

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
29 December 2005 (29.12.2005)

PCT

(10) International Publication Number
WO 2005/123775 A1

- (51) International Patent Classification⁷: **C07K 16/18**, A61K 39/395, A61P 25/28, C07K 14/47
- (21) International Application Number:
PCT/SE2005/000993
- (22) International Filing Date: 21 June 2005 (21.06.2005)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
0401601-0 21 June 2004 (21.06.2004) SE
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- (81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.
- (84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
- Published:**
— with international search report
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(54) Title: ANTIBODIES SPECIFIC FOR SOLUBLE AMYLOID BETA PEPTIDE PROTOFIBRILS AND USES THEREOF

(57) Abstract: The invention pertains to the development of antibodies that specifically bind amyloid beta protein (Abeta) in its protofibril conformation. The invention also comprises methods of using anti-Abeta protofibril antibodies to diagnose or treat Alzheimer's disease, Down's syndrome Lewybody dementia, vascular dementia, and other neurodegenerative disorders. Furthermore, the invention pertains to the use of anti-Abeta protofibril antibodies to screen and identify substances that will modulate protofibril activity or formation in vitro or in vivo. The invention also pertains to methods for synthesising pure Abeta protofibril antigens as well as to a method for stabilising Abeta protofibril antigens as well as to a method for stabilising Abeta protofibrils.

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Antibodies specific for soluble amyloid beta peptide protofibrils and uses thereof

1. FIELD OF INVENTION

This invention pertains to the diagnosis, prevention and treatment of neurodegenerative diseases, in particular Alzheimer's disease, and other similar disease. More precisely, to antibodies that specifically bind amyloid beta protein (A β) in its protofibril conformation.

2. BACKGROUND OF THE INVENTION

Alzheimer's disease (AD) is a progressive and irreversible neurodegenerative disorder causing cognitive, memory and behavioural impairments. It is the most common cause of dementia in the elderly population affecting roughly 5% of the population above 65 years and 20% above 80 years of age. AD is characterized by an insidious onset and progressive deterioration in multiple cognitive functions. The neuropathology involves both extracellular and intracellular argyrophillic proteineous deposits. The extracellular deposits, referred to as neuritic plaques, mainly consist in amyloid beta protein (A β) surrounded by dystrophic neurites (swollen, distorted neuronal processes). A β within these extracellular deposits are fibrillar in their character with a β -pleated sheet structure. A β in these deposits can be stained with certain dyes e.g. Congo Red and display a fibrillar ultrastructure. These characteristics, adopted by A β in its fibrillar structure of neuritic plaques, are the definition of the generic term amyloid. The classic intracellular AD pathologic lesion is the neurofibrillary tangle (NFT) which consists of filamentous structures called paired helical filaments (PHFs) composed of twisted strands of hyperphosphorylated microtubule-associated protein tau. Frequent neuritic plaques and neurofibrillary tangle deposits in the brain are diagnostic criteria for AD, as carried out post mortem. AD brains also display macroscopic brain atrophy, nerve cell loss, local inflammation (microgliosis and astrocytosis) and often congophilic amyloid angiopathy (CAA) in cerebral vessel walls.

Two forms of A β peptides, A β 40 and A β 42, are the dominant species in AD neuritic plaques (*Masters 1985*), while A β 40 is the prominent species in cerebrovascular amyloid associated with AD (*Glennner 1984*). Enzymatic activities allow A β to be

continuously formed from a larger protein called the amyloid precursor protein (APP) in both healthy and AD afflicted subjects in all cells of the body. Two major APP processing events through β - and γ -secretase activities enables A β production, while a third enzyme called α -secretase activities prevents A β generation by cleavage inside the A β sequence (*Selkoe, 1994; US5604102*). The A β 42 is a forty two amino acid long peptide i.e. two amino acids longer at the C-terminus, as compared to A β 40. A β 42 is more hydrophobic, and does more easily aggregate into larger structures of A β peptides such as A β dimers, A β tetramers, A β oligomers, A β protofibrils or A β fibrils. A β fibrils are hydrophobic and insoluble, while the other structures are all less hydrophobic and soluble. All these higher molecular structures of A β peptides are individually defined based on their biophysical and structural appearance e.g. in electron microscopy, and their biochemical characteristics e.g. by analysis with size-exclusion chromatography/western blot. These A β peptides, particularly A β 42, will gradually assemble into a various higher molecular structures of A β during the life span. AD, which is a strongly age-dependent disorder, will occur earlier in life if this assembly process occurs more rapidly. This is the core of the "amyloid cascade hypothesis" of AD which claims that APP processing, the A β 42 levels and their assembly into higher molecular structures is a central cause of AD. All other neuropathology of AD brain and the symptoms of AD such as dementia are somehow caused by A β or assembled forms thereof.

A β can exist in different lengths i.e. 1-39,1-40,1-42 and 1-43 and fragments sizes i.e. 1-28, 3-40/42, 11-40/42, 17-40/42 and 25-35. All these peptides can aggregate and form soluble intermediates and insoluble fibrils, each molecular form having a unique structural conformation and biophysical property. Monomeric A β 1-42 for example, is a 42 amino acid long soluble and non-toxic peptide, that is suggested to be involved in normal synapse functions. Under certain conditions, the A β 1-42 can aggregate into dimers, trimers, tetramers, pentamers up to 12-mer and higher oligomeric forms, all with its distinct physicochemical property such as molecular size, EM structure and AFM (atomic force microscopy) molecular shape. An example of a higher molecular weight soluble oligomeric A β form is the protofibril (*Hartley 1999, Walsh 1999*), which has an apparent molecular weight >100 kDa and a curvilinear structure of 4-11 nm in diameter and < 200 nm in length. It has

recently been demonstrated that soluble oligomeric A β peptides such as A β protofibrils impair long-term potentiation (LTP) (Hartley, 1999), a measure of synaptic plasticity that is thought to reflect memory formation in the hippocampus (Walsh 2001). Furthermore, oligomeric Arctic A β peptides display much more profound inhibitory effect than wtA β on LTP in the brain, likely due to their strong propensity to form A β protofibrils (Klyubin 2003).

There are also other soluble oligomeric forms described in the literature that are distinctly different from protofibrils. One such oligomeric form is ADDL (Amyloid Derived Diffusible Ligand) (Lambert 1998). AFM analysis of ADDL revealed predominantly small globular species of 4.7-6.2 nm along the z-axis with molecular weights of 17-42 kDa. (Stine 1996). Another form is called ASPD (Amyloidspheroids). ASPD are spherical oligomers of A β 1-40. Toxicity studies showed that spherical ASPD >10 nm were more toxic than lower molecular forms (Hoshi 2003). The A β fibril as the main neurotoxic species is inconsistent with the poor correlation between neuritic plaque density and AD dementia score and also with the modest signs of neurodegeneration in current APP transgenic mice. Soluble neurotoxic A β -intermediate species and their appropriate subcellular site of formation and distribution could be the missing link that will better explain the amyloid hypothesis. This idea has gained support from recent discovery of the Arctic (E693) APP mutation, which causes early-onset AD (US 2002/0162129 A1; Nilsberth et al., 2001). The mutation is located inside the A β peptide sequence. Mutation carriers will thereby generate variants of A β peptides e.g. Arctic A β 40 and Arctic A β 42. Both Arctic A β 40 and Arctic A β 42 will much more easily assemble into higher molecular structures (protofibrils) that are soluble and non-fibrillar. Thus the pathogenic mechanism of the Arctic mutation suggests that the soluble higher molecular protofibrils are causing AD.

2.1 Diagnosis of Alzheimer's disease

2.1.1 Clinical diagnosis

The clinical diagnosis of Alzheimer's disease (AD) is difficult to make, especially in early stages of the disease. Today, the diagnosis is based on a typical medical history combined with the exclusion of other causes of dementia. Clinical centres

with high specialization can have a diagnostic accuracy of 85-90% compared with the neuropathological diagnosis. In the early stages of the disease the clinical picture is vague and definite diagnostic markers have not yet been identified (*McKhann 1984*). The development of biochemical diagnostic markers is important for a number of reasons: to support the clinical diagnosis, to allow clinicians to give adequate information to patients and their relatives, to initiate pharmacological treatment and care-giving, and in various aspects of clinical research.

2.1.2 Amyloid β -peptide

Pathogenic mutations in the APP and presenilin (PS) genes have been discovered in families with early-onset AD inherited as a dominant trait (*Hardy 1992*). The effects of some of these mutations are now fairly well understood. The Swedish AD mutation (*Mullan 1992; Axelman 1994; Lannfelt 1994*) has revealed one pathogenic mechanism for the development of AD. When a cDNA construct with this mutation was transfected into human cell-lines it gave rise to approximately six times higher release of soluble A β (*Citron 1992, Cai 1993*). Furthermore, fibroblasts from individuals with the Swedish mutation secreted three times more A β into the media compared to fibroblasts from non-carriers (*Johnston 1994*). Overproduction of A β therefore seemed to be an important factor in the disease pathogenesis in this Swedish family. Thus, it was expected that A β levels measured in cerebrospinal fluid (CSF) from family members would differentiate carriers from non-carriers of the mutation. However, no difference was found in levels of total A β between the groups (14.5 \pm 3.3 ng/ml versus 14.9 \pm 2.3 ng/ml) (*Lannfelt 1995*). One explanation for this result may be that A β is cleared from CSF by aggregating to amyloid in the brain. However, there was a strong correlation between duration of dementia and decreasing A β levels. These measurements were done with antibodies recognizing soluble monomeric A β . With protofibril specific monoclonal antibodies more accurate measurements of the toxic species will be possible.

2.1.3 A β in plasma

A β is found in a soluble form in plasma and other tissues (*Seubert 1992*), and not as previously presumed, only in the brains of AD cases. A β plasma levels in members of the Swedish mutation family revealed that both A β 40 and A β 42 were 2-3 times increased in mutation-carriers (*Scheuner 1996*). The proportion of A β 42 of total A β was approximately 10% in both groups, which is in agreement with

experiments performed in cell cultures with the Swedish mutation. Mutation-carriers below the age of expected onset of the disease had the same levels of A β as already affected cases. This indicates that APP mismetabolism may play an important role early in the pathogenesis of the disease.

2.1.4 A β 42 in CSF in Alzheimer's disease

ELISAs specifically measuring A β 40 and A β 42 in CSF in AD cases have given different results. Some researchers (*Pirtilä 1994; Motter 1995*) have found decreased A β 42 in AD, while one group have found elevated levels in cases early in the disease progression. The most demented cases in one study had all very low levels of A β 42. In conclusion, A β 42 is most likely increased early in the disease process and levels of A β 42 and A β 40 decreased during progression of the disease. The development of accurate biochemical markers of early AD is important especially when efficient pharmacological treatments will be available in the future. Pharmacological therapy should most likely be initiated at an early stage of disease, before severe brain damage has occurred. A therapy making it possible to prevent the progression of the disease to its later stages would therefore be much desired.

2.2 Prevention and treatment of Alzheimer's disease

Antibodies that are specific towards different conformations of A β , such as A β fibrils (*O'Nuallain 2002*), micellar A β (*Kayed 2003*), ADDL (M93, M94) (*Lambert 2001*) have also been described.

Several pre-clinical studies in transgenic animal models have shown decreased plaque burden and improvements in memory function after active or passive immunization with antibodies raised against fibrils (*Shenk 1999, Janus 2000, Morgan 2000, Weiner 2000, Sigurdson 2001*). Since fibrils are present in pathological deposits occurring late in the Alzheimer disease process, the Shenk antibodies may only be used to slow the progression of Alzheimer's disease when it has already reached its later stages.

Recently, a phase II clinical trial in Alzheimer patients with mild to moderate dementia was performed by ELAN Pharmaceuticals with their vaccine AN 1792, which is an aggregated preparation of human wtA β 42. The study had to be stopped

due to side effects in 5% of the patients. The side effect was considered to be due to T-lymphocyte-induced meningoencephalitis (*Nicholl 2003*). The drug targets of the ELAN vaccine were insoluble fibrils found in plaques inside the brain and deposits on the brain blood vessel walls (Congophilic Amyloid Angiopathy, CAA), which are common features of Alzheimer's disease. Thus, an immune response towards insoluble fibrils could be responsible for the invasive inflammation in the brain blood vessel walls leading to meningoencephalitis.

WO02/203911 disclose the discovery of the Arctic mutation in a Swedish family leading to early onset of Alzheimer's disease (55.6 years). The Arctic mutation (Glu>Gly), which is located at position 22 in the beta amyloid peptide (A β), in combination with various experiments led to the insight that the A β peptide was much more prone to oligomerize and form protofibrils compared to wild type A β 40. The discovery indicated for the first time that the protofibril is a central component in the disease process, and that AD could be treated by reducing the amount of protofibrils in the brain. This unique property of the Arctic mutation could then be used to generate protofibrils. WO02/203911 then suggested that said protofibrils could be used to immunize a mouse or other animal, in order to generate antibodies, after which any protofibril-specific monoclonal antibody could be identified by screening. Said antibodies should then be specific towards an A β peptide of SEQ ID No 1 (page 7, third paragraph), i.e. a peptide carrying the Arctic mutation and having a protofibril conformation.

Thus, in view of the prior art techniques for preventing and treating Alzheimer's disease, there is a need for a technique that enables earlier detection of markers of Alzheimer's disease. If said markers could be prevented without causing negative side-effects, this would be a means to prevent and treat Alzheimer's disease at an early stage. Any treatment of Alzheimer's disease that would reduce the amount of protofibrils in the brain of AD patients, would be of significant therapeutic value.

3. DESCRIPTION OF THE INVENTION

Protofibrils occur early in Alzheimer's disease, when little brain damage has occurred. An antibody treatment that reduce protofibril levels in the brain will save the brain from neuronal destruction and hence be more advantageous for Alzheimer

patients. The present invention describes the development of such therapeutic protofibril specific antibodies.

According to one aspect, the present invention relates to antibodies that have the property to bind both wild type A β 42/40 and A β 42/40arc protofibrils (conformation-specific antibodies) and which are suitable for development into pharmaceuticals for Alzheimer's disease, targeting wild type A β 42/40 protofibrils. Current ideas in the field of Alzheimer's disease research is that A β fibrils are the main cause of the disease. Hence, the approach of the present invention contradicts the general opinion within the field.

According to another aspect, the present invention also relates to a composition comprising said antibody and optionally a carrier or excipient.

To immunise and screen for conformation-specific anti-protofibril antibodies, it is necessary to produce pure A β 42arc and A β 42 protofibrils (>95% degree of purity). In order to obtain said pure protofibril preparation, it is also necessary to be able to stabilise the protofibrils, such that they stay protofibrils and do not separate into monomers or aggregate into fibrils. It is also necessary to find a solvent that made it possible to separate the protofibrils by column chromatography. Another necessity is the ability to test said purity before immunisation. The above-mentioned abilities are essential, since the degree of purity of the antigen (protofibrils) decide the possibility to produce conformation-specific anti-protofibril antibodies, which in turn decide the possibility to screen for antibodies with useful specificity. Should the preparation for example contain 50% A β monomer and 50% A β protofibril, it is only possible to claim that the antibody binding said preparation may bind both forms or only one. All of said necessary features are provided by the present invention.

The above-mentioned features were not possible when WO02/203911 was filed. The HPLC analysis method disclosed in the present application or any other analysis method had not been developed, wherefore it was not possible to determine whether the A β 42arc peptide actually formed protofibrils, probably due to the lack of binding to protofibrils. It was only a hypothesis at the time. The major reasons why WO02/203911 could not analyse and purify protofibril preparation to a degree of

purity of >95%. Also, to the fact that they did not know how to stabilise protofibrils. In that connection, it should also be mentioned that protofibrils are very difficult to handle in comparison with for example A β 40 peptides. A β 42, but in particular the A β 42Arc peptide, is extremely difficult to handle, since they stick to test tubes and columns and aggregate into fibrils. The latter problems has been solved by the present invention by the provision of methods to stabilise the protofibrils involving low temperatures and selected solvents. WO02/203911 also had no means for testing the purity of the protofibrils before immunisation. This is possible with the present invention by the provision of a new HPLC (size exclusion) method. In addition to this, WO02/203911 did not provide any means to check the protofibril specificity of the screened antibodies. The present invention makes this possible by the provision of a new Elisa method. WO02/203911 could only produce impure and transient A β 40arc protofibrils, not useful for monoclonal development of protofibril specific antibodies (WO02/203911 only describes analysis of A β 40 and A β 40Arc peptides, see Example 3).

The antibodies according to the present invention are particularly suitable for treating Alzheimer's disease since they will: i) target and eliminate the most toxic A β form (protofibril), ii) provide an opportunity to treat the disease early since protofibrils develops early in the disease process, iii) avoid side-effects since they will not significantly cross-react with A β fibrils present in the blood vessel wall of the brain (CAA= Congophilic Amyloidogenic Angiopathy).

The antibodies according to the present invention may be human or humanised, monoclonal or polyclonal. The present invention also relates to biologically active fragments of said antibodies, with the proviso that they still have the claimed properties.

The A β protofibril specific antibodies according to the present invention can also be used to quantitate wild type A β 42/40 protofibrils in biological fluids, thus the antibodies will be suitable for clinical diagnosis at an early stage of the disease and suitable as a biomarker to monitor efficacy in clinical studies.

Furthermore, the invention describes procedures to generate wild type A β 42 and A β 42arc protofibrils as antigens for immunization and for reagents to screen for antibodies that binds A β 42arc and wild type A β 42 protofibrils.

Antigen (protofibril) purity is very important to determine in antibody screening experiments, since it affects antibody specificity determinations by ELISA.

To assess the purity of antigen (A β 42arc and wild type A β 42 protofibrils) preparation, a size exclusion HPLC method has been developed. The HPLC method invented uses a detergent, with low optical interference in chromatography. The detergent has the advantage that it eliminates interactions of A β 42arc and wild type A β 42 protofibrils with the column matrix, preventing loss of material and material from adhering to the column, and erroneous estimations of protofibril purity. Furthermore, the detergent is compatible with A β protofibrils and does not solubilize them into A β monomers or alter their biological profile. Polysorbate (Tween) has been found to be a suitable detergent, in particular Polysorbate-80 (Tween-80). Other detergents with similar properties are also useful.

The antibody according to the present cross-reacts less than 50% with A β fibrils. Said antibody can detect both wild type A β protofibrils and Arctic A β protofibrils in an ELISA in a concentration range of 1000-10 ng/ml (see Example 5 and Figures 4A-C). In an optimised ELISA or by other detection systems with higher sensitivity, for example proximity ligation, said detection level could probably be brought down to a detection level of 1-0.1 ng/ml.

The present invention provides a method to raise antibodies that are specific to amyloid β (A β) protofibrils (high molecular weight soluble non-fibrillar A β oligomers). The antibodies are raised against A β in its protofibril conformation. These antibodies will be administered to AD patients to reduce protofibril levels in the brain, which will be of significant therapeutic value.

The lowering of protofibril levels might occur through the elimination of the antigen when bound to an antibody, through microglia-mediated phagocytosis. This is a well documented biological process, which occurs through the binding of the antibody's constant region (Fc) to an Fc receptor on microglial cells. Binding induces phagocytosis (internalisation and destruction) of the antibody and its

bound antigen (protofibril) leading to reduced levels of protofibrils in the brain. Other non-Fc receptor mediated processes might also occur to eliminate the antigen (protofibril).

Another aspect of the invention pertains to a method to synthesize A β protofibrils which are to be used as antigen for immunization. The synthesis can be made from wild type A β (wtA β) or alternatively, from non-wild type, mutated or modified forms of A β . In a preferred embodiment of the invention, but not limited to said embodiment, the synthesis is initiated by dissolving wtA β 1-42 in a dissociating agent, for example NaOH, to achieve a homogenous A β solution, of about 50-500 μ M in peptide concentration, but not limited to this concentration. Alternative agents with dissociating capacity are for example dimethylsulfoxide, DMSO; hexafluoroisopropanol, HFIP; trifluoroacetic acid, TFA. Wild type protofibrils can also be made from wtA β peptides of different lengths i.e. wtA β 1-39, wtA β 1-40, wtA β 1-43 or N-terminal truncated forms of these peptides. The truncation can be 1-10 amino acids, giving wtA β forms such as: wtA β 2-42, wtA β 3-42, wtA β 4-42, wtA β 5-42 and so on. The dissolved wtA β 1-42 peptide is subsequently neutralized by PBS or similar physiological buffer and incubated at higher temperature, preferably 37°C, for a period of time sufficient for protofibril-formation to occur, for example over night. This will yield wild type A β protofibrils. The invention also provide a molecular size (molecular weight) determination method to asses protofibril formation and purity, preferably, but not limited to, a size-exclusion chromatographic method (SEC).

The method of synthesizing pure wild type (wtA β 42) protofibril antigen, comprises the steps of dissolving an wtA β 42 by using a dissociating agent, such as NaOH (pH>10), dimethylsulfoxide, DMSO; hexafluoroisopropanol, HFIP; trifluoroacetic acid and TFA, to achieve a monodisperse solution, neutralizing the solution by PBS (pH 7-8) or similar biocompatible buffers, to achieve a physiological solution, incubating the neutralized wtA β 42 peptide solution at an elevated protein concentration between 1-1000 μ M, preferably 440 μ M, for 6 hours or longer at 20-40 °C, preferably 37 °C, to form A β 42wt protofibrils, diluting the protofibrils to approximately 1-500 μ M, preferably 50 μ M centrifuging at sufficient speed and time to sediment wtA β 42 fibrils, which normally takes 5 minutes at 17.000 x g at +4°C,

assessing the purity of the protofibril preparation by HPLC to control that the purity is >95%, using for example size exclusion HPLC, using a physiological buffer such as PBS at neutral pH as running buffer, including a detergent, such as Polysorbate (Tween), or similar detergent, to avoid sticking of the protofibrils to the column matrix and dissociation.

Another aspect of the invention pertains to the synthesis of non-wild type A β protofibrils using A β 1-42, A β 1-41, A β 1-40 or A β 1-39 or N-terminal truncated forms (1-10 truncations) with either the Arctic (G22E) mutation (US 09/899,815), the Dutch mutation (E22Q), the Flemish (A21G) mutation, the Italian (E22K) mutation, the Iowa (D23N) mutation, and combinations thereof. In a preferred embodiment of the invention A β 42arc peptide (i.e., comprising the arctic mutation) is used. The method to make A β 42arc protofibrils is similar to that for wtA β 42 protofibrils except that A β 42arc protofibrils are not incubated at 37° C over night since they are spontaneously formed after the neutralization step.

The method of synthesizing pure A β 42arc protofibril antigen comprises the steps of dissolving an A β 42arc peptide by using a dissociating agent, such as NaOH (pH>10), dimethylsulfoxide, DMSO; hexafluoroisopropanol, HFIP; trifluoroacetic acid and TFA to achieve a monodisperse solution, neutralizing the solution by PBS (pH 7-8) or similar biocompatible buffers, to achieve a physiological solution, stabilizing the spontaneously formed protofibrils by keeping them at below 20° C, preferably at 0-5° C, centrifuging the protofibrils, at the same temperature as in the stabilising step, at a sufficient speed and time to sediment A β 42arc fibrils, which normally takes 5 minutes at 17.000 x g at +4°C, assessing the purity of the protofibril preparation by HPLC to control that the purity is >95%, using for example size exclusion HPLC, using a physiological buffer such as PBS at neutral pH as running buffer, including a detergent, such as Polysorbate (Tween), or similar detergent, to avoid sticking of the protofibrils to the column matrix and dissociation.

Another aspect of the invention pertains to the development of antibodies that cross-react with wild type A β 42 protofibrils after immunization with A β 42arc protofibrils.

Another aspect of the invention pertains to a method to stabilize A β protofibril and where stabilization can be assessed by size-exclusion chromatography (SEC). A β protofibrils elutes after 12-13 minutes in a uniform peak on a Superdex 75 or similar size-exclusion column. Conformation stability can also be assessed by staining with Congo Red and (electron microscopy), where protofibrils attain a curve-linear structure of 6-10 nm in diameter and <200 nm in length. Lowered temperature has a significant effect on A β protofibril conformational stability. Samples should preferably be kept below 20° C, preferably below 5° C, and most between 0° C and 5° C. Furthermore, agents that decrease the polarity or surface tension or increase viscosity have stabilizing effects on A β protofibril conformation. For example, 10-50% glycerol or 0.6-5% Polysorbate (Tween), have stabilizing effects on A β protofibrils. These agents and treatments can be added to the A β protofibril preparation preferably after the neutralization step in the method to synthesize A β protofibrils described above. Addition of these agents before this step is also possible. Increased stability of protofibrils is advantageous when developing monoclonal antibodies and when A β protofibrils are used as reagents in immunoassays, such as ELISA, radio-immunoassay (RIA), Western blotting or dot blotting.

The method of stabilizing the A β protofibrils includes mixing them with an agent that decreases the solvent polarity or one that lowers the surface tension, such as glycerol or Polysorbate (Tween), or a combination of said agents. The stabilisation can also be achieved by keeping the protofibrils at a temperature below 20° C, preferably 0-5 °C. Said methods can also be combined.

The invention further pertains to the use of anti-A β protofibril specific antibodies for determinations of A β protofibrils in human and animal tissues, for example, cerebrospinal fluid, blood, serum, urine and brain tissue, but is not limited to these tissues, providing for a possible diagnostic method for Alzheimer's disease. Suitable methods for assaying A β protofibrils in these tissues as well as in cell cultures using an anti-A β protofibril antibody are immunoassays such as ELISA, RIA, Western blotting dot blotting or proximity ligation. The method would be suitable to

follow treatment efficacy (protofibril reduction) in clinical trials and suitable as a diagnostic test for Alzheimer's disease or Down's syndrome.

The method of diagnosing or monitoring Alzheimer's disease (AD) or Down's syndrome comprises the steps of labelling the antibody according to the present invention with an agent that can generate a measurable signal, administering said antibody according to a subject having or suspected of having AD or Down's syndrome, measuring the amount of protofibrils bound to the antibody by measuring the signal generated by the agent.

The invention further pertains to the use of an anti-A β protofibril antibody in imaging for detection, localization and quantitation of A β protofibrils in human and animal tissues. The anti-A β protofibril antibody could be label with a radioactive ligand such as I¹³¹, C¹⁴, H³ or Gallium⁶⁸, but it is not limited to these radioisotopes, for detection purposes. In addition to labelling with radioactive markers, DNA, fluorescent molecules, enzymes which converts a substrate such that its absorbance can be measured, could also be used. The method will be suitable as a diagnostic tool for Alzheimer's disease or Down's syndrome, Lewybody dementia, vascular dementia, and other neurodegenerative disorders.

A further aspect of the invention pertains to the use of an anti-A β protofibril antibody for the prevention or treatment of Alzheimer's disease. Pre-clinical studies in transgenic animal models have demonstrated effects on plaque burden (*Bard 2000*), reversal of memory deficits (*Dodard 2002*) and drainage of A β from CNS after anti-A β antibody treatment. However, the antibodies used in these studies have not been specific to A β protofibrils. Administration of an anti-A β protofibril antibody, with no or little cross-reactivity towards A β monomers and fibrils would be particular suitable for treatment and prevention of Alzheimer's disease. Firstly, it would not bind significantly to fibrillar forms of A β and avoid interaction with fibrillar deposits which are prevalent in blood vessel walls, avoiding severe immunoreactions with concomitant serious brain inflammation and encephalitis, which was encountered in the ELAN vaccination study (see at the end of the Background) with their vaccine AN-1792 (*Nicoll 2004*). Secondly, an anti-A β protofibril antibody with low A β 1-40 and A β 1-42 monomer cross-reactivity would

not bind and interfere with their normal biological functions, thus avoiding side effects.

In said method of prevention or treatment of Alzheimer's disease or Down's a subject having or suspected of having Alzheimer's disease, Down's syndrome, Lewybody dementia, vascular dementia, and other neurodegenerative disorders, is administered the antibody or composition according to the present invention.

The invention also provides a technique whereby anti-A β protofibril antibodies can be used to identify and select for epitopes present on A β protofibrils but less on A β monomers and A β fibrils or other A β conformational forms. The method is general and is applicable to other amyloids forming protofibrils such as, but not limited to, islet amyloid protein peptide (IAPP, amylin) associated with Type-2 diabetes, prion protein (PrP), alpha-synuclein (Parkinson). Considering the central role of amyloid beta (A β) protofibrils in Alzheimer's disease, these antibodies can be used to diagnose or treat Alzheimer's disease. There is a need for a method that specifically can determine protofibrils in different human and animal tissues such as cerebral spinal fluid (CSF), plasma, blood, urine and brain tissue, but is not limited to these tissues. Such a method could be used as a diagnostic method for Alzheimer's disease but also for similar diseases that forms amyloid protofibrils including Parkinson (alpha-synuclein) (*Stihlerman 2002*), Type-2 diabetes (Islet amyloid polypeptide, IAPP) (*WO03/092619 A2*), and Creutzfeldt-Jacob Disease and the corresponding animal disease called mad cow disease (Prion protein) (*DeMarco 2004*), but is not limited to these diseases.

According to another aspect, the present invention relates to the use of anti-A β protofibril antibodies for *in vitro* or *in vivo* screening of substances that inhibit or modulate A β protofibril levels and/or activity in cell cultures or animal models, being potential Alzheimer drugs. Suitable screening systems would be, but are not limited to, cell cultures (HEC or neuroblastoma cells) or for example the Thy-1 APPSweArc transgenic mouse model, (Nilsson L. et al. Swedish patent application 0400707-6, 2004) that expresses human Amyloid Precursor Protein (APP) with the Arctic mutation (E693G) providing increased production of A β protofibrils. Alzheimer drug candidates can be administered to these cell cultures or transgenic

animal models and their effects on A β protofibril levels measured by an immunoassay, using an anti-A β protofibril antibody as reagent. Such method would be ideally suitable for identifying potent Alzheimer drug candidates.

Said method for *in vitro* or *in vivo* screening of substances that inhibit or modulate A β protofibril levels and/or activity in cell cultures or animal models, comprises the steps of administering potential drug candidates to a cell culture or an animal model, administering the antibody according to any the present invention, labelled with an agent that can generate a measurable signal, to said cell culture or animal model, evaluating the effect of said drug candidates by measuring the amount of protofibrils bound to the antibody by measuring the signal generated by the agent.

According to another aspect, the present invention relates to a method of detecting A β protofibrils *in vitro*, comprising labelling the according to the present invention with an agent that can generate a measurable signal, contacting said antibody or the composition comprising the antibody with a biological sample suspected of containing soluble protofibrils, measuring the amount of protofibrils bound to the antibody by measuring the signal generated by the agent. Said method may be an immunoassay. The biological samples tested may be selected from plasma, CSF, brain and other tissues of animal or human origin. The labelling includes labelling with radioactive markers, DNA, fluorescent molecules, enzymes which converts a substrate such that its absorbance can be measured.

All percentages in the description relates to percent volume, unless stated otherwise.

4. DESCRIPTION OF THE DRAWINGS

Figure 1. Size exclusion chromatography (SEC) of Human Monomer A β 40, Protofibril A β 42Arc, Protofibril wtA β 42 and Fibril wtA β 42 preparations

Human A β monomers and A β protofibrils are eluting in 19-20 minutes and at 12-13 minutes, respectively. The protofibril preparations (B and C) are essentially free (<3%) from contamination of other conformational A β forms. The analysis of the fibril preparation (D) was done after centrifugation at 17.900 x g for 5 minutes at

room temp. The supernatant was analysed demonstrating the almost complete absence of any soluble A β forms in the fibril preparation.

Figure 2. Stability study of protofibrils

The stability of Protofibril A β 42Arc and Protofibril wt42 preparations was measured by SEC. A conversion of protofibrils to fibrils was assessed by a decrease in the protofibril peak area (decreased absorbance (AU) at 214 nm elution time 12-13 minutes). The addition of 10-50% glycerol, 0.6% Tween-20 or storage at 0-5° C, all increased the protofibril stability significantly.

Figure 3. Titration of mouse serum after immunization with protofibril wtA β 42

Mice were immunized with a protofibril wtA β 42 preparation in Freund complete adjuvant (first injection) and incomplete Freund adjuvant (5-6 boosters). Blood was collected and the antibody titers against A β protofibrils were determined. Mouse #2 and #4 showed the highest titers.

Figure 4. Sandwich-ELISA method using anti-mAb 258 for A β protofibril determination

mAb 258 was coated over night in a microtiterplate. Preparations of wtA β 42 protofibrils, A β 42Arc protofibrils, wtA β 40 monomers and wtA β 42 fibrils were added in decreasing concentrations and incubated for 1 hour at +4° C. Secondary anti-A β antibody (6E10) was added. Finally, a detection ALP (alkaline phosphatase) conjugated antibody (anti-IgG) was added to each well. Detection was achieved by adding ALP substrate. The colour formation was measured in at 405 nm and 492 nm.

mAb 258 bound wtA β 42 protofibrils and slightly less A β 42Arc protofibrils.

No binding was observed to wtA β 40 monomers. The slight binding to wtA β 42 fibrils is probably due to a slight contamination with wtA β 42 protofibrils (see Figure 1 D).

5. EXAMPLES

The following examples are provided for illustration and are not intended to limit the invention to the specific examples provided.

Example: 1 *Synthesis of human amyloid beta peptide (A β) of different conformations*

Synthetic human wild type or mutant forms of A β 1-42 or A β 1-40 peptides were purchased from Polypeptide Laboratories GmbH. The peptides had been synthesized by a standard solid phase peptide synthesis procedure. The peptides were subsequently purified with RP-HPLC to a purity in the range of 90-95%. Alternative vendors producing A β peptides with similar purity are possible to use as well.

Synthesis of human wild type A β 1-40 monomers

A synthetic wtA β 1-40 peptide was dissolved in 1 volumes of 10 mM NaOH pH>10, and 1 volume of cold 2 x PBS (pH 7-8) to a concentration of 50 μ M. The wtA β 1-40 monomer preparation was centrifuged at 17.900 x g at +4 $^{\circ}$ C for 5 minutes prior to analysis (Figure 1).

Synthesis of human wild type A β 1-42 protofibrils

A synthetic wtA β 1-42 peptide was dissolved in 9 volumes of 10 mM NaOH pH>10, vortexed for 2 minutes and diluted with 1 volume of 10 x PBS (pH 7-8). The final peptide concentration was at this point 443 μ M. The peptide was further incubated over night at 37 $^{\circ}$ C. After the overnight incubation, the peptide was further diluted with PBS to 50 μ M. The sample was centrifuged for 17.900 x g for 5 minutes prior to analysis and immunisation.

Synthesis of human A β 1-42Arc protofibrils

A synthetic A β 1-42Arc (E22G) peptide was dissolved in 1 volumes of 10 mM NaOH pH>10 and 1 volume cold 2 x PBS (pH 7-8) to a final peptide concentration of 50 μ M. The A β 1-42Arc protofibrils were formed immediately and the A β 1-42Arc protofibrils were stabilized by keeping the solution at 0-4 $^{\circ}$ C before analysis or immunization. The sample was centrifuged at 17.000 x g for 5 minutes at +4 $^{\circ}$ C prior to analysis. Alternative ways to stabilize the A β 1-42Arc protofibrils were to add glycerol to a final concentration of 5-50 % or to add Tween-20 to a final concentration of 0.6%.

Synthesis of human wild type A β 1-42 fibrils

A synthetic wtA β 1-42 peptide was dissolved in 1 volumes of double distilled water, vortexed for 2 minutes and diluted with 1 volume of 2 x PBS (pH 7-8) and vortexed

again for 2 minutes. The final peptide concentration at this point was 50 μ M. The wtA β 1-42 protofibril preparation was incubated at 37° C, for 48 hours before analysis. The sample was centrifuged at 17.900 x g for 5 minutes prior to SEC analysis. The supernatant was analysed after centrifugation. No centrifugation was performed when A β 1-42 fibril preparations were analysed by ELISA or dot blotting.

Analysis of A β preparation by Size Exclusion Chromatography (SEC)

A Merck Hitachi D-7000 HPLC LaChrom system, having a diode array detector (DAD) model L-7455 and a model L-7100 pump, was used for the chromatographic analysis of protofibril preparations in combination with a Superdex 75 PC3.2/30 column (Amersham Pharmacia Biotech, Uppsala, Sweden). The chromatographic system separates A β monomers from protofibrils, which are eluting at the void volume of the column. The column was equilibrated with 50 mM Na₂HPO₄ · NaH₂PO₄ (pH 7.4) with 0.15 M NaCl (PBS) and 0.6% Tween 20 and eluted with the same buffer at a flow rate of 0.08 ml/min (pressure was 5-6 bars) at ambient temperature (22°C). Ten (10 μ l) of a 50 μ M-100 μ M A β sample was subjected analysis using a wavelength scan between 200-400 nm. Tween-20 was added to the sample to give a final concentration of 0.6% prior to chromatography. Data was extracted from measurements at 214 and 280 nm. Peak areas were integrated using Merck Hitachi model D-7000 Chromatography Data Station Software. Figure 1 shows chromatograms of wtA β 1-40 monomer, wtA β 1-42 protofibril, A β 1-42Arc protofibril and wtA β 1-42 fibril preparations.

Each preparation was essentially free (< 3%) of other contaminating conformational A β forms except the wtA β 1-40 monomer preparation which contained 6.4 % protofibrils (elution time 12-13 minutes).

Example 2: Protofibrils specific monoclonal antibody development.

A standard procedure was used for monoclonal development. Mice were injected with wtA β 1-42 protofibril preparation. Alternatively, A β 1-42Arc protofibrils could be used. Every wtA β 1-42 protofibril batch was assayed by SEC and Congo Red (binds β -sheet structures in proteins) before immunisation to ascertain protofibril purity and that the preparation contained β -sheet structure. The procedure used to immunise mice was a standard protocol involving s.c injections in the presence of

Freund adjuvant. Each of six mice were immunized with 10 ug wtA β 1-42 protofibrils and subsequently boosted with 30 ug. The sample was mixed 1:1 with Freund complete adjuvant prior to the first injection. For subsequent 5 booster injections incomplete Freund adjuvant was used. Mouse n was not immunized (control). Mice were bled and blood collected for antibody titer determinations (Figure 3). Mice were given one more booster (30ug) and then sacrificed and the spleen collected for hybridom development. The hybridom preparation method used was according to standard procedure (*Harlow 1988*).

Example 3: Screening for wtA β 1-42 protofibril specific antibodies

An ELISA method was developed where by hybridom supernatants were screened for antibodies that bind A β 1-42 protofibrils. Hybridom supernatant #258 showed high protofibril specificity (Figure 3). The hybridom #258 was reseeded and screened again to ascertain a homogenous cell line. The monoclonal antibody that was produced from the #258 hybridom was defined as monoclonal antibody 258 (mAb258). Alternatively, screening (binding to A β protofibrils but not to A β monomers or fibrils) can be performed against an antibody phage display library, where the library has been made from RNA isolated from animals immunized with protofibril.

Example 4. Characterization of mAb 258 by Western blot analysis.

The aim of the experiment was to determine if mAb 258 cross-reacts with wild type human amyloid precursor protein (wtAPP) or mutated human APP, APP^{swe}, APP^{swe-arc} (*Nilsberth 2001, Mullan 1992*) all of which contains uncleaved A β 1-42. Cells were transfected with plasmids expressing human wtAPP, APP^{swe} and APP^{swe-arc}. Cells were subsequently harvested and solubilized and cellular proteins separated by SDS-PAGE and transferred to nitrocellulose filter papers and subsequently incubated with either mAb 258 or mAb 6E10. Specific binding of these antibodies to separated cellular proteins was detected by incubating the filter papers in a secondary anti-mouse IgG/IgM antibody solution and subsequent developed by incubation with Pierce super signal (art nr 34080-P) and a light sensitive film.

mAb 258 showed no binding to wtAPP, APP^{swe} or APP^{swe-arc} nor to wtA β 40 monomer. The commercial mAb 6E10 bound all these forms.

Example 5: Sandwich ELISA for protofibril characterization and determinations

The specificity of mAb 258 was determined by a sandwich-ELISA.

An 96-well ELISA plate was coated with mAb 258 over night at +4°C. After coating, wells were blocked with BSA for 1 hour at room temp. A β samples i.e wtA β 40

monomers, wtA β 42 protofibrils, wtA β 42-Arc protofibrils wtA β 42 fibrils, were added to the microtiterplate in 5 x dilutions starting with 10 ug/ml. Samples were incubated for 1 hour at +4°C, after which 10 ng/well of a commercial secondary antibody, 6E10 (Signet) was added and incubated for 1 hour at room temp.

Detection was achieved by incubation with a commercial ALP-conjugated anti-IgG antibody for 1 hour at room temp. and subsequent incubation with the substrate at room temp. for one hour according to the manufacturer's procedure. Samples were read in a microtiterplate reader (Spectra max 190 Molecular Devices, Sunnyvale, USA) at 405 nm and 492 nm. Figure 4A.

mAb 258 showed little or no cross-reactivity towards wtA β 40 monomers or wtA β 42 fibrils. Concentrations of wtA β 42 protofibrils down to 10 ng/ml were measurable.

Binding of an anti-A β 42arc protofibril specific antibody (7E4)(A β 42arc in protofibril conformation was used as a antigen during immunization) was measured in the sandwich-ELISA coated with increasing concentrations of wtA β 42 protofibrils.

Detection was achieved by either an A β -specific mAb (1C3) as a secondary detection antibody (Figure 4B) or by the commercial mAb 6E10, as secondary antibody (Figure 4C). Strong binding of mAb 7E4 was achieved to wtA β 42 protofibrils. Concentration levels as low as 2-5 ng/ml of wtA β 42 protofibrils were measurable. The monoclonal antibodies 4E11 and 10F7 show less strong binding to wtA β 42 protofibrils (Figure 4B and 4C).

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CLAIMS

1. An antibody specific for soluble amyloid beta peptide (A β) protofibrils and/or biologically active fragments thereof.
2. The antibody according to claim 1, wherein said antibody is specific for wild type A β protofibrils.
3. The antibody according to claim 2, wherein said antibody is specific for human wild type A β protofibrils.
4. The antibody according to claim 3, wherein said antibody is specific for human wild type A β 42 protofibrils.
5. The antibody according to any of claims 1-4, wherein said antibody cross-reacts less than 50% with A β fibrils.
6. The antibody according to claims any of claims 1-5, wherein said antibody can detect both wild type A β protofibrils and Arctic A β protofibrils in an ELISA in the concentration range of 1000-10 ng/ml.
7. The antibody according to claims any of claims 1-5, wherein said antibody can detect both wild type A β protofibrils and Arctic A β protofibrils in an ELISA in the concentration range of 1000-0.1 ng/ml.
8. The antibody according to any of claims 1-7, wherein said antibody is polyclonal.
9. The antibody according to any of claims 1-7, wherein said antibody is monoclonal (mAb).
10. The antibody according to any of claims 1-7, wherein said antibody is mAb 7E4, mAb 4E11 or mAb 10F7.

11. The antibody according to any of claims 1-10, wherein said antibody has been raised against protofibrils comprising A β selected from the group consisting of A β 1-39, 1-40, 1-42, 1-43, and combinations thereof.
12. The antibody according to any of claims 1-10, wherein said antibody has been raised against protofibrils comprising A β selected from the group consisting of A β 1-39, 1-40, 1-42, 1-43 truncated at the N-terminal end with up to ten (10) amino acids, and combinations thereof.
13. The antibody according to claims 1-12, wherein said antibody has been raised against protofibrils comprising said A β peptides containing one or several mutations selected from the group consisting of the Arctic (G22E mutation, the Dutch mutation (E22Q), the Flemish (A21G) mutation, the Italian (E22K) mutation, the Iowa (D23N) mutation, and combinations thereof.
14. The antibody according to claims 1-13, wherein said antibody is human or has been humanized.
15. A composition comprising one or more antibodies according to any of claims 1-14 and optionally a carrier or excipient.
16. A method of detecting A β protofibrils *in vitro*, comprising:
 - labelling the antibody according to any of claims 1-14 or in the composition according to claim 15 with an agent that can generate a measurable signal,
 - contacting said antibody or the composition comprising the antibody with a biological sample suspected of containing soluble protofibrils,
 - measuring the amount of protofibrils bound to the antibody by measuring the signal generated by the agent.
17. The method according to claim 16, wherein said detection method is an immunoassay.

18. The method according to any of claims 16-17, wherein said biological sample is selected from plasma, CSF, brain and other tissues of animal or human origin.
19. The method according to any of claims 16-17, wherein the labelling includes labelling with radioactive markers, DNA, fluorescent molecules, enzymes which converts a substrate such that its absorbance can be measured.
20. Use of the antibody according to any of claims 1-14 or the composition according to claim 15 for prevention or treatment of Alzheimer's disease or Down's syndrome in a subject of having or suspected of having Alzheimer's disease, Down's syndrome, Lewybody dementia, vascular dementia, and other neurodegenerative disorders.
21. A method for *in vitro* or *in vivo* screening of substances that inhibit or modulate A β protofibril levels and/or activity in cell cultures or animal models, comprising the steps of:
 - administering potential drug candidates to a cell culture or an animal model,
 - administering an antibody according to any of claims 1-8 or in the composition according to claim 9, labelled with an agent that can generate a measurable signal, to said cell culture or animal model
 - evaluating the effect of said drug candidates by measuring the amount of protofibrils bound to the antibody by measuring the signal generated by the agent.
22. A method of synthesizing pure A β 42arc protofibril antigen, comprising the steps of:
 - dissolving an A β 42arc peptide by using a dissociating agent, to achieve a monodisperse solution,
 - neutralizing the solution by PBS (pH 7-8) or similar biocompatible buffers, to achieve a physiological solution,

- stabilizing the spontaneously formed protofibrils by keeping them at below 20° C,
- centrifuging the protofibrils, at the same temperature as in the stabilising step, at a sufficient speed and time to sediment A β 42 arc fibrils,
- assessing the purity of the protofibril preparation by HPLC to control that the purity is >95%.

23. The method according to claim 22, wherein the dissociating agent is selected from NaOH (pH>10), dimethylsulfoxide, DMSO; hexafluoroisopropanol, HFIP; trifluoroacetic acid and TFA.
24. The method according to claim 22 or 23, wherein the temperature is the stabilising step is 0-5° C.
25. The method according to any of claims 22-24, wherein the purity assessment is achieved by using size exclusion HPLC, using a physiological buffer at neutral pH as running buffer, including a detergent, to avoid sticking of the protofibrils to the column matrix and dissociation.
26. The method according to claim 25, wherein the detergent in the purifying step is Polysorbate (Tween) or similar detergent.
27. A method to synthesize pure wild type A β 42 protofibril antigen, comprising the steps of:
 - dissolving an A β 42arc peptide by using a dissociating agent, to achieve a monodisperse solution,
 - neutralizing the solution by PBS (pH 7-8) or similar biocompatible buffers, to achieve a physiological solution,
 - incubating the neutralized A β 42wt peptide solution at an elevated protein concentration between 1-1000 uM, for 6 hours or longer at 20-40 °C, to form A β 42wt protofibrils,
 - diluting the protofibrils to 1-500uM,
 - centrifuging at sufficient speed and time to sediment A β 42 wt fibrils,

-assessing the purity of the protofibril preparation by HPLC to control that the purity is >95%.

28. The method according to claim 27, wherein the dissociating agent is selected from NaOH (pH>10), dimethylsulfoxide, DMSO; hexafluoroisopropanol, HFIP; trifluoroacetic acid and TFA.
29. The method according to any of claims 27-28, wherein the protofibrils are diluted to approximately 50uM in the dilution step.
30. The method according to any of claims 27-29, wherein the purity assessment is achieved by using size exclusion HPLC, using a physiological buffer at neutral pH as running buffer, including a detergent, to avoid sticking of the protofibrils to the column matrix and dissociation.
31. The method according to claim 30, wherein the detergent in the purifying step is Polysorbate (Tween) or similar detergent.
32. A method of diagnosing or monitoring Alzheimer's disease (AD) or Down's syndrome, comprising the steps of:
 - labelling the antibody according to any of claims 1-14 or in the composition according to claim 15 with an agent that can generate a measurable signal,
 - administering said antibody to a subject having or suspected of having AD or Down's syndrome,
 - measuring the amount of protofibrils bound to the antibody by measuring the signal generated by the agent.
33. A method of prevention or treatment of Alzheimer's disease (AD) or Down's syndrome comprising the step of administering the antibody thereof according to any of claims 1-14 or the composition according to claim 15 to a subject having or suspected of having AD or Down's syndrome.

34. A method of stabilizing A β protofibrils by mixing them with an agent that decreases the solvent polarity or one that lowers the surface tension, or a combination of said agents, and/or keeping the protofibrils at a temperature below 20° C.
35. The method according to claim 34, wherein said agent is glycerol or Polysorbate (Tween), or combinations thereof.
36. The method of stabilizing A β protofibrils according to claim 34 or 37, wherein the A β protofibrils are kept at a temperature between 0-5 °C.

FIG. 1

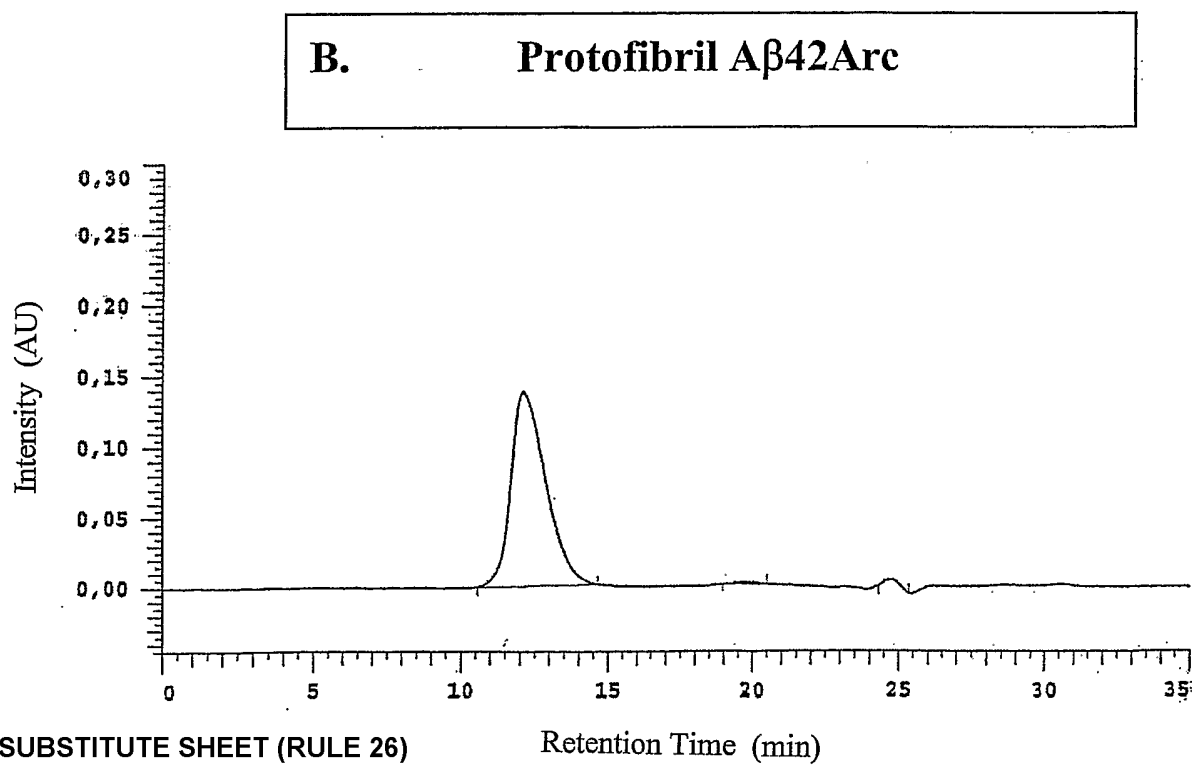
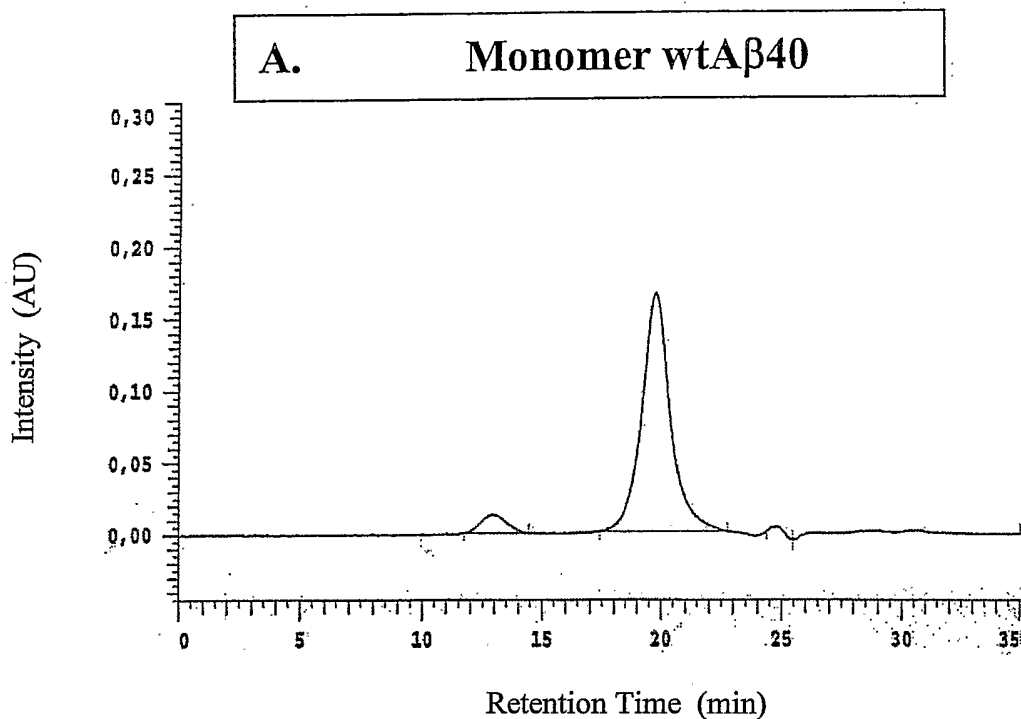


FIG. 1 (cont.)

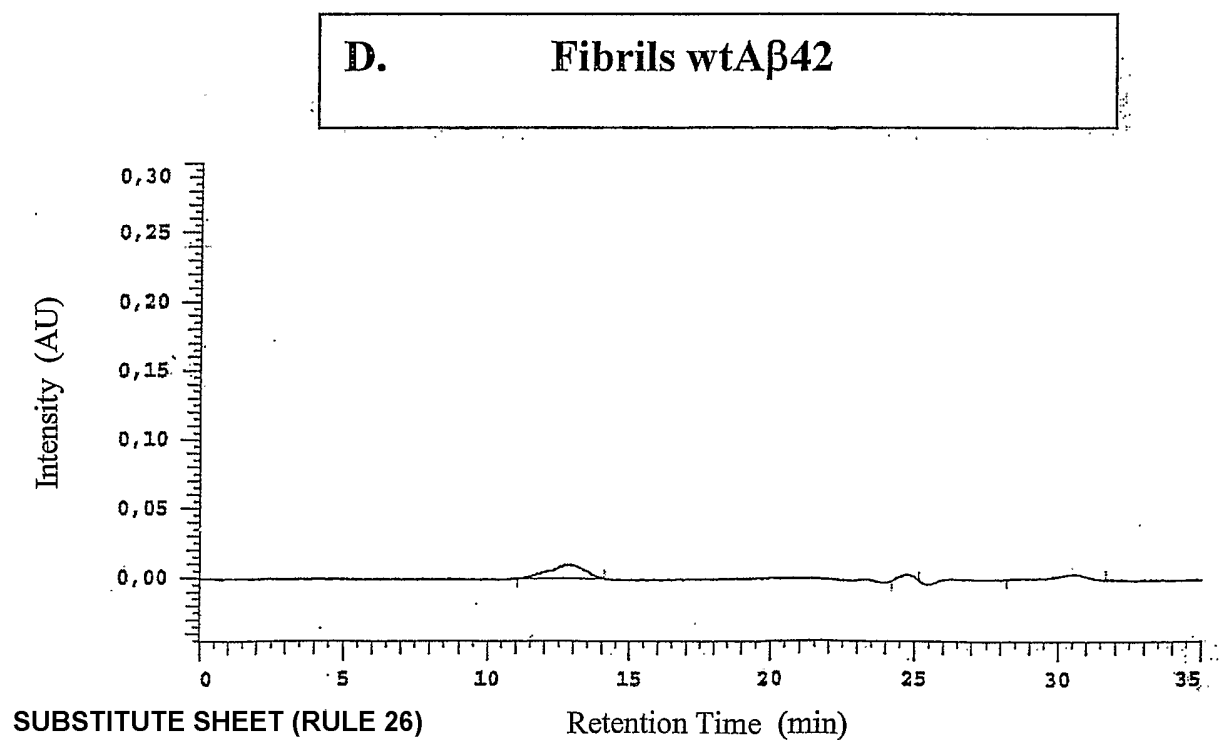
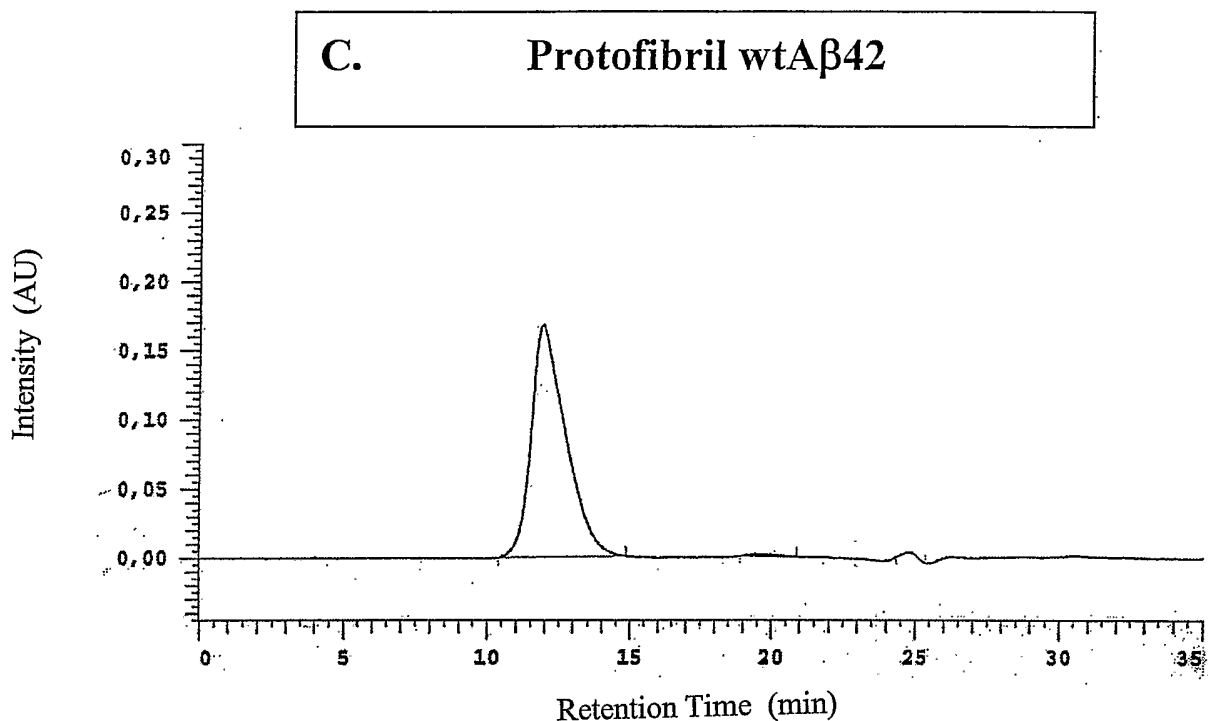


FIG. 2

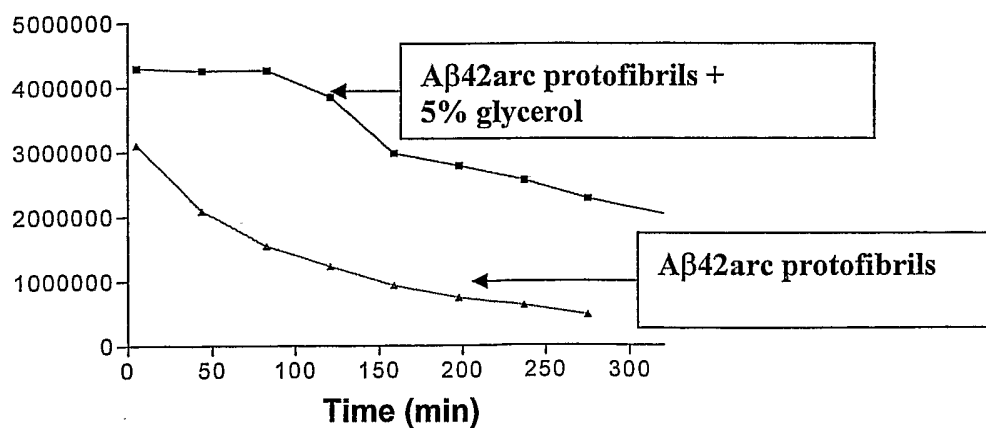
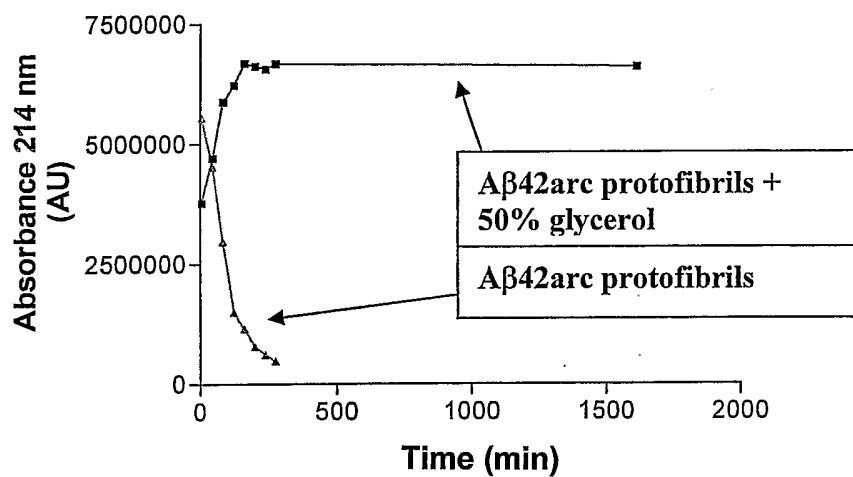


FIG. 2 (cont.)

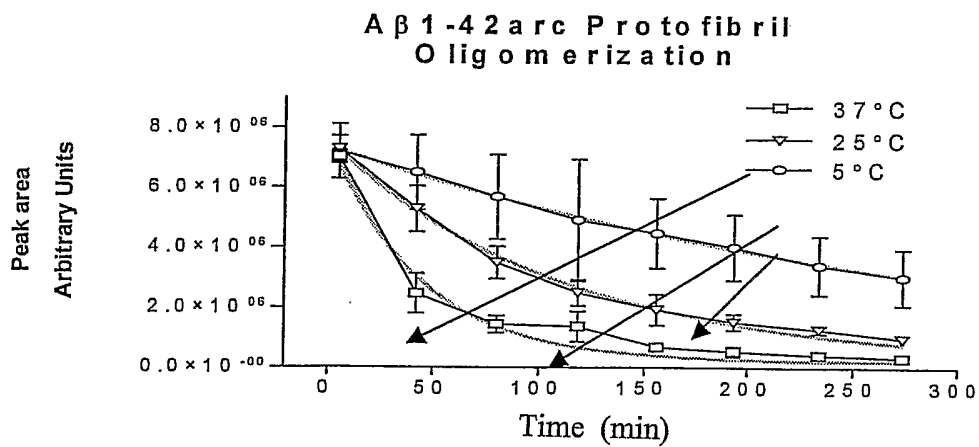
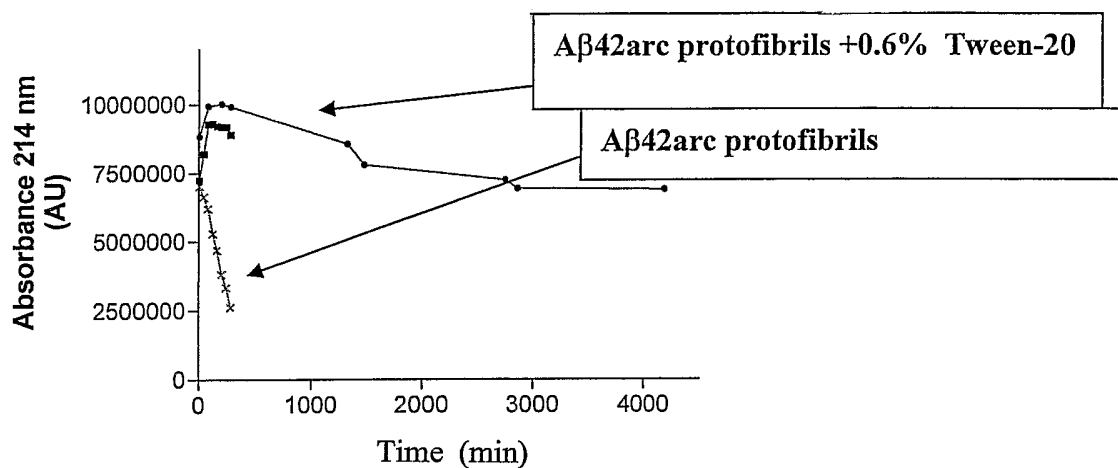
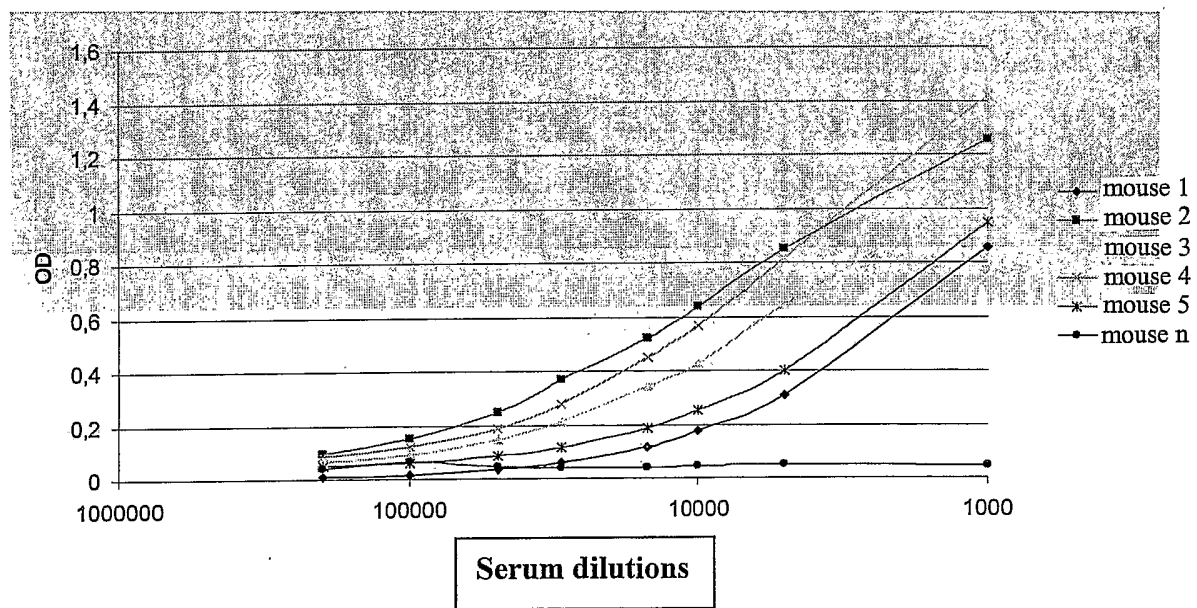


FIG. 3



Mouse *n*, non-immunized mouse (control)

FIG. 4, A

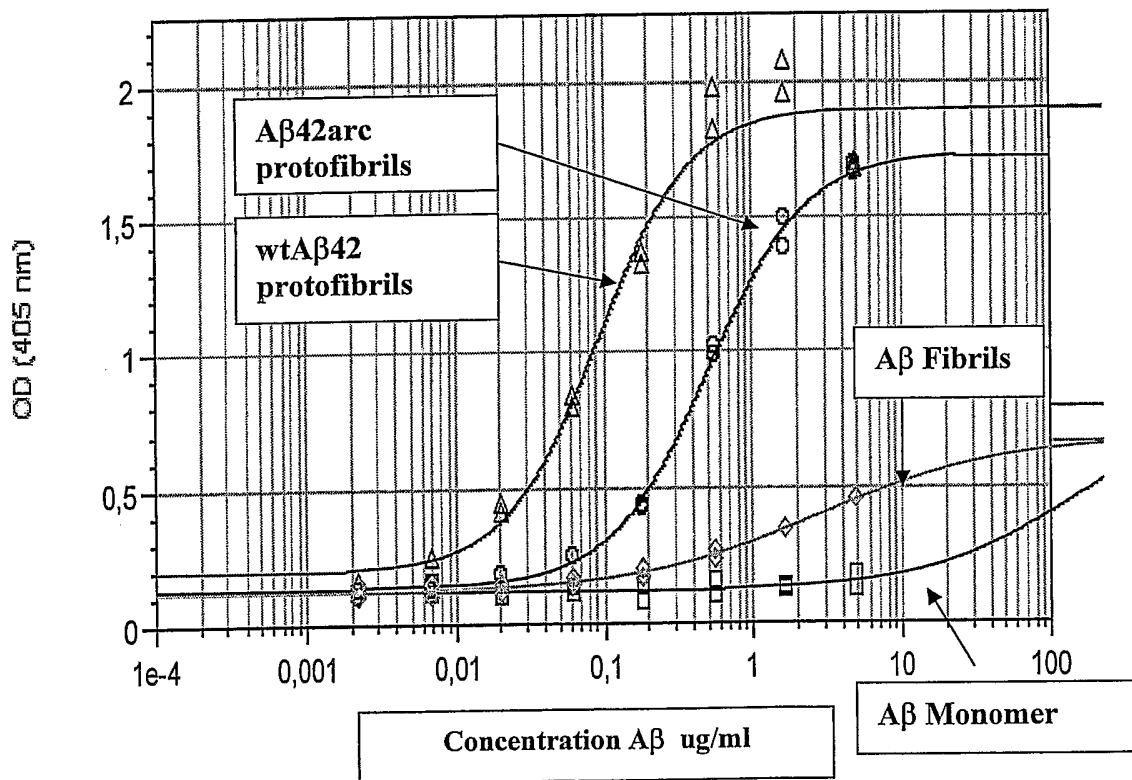


FIG. 4 B

Titration by a sandwich-ELISA of 4 monoclonal antibodies (258, 7E4, 4E11 and 10F7) against wtA β 42 protofibril concentration.

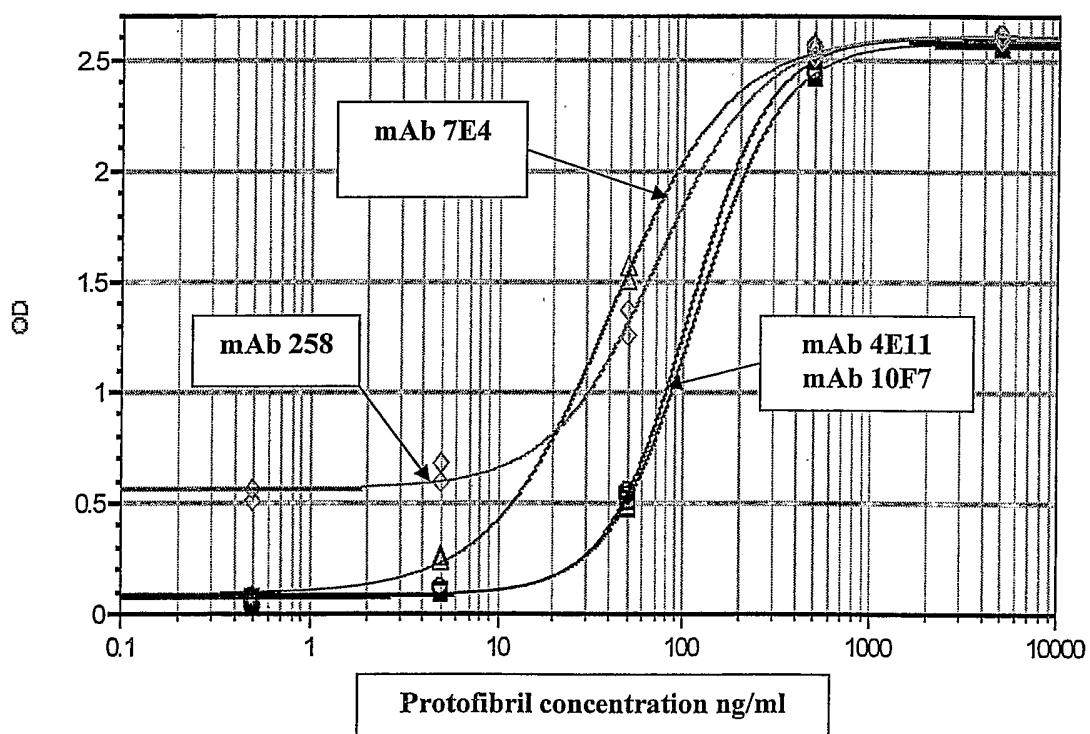
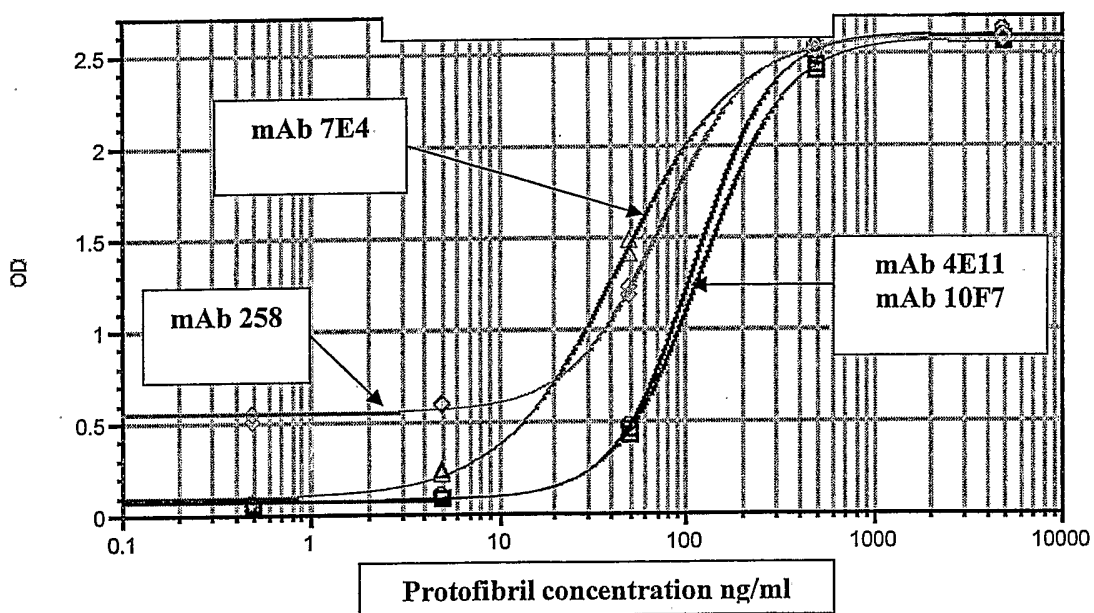


FIG. 4 C

Titration by a sandwich-ELISA of 4 monoclonal antibodies (258, 7E4, 4E11 and 10F7) against wtA β 42 protofibril concentration.



INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 2005/000993

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: C07K 16/18, A61K 39/395, A61P 25/28, C07K 14/47
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)

IPC7: C07K, A61K, A61P, C07K

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

SE,DK,FI,NO classes as above

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

EPO-INTERNAL, WPI DATA, PAJ, BIOSIS, MEDLINE, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|-----------|--|-----------------------|
| X | KAYED, RAKEZ ET AL, "Common Structure of Soluble Amyloid Oligomers Implies Common Mechanism of Pathogenesis", Science, 18 April 2003, volume 300, page 486 - page 489, page 486, column 2, paragraphs 1-2; page 487, column 1; figures 1-2 -- | 1-10,14-15 |
| Y | WO 0203911 A2 (LANNFELT, LARS), 17 January 2002 (17.01.2002), page 8, line 6 - line 16 -- | 1-21,32-33 |

Further documents are listed in the continuation of Box C. See patent family annex.

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| * Special categories of cited documents: | "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention |
| "A" document defining the general state of the art which is not considered to be of particular relevance | "X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone |
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| "O" document referring to an oral disclosure, use, exhibition or other means | |
| "P" document published prior to the international filing date but later than the priority date claimed | |

Date of the actual completion of the international search
28 Sept 2005

Date of mailing of the international search report
04-10-2005

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INTERNATIONAL SEARCH REPORT

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| International application No. PCT/SE 2005/000993 |
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| C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT | | |
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| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
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| X | WARD, ROBIN V. ET AL, "Fractionation and characterization of oligomeric, protofibrillar and fibrillar forms of Beta-amyloid peptide", 2000, vol. 348, page 137 - page 144, page 138, column 1, paragraphs 2-3 | 27-29 |
| Y | -- | 1-21,32-33 |
| X | US 6054114 A (PETER T. LANSBURY, JR. ET AL), 25 April 2000 (25.04.2000), column 103, last paragraph - column 104, paragraph 2 | 27-29 |
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| A | CHROMY, BRETT A. ET AL, "Self-Assembly of A Beta 1-42 into Globular Neurotoxins", Biochemistry, 2003, vol. 42, page 12749 - page 12760 | 1-21,32-33 |
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 2005/000993

| C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT | | |
|---|---|-----------------------|
| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
| A | WO 03089460 A1 (THE GOVERING COUNCIL OF THE UNIVERSITY OF TORONTO), 30 October 2003 (30.10.2003), page 17, line 1 - line 4; page 69, line 10 - line 15 -- | 1-21, 32-33 |
| A | US 20030187011 A1 (HILAL A. LASHUEL ET AL), 2 October 2003 (02.10.2003), paragraphs (0046) - (0051) -- | 34-36 |
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE2005/000993**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: 20, 21 (partly), 32-33
because they relate to subject matter not required to be searched by this Authority, namely:
Claims 20, 32-33 and the part of claim 21 that refers to in vivo screening relate to a method of treatment of the human or animal body by surgery or by therapy, as well as diagnostic methods /Rule 39.1(iv). .../...
2. Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE2005/000993

Box II

1) Nevertheless, a search has been executed for these claims. For claims 20 and 32-33, the search has been based on the alleged effects of the antibodies. For claim 21, only the part relating to the screening being performed in vitro has been searched.

INTERNATIONAL SEARCH REPORT
Information on patent family members

31/08/2005

International application No.
PCT/SE 2005/000993

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