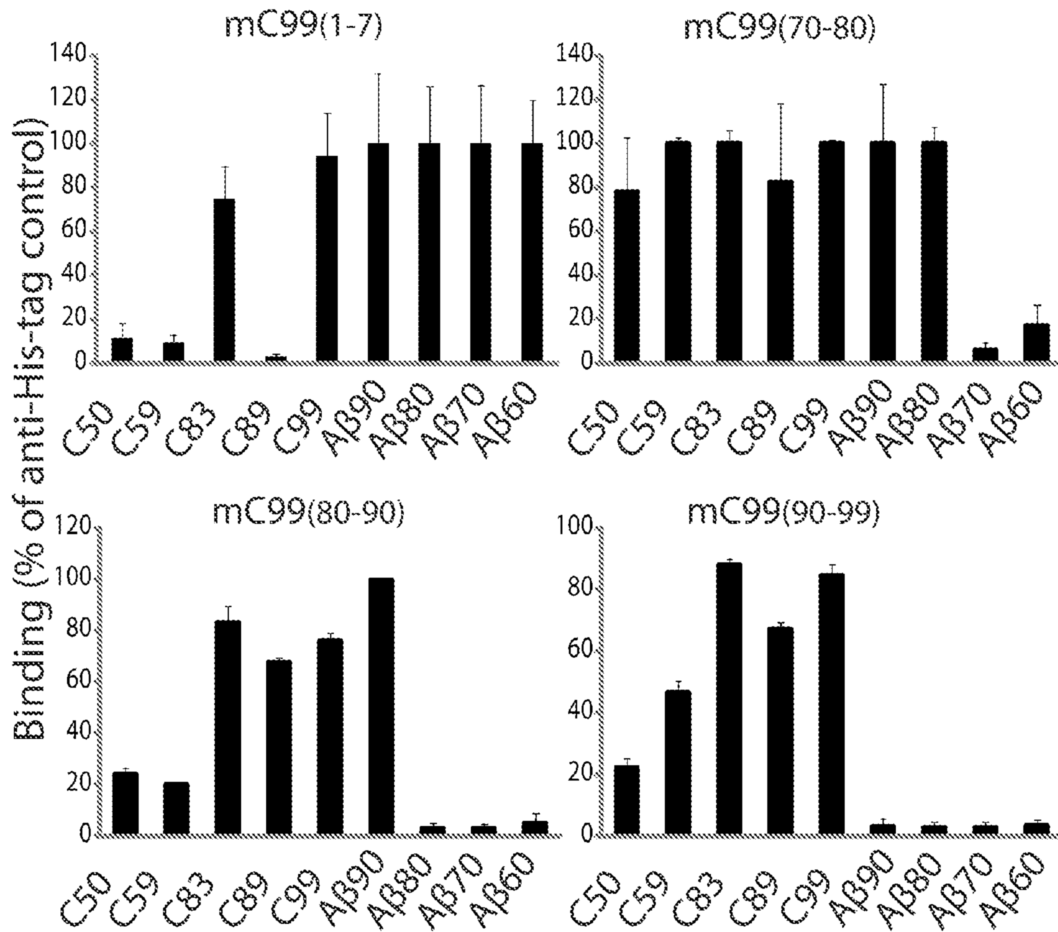


Fig. 1C
mC99(1-7)
mC99(70-80)
mC99(80-90)
mC99(90-99)
Binding (% of anti-His-tag control)
C50
C59
C83
C89
C99
Aβ90
Aβ80
Aβ70
Aβ60

Fig. 1D

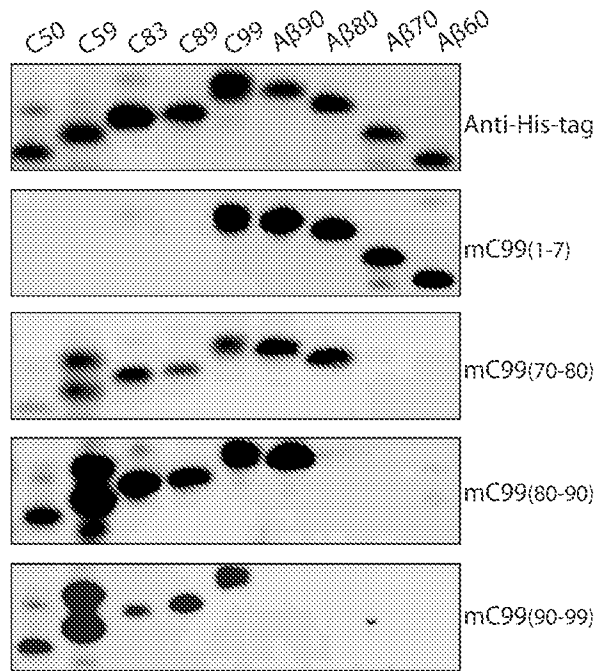
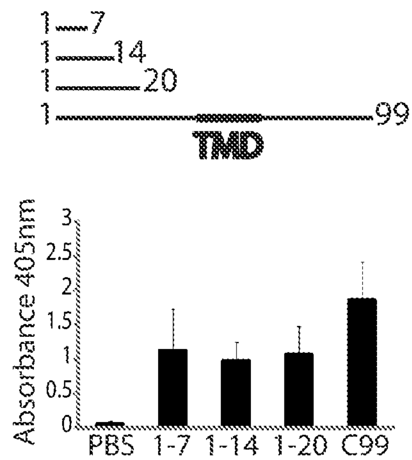


Fig. 1E



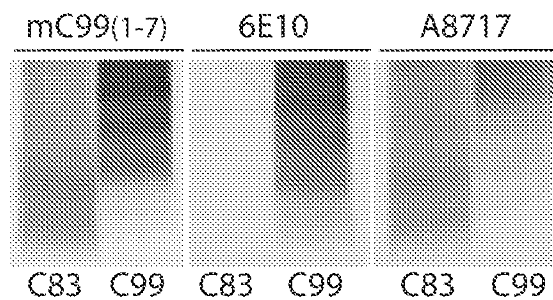


Fig. 1F

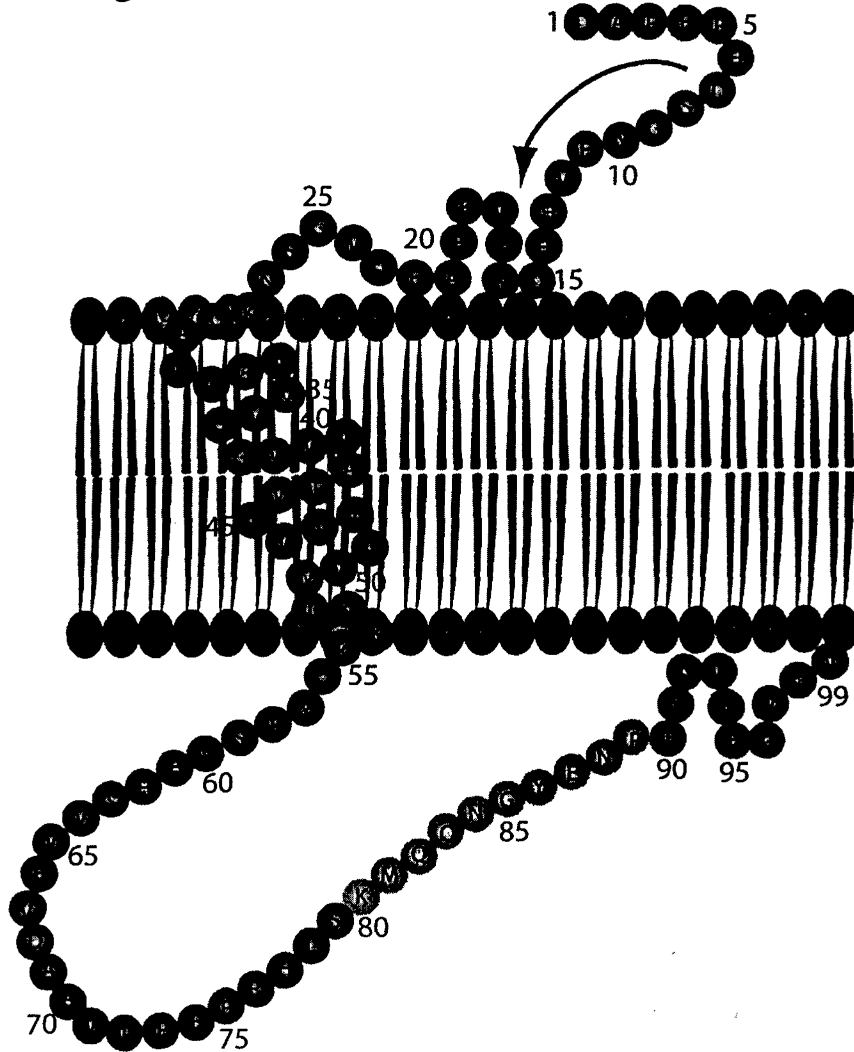
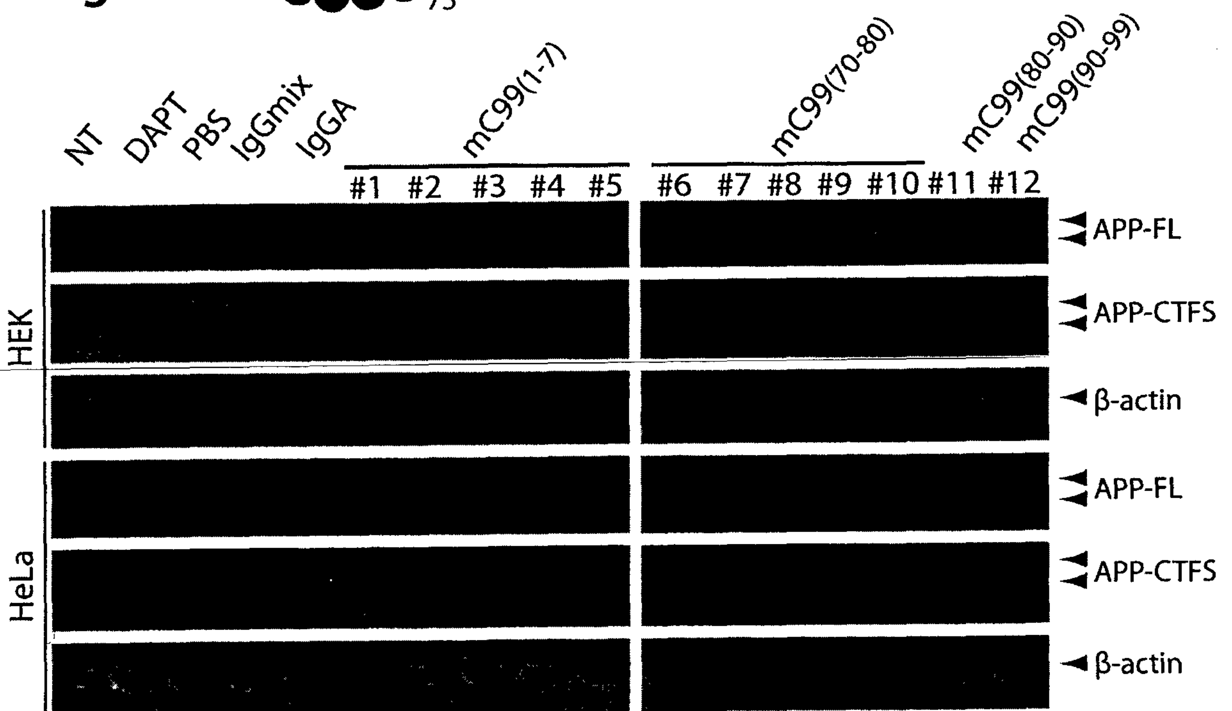


Fig. 2A



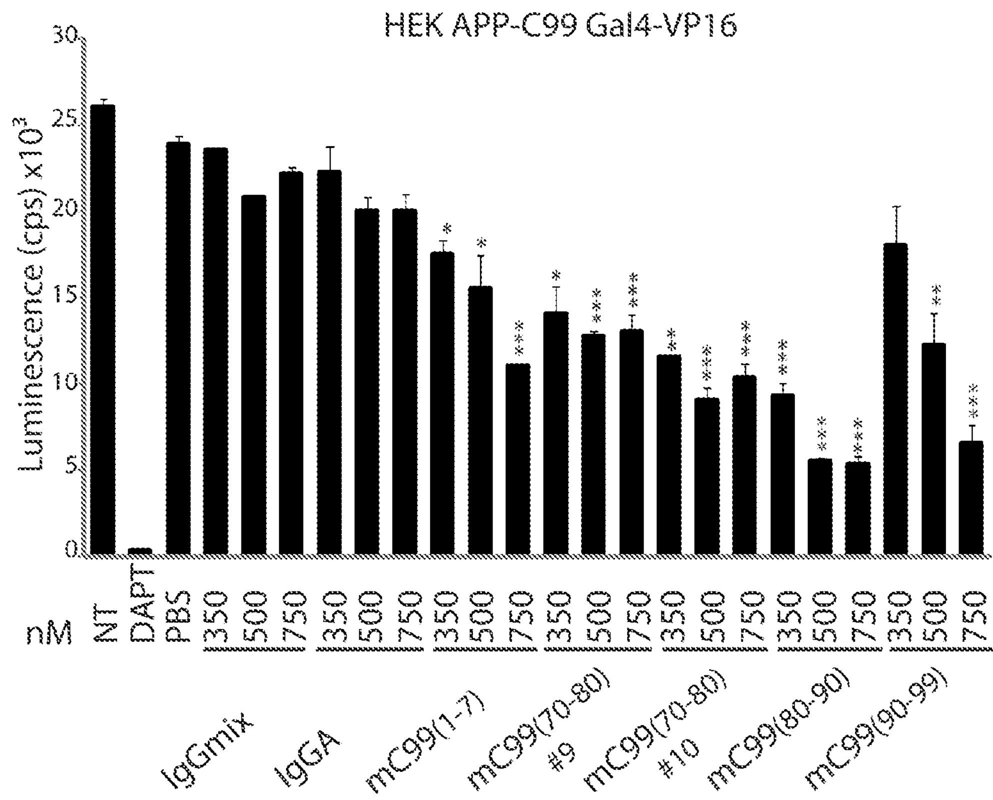


Fig. 2B
HEK APP-C99 Gal4-VP16
Luminescence (cps) x10^3
30
25
20
15
10
5
0
nM
NT
DAPT
PBS
350
500
750
IgGmix
IgGA
mC99(1-7)
mC99(70-80) #9
mC99(70-80) #10
mC99(80-90)
mC99(90-99)
*
**
***

Fig. 2C

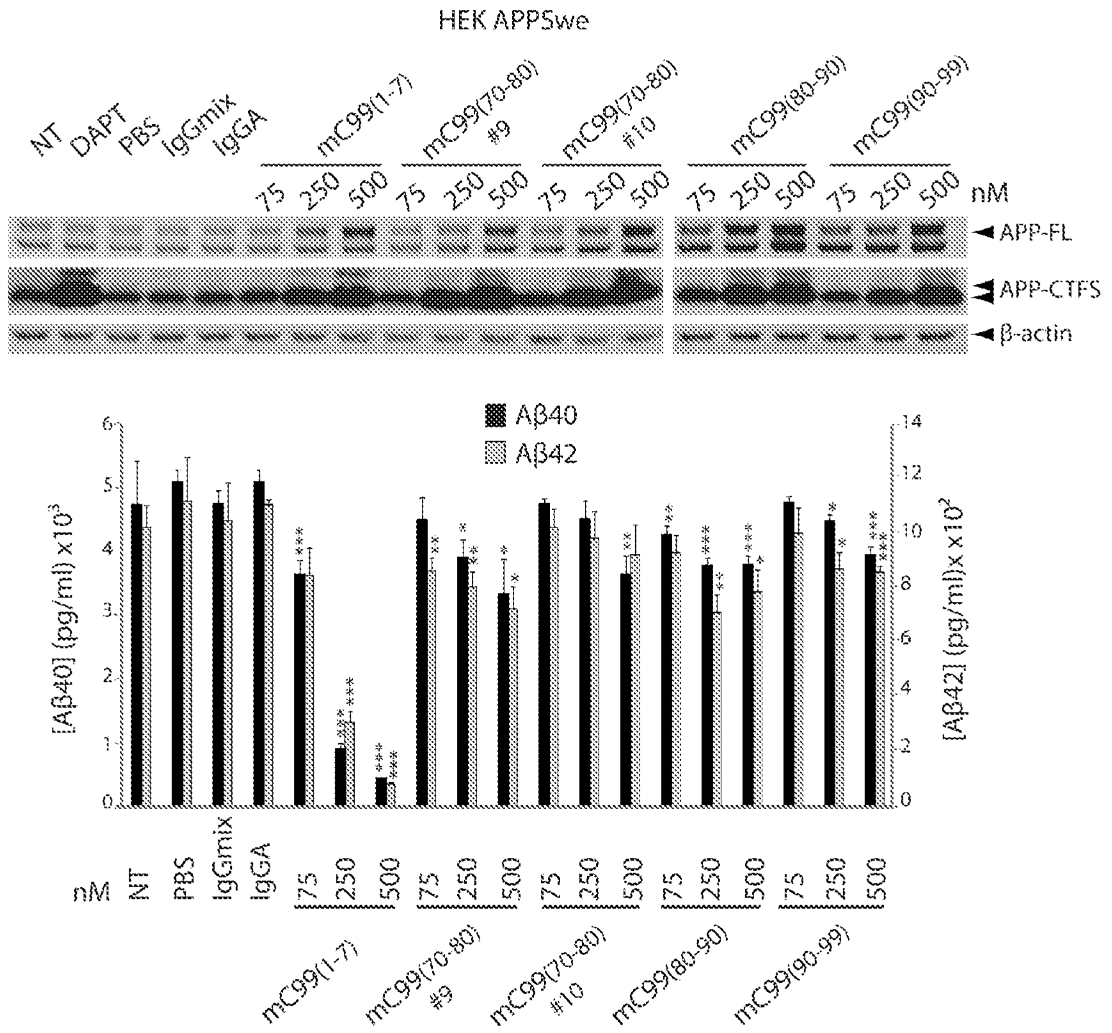


Fig. 2D

HEK NotchΔE

NT DAPT PBS IgGmix IgGA mC99(1-7) mC99(70-80) #9 mC99(70-80) #10 mC99(80-90) mC99(90-99)

APP-FL
APP-CTFs
NICD
β-actin

Fig. 2E

PBS IgG A mC99(1-7) mC99(70-80)

start unbound IP start unbound IP start unbound IP start unbound IP C83 C99

HEK APPSwe

APP-FL
APP-CTFs

Fig. 3A

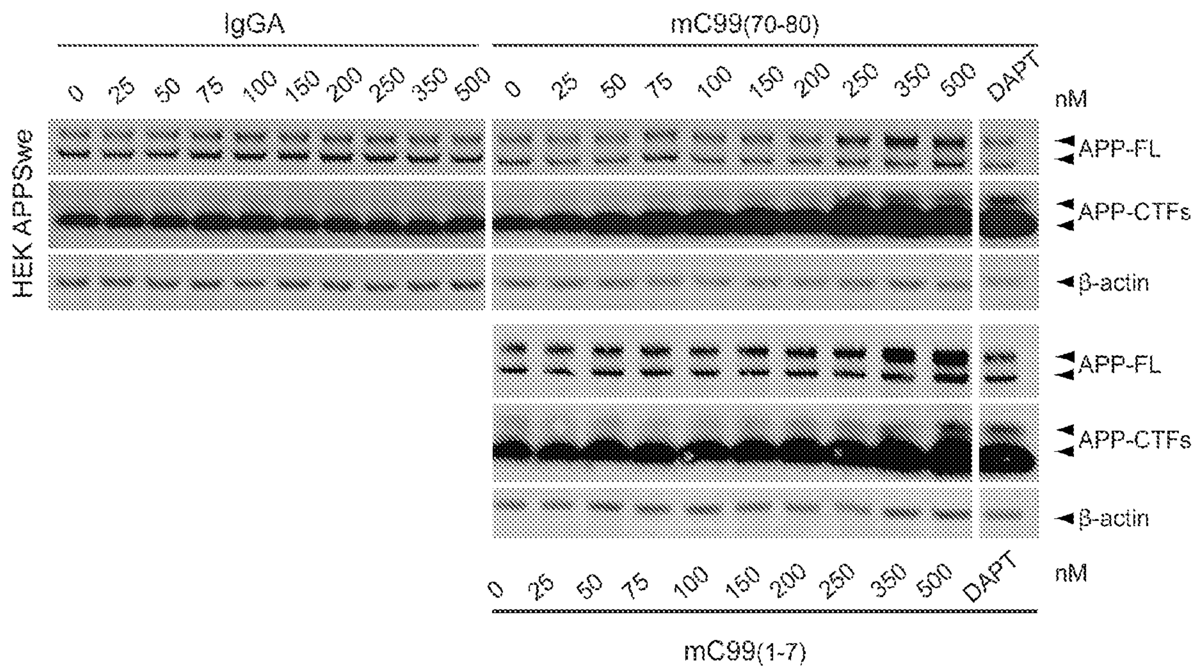


Fig. 3B

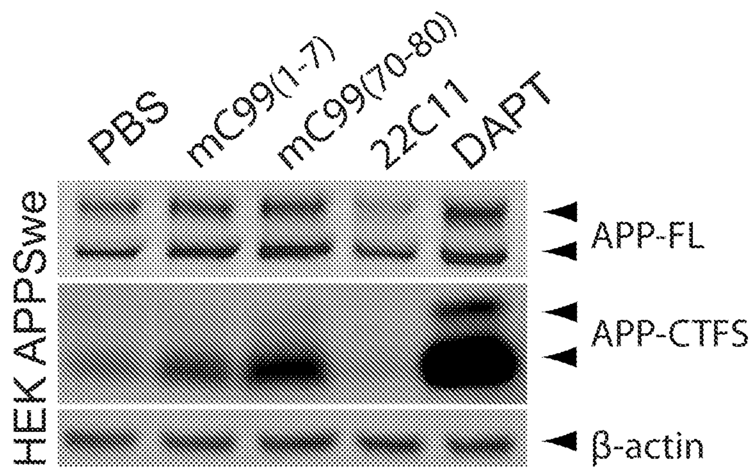
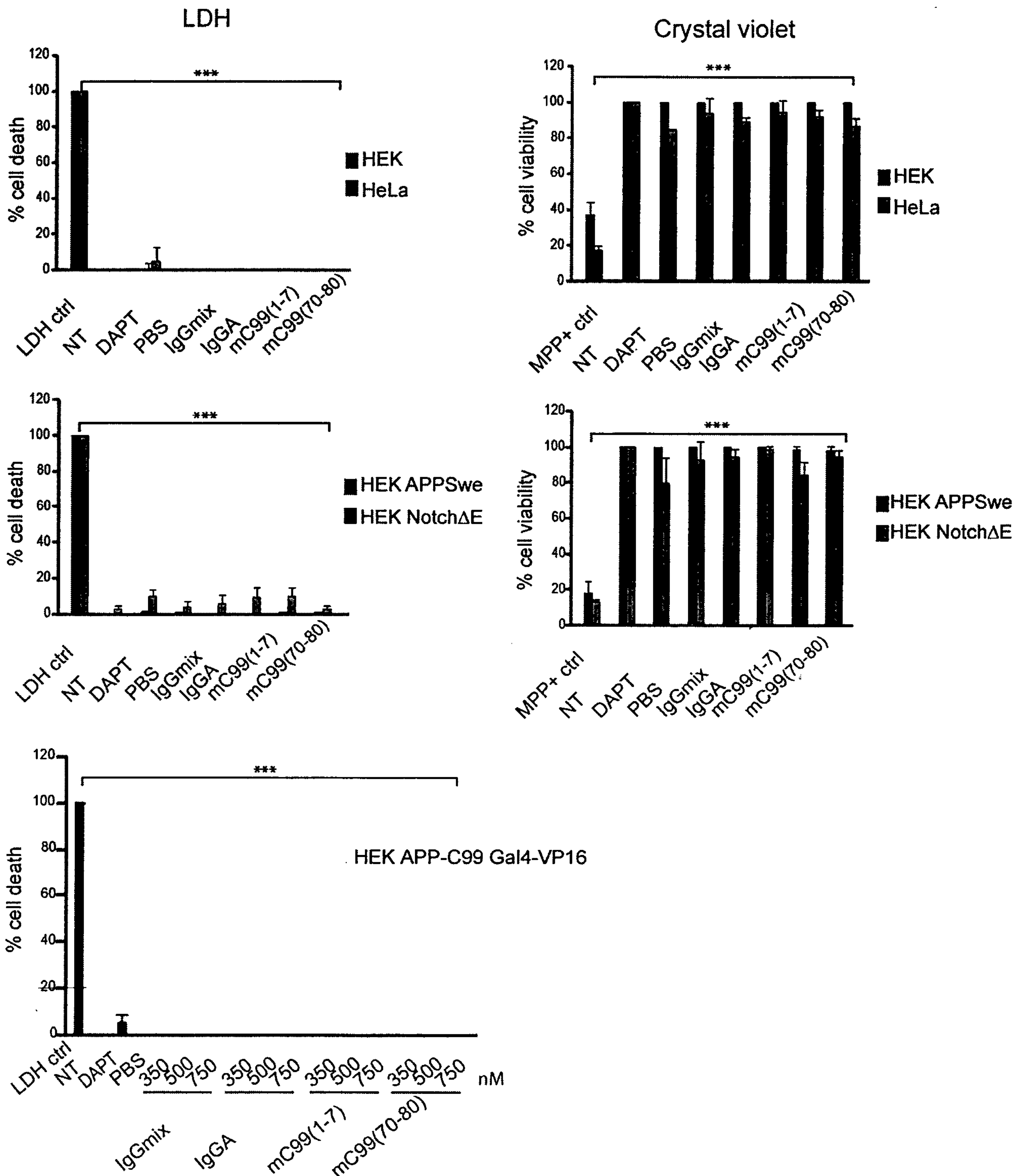


Fig. 3C



INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2012/050515

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/18
ADD.

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

B. FIELDS SEARCHED

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

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

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

EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A,P	Anonymous: "Datasheet: beta Amyloid, 1-16 (6E10) Monoclonal Antibody", Covance , 6 November 2011 (2011-11-06), XP002674264, Retrieved from the Internet: URL:https://store.crpinc.com/pdfdatasheet.aspx?catalogno=SIG-39320 [retrieved on 2012-04-19] the whole document -----	1-21
X	EP 2 224 000 A1 (UNIV KYOTO [JP]) 1 September 2010 (2010-09-01) paragraph [0109] figure 5 -----	1-10

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

[X] See patent family annex.

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
8 May 2012	29/05/2012

Name and mailing address of the ISA/
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Fax: (+31-70) 340-3016

Authorized officer
Bumb, Peter

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2012/050515

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A,P	Anonymous: "Anti-Amyloid Precursor Protein antibody (ab2072)", Abcam , 12 April 2012 (2012-04-12), XP002674265, Retrieved from the Internet: URL:http://www.abcam.com/Amyloid-Precursor-Protein-antibody-ab2072.pdf [retrieved on 2012-04-19] the whole document -----	1-21
X	UTSUKI TADA ET AL: "Identification of novel small molecule inhibitors of amyloid precursor protein synthesis as a route to lower Alzheimer's disease amyloid-beta peptide.", THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS, vol. 318, no. 2, August 2006 (2006-08), pages 855-862, XP002674266, ISSN: 0022-3565 page 857 - page 858 -----	1-7,9,10
A	JIANG YING ET AL: "Alzheimer's-related endosome dysfunction in Down syndrome is Abeta-independent but requires APP and is reversed by BACE-1 inhibition.", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, vol. 107, no. 4, 26 January 2010 (2010-01-26), pages 1630-1635, XP002674267, ISSN: 1091-6490 the whole document -----	1-21
A,P	Anonymous: "Anti-Amyloid beta precursor protein antibody (ab18813)", Abcam , 12 April 2012 (2012-04-12), XP002674268, Retrieved from the Internet: URL:http://www.abcam.com/Amyloid-beta-precursor-protein-antibody-ab18813.pdf [retrieved on 2012-04-19] the whole document ----- -/--	1-21

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2012/050515

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WON J S ET AL: "Involvement of AMP-activated-protein-kinase (AMPK) in neuronal amyloidogenesis", BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 399, no. 4, 3 September 2010 (2010-09-03), pages 487-491, XP027259107, ACADEMIC PRESS INC. ORLANDO, FL, US ISSN: 0006-291X, DOI: 10.1016/J.BBRC.2010.07.081 [retrieved on 2010-07-24] page 488, last line figure 2 -----	1-7,9,10
X	SERGEANT NICOLAS ET AL: "Progressive decrease of amyloid precursor protein carboxy terminal fragments (APP-CTFs), associated with tau pathology stages, in Alzheimer's disease.", JOURNAL OF NEUROCHEMISTRY, vol. 81, no. 4, May 2002 (2002-05), pages 663-672, XP002674269, ISSN: 0022-3042 page 664 -----	1-10
X	MATHEWS P M ET AL: "Calpain activity regulates the cell surface distribution of amyloid precursor protein. Inhibition of clapains enhances endosomal generation of beta-cleaved C-terminal APP fragments", JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 277, no. 39, 27 September 2002 (2002-09-27), pages 36415-36424, XP002995626, THE AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, INC, US ISSN: 0021-9258, DOI: 10.1074/JBC.M205208200 page 36416, right-hand column figure 3 page 36419, right-hand column figure 4 -----	1-7,9,10

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INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2012/050515

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SELKOE D J ET AL: "BETA-AMYLOID PRECURSOR PROTEIN OF ALZHEIMER DISEASE OCCURS AS 110- TO 135-KILODALTON MEMBRANE-ASSOCIATED PROTEINS IN NEURAL AND NONNEURAL TISSUES", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, NATIONAL ACADEMY OF SCIENCES, vol. 85, 1 October 1988 (1988-10-01), pages 7341-7345, XP000867435, ISSN: 0027-8424, DOI: 10.1073/PNAS.85.19.7341 page 7341, right-hand column figure 2 page 7343 - page 7344 -----	1-7,9,10
X	THAKKER DEEPAK R ET AL: "Intracerebroventricular amyloid-beta antibodies reduce cerebral amyloid angiopathy and associated micro-hemorrhages in aged Tg2576 mice.", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, vol. 106, no. 11, 17 March 2009 (2009-03-17), pages 4501-4506, XP002674270, ISSN: 1091-6490 page 4502 - page 4503 -----	1-7, 9-11,13, 17-21
X	MORROW MATTHEW P ET AL: "Design and characterization of a plasmid vector system capable of rapid generation of antibodies of multiple isotypes and specificities.", BIOTECHNOLOGY LETTERS, vol. 31, no. 1, January 2009 (2009-01), pages 13-22, XP002674271, ISSN: 1573-6776 table 1 -----	12,14-16

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IB2012/050515

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		EP 2224000 A1	01-09-2010
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		WO 2009057664 A1	07-05-2009