

ANTI-PYROGLUTAMATED AMYLOID BETA HUMANIZED ANTIBODIES

Field of the Invention

The invention relates to humanized antibodies that bind to an epitope at the N-terminus of pyroglutamated amyloid beta (A β N3pE) peptide and to preventive and therapeutic treatment of diseases and conditions that are related to accumulation and deposition of amyloid peptides, such as amyloidosis, a group of disorders and abnormalities associated with pyroglutamated amyloid peptide, like Alzheimer's disease, Down's syndrome, cerebral amyloid angiopathy and other related aspects. More specifically, it pertains to the use of humanized monoclonal antibodies to bind pyroglutamated amyloid beta peptide in plasma, brain, and cerebrospinal fluid to prevent accumulation or to reverse deposition of A β N3pE within the brain and in various tissues in the periphery, and to alleviate amyloidosis. The present invention further pertains to diagnostic assays for the diagnosis of amyloidosis using the humanized antibodies of the invention.

Background Art

Amyloidosis is not a single disease entity but rather a diverse group of progressive disease processes characterized by extracellular tissue deposits of a waxy, starch-like protein called amyloid, which accumulates in one or more organs or body systems. As the amyloid deposits accumulate, they begin to interfere with the normal function of the organ or body system. There are at least 15 different types of amyloidosis. The major forms are primary amyloidosis without known antecedent, secondary amyloidosis following some other condition, and hereditary amyloidosis.

Secondary amyloidosis occurs during chronic infection or inflammatory disease, such as tuberculosis, a bacterial infection called familial Mediterranean fever, bone infections (osteomyelitis), rheumatoid arthritis, inflammation of the small intestine (granulomatous ileitis), Hodgkin's disease and leprosy.

Amyloid deposits include amyloid P (pentagonal) component (AP), a glycoprotein related to normal serum amyloid P (SAP), and sulphated glycosaminoglycans (GAG), complex carbohydrates of connective tissue. Amyloid protein fibrils, which account for about 90% of the amyloid material, comprise one of several different types of proteins. These proteins are capable of folding into so-called "beta-pleated" sheet fibrils, a unique protein configuration which exhibits binding sites for Congo red resulting in the unique staining properties of the amyloid protein.

Many diseases of aging are based on or associated with amyloid-like proteins and are 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 mild cognitive impairment (MCI), Alzheimer's disease (AD), like for instance sporadic Alzheimer's disease (SAD) or Familial Alzheimer's dementias (FAD) like Familial British Dementia (FBD) and Familial Danish Dementia (FDD), neurodegeneration in Down Syndrome, Lewy body dementia, 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; Creutzfeldt Jacob disease, Parkinson's disease, HIV-related dementia, ALS (amyotrophic lateral sclerosis), Adult Onset Diabetes; senile cardiac amyloidosis; endocrine tumors, and others, including macular degeneration.

Although pathogenesis of these diseases may be diverse, their characteristic deposits often contain many shared molecular constituents. To a significant degree, this may be attributable to the local activation of pro-inflammatory pathways thereby leading to the concurrent deposition of activated complement components, acute phase reactants, immune modulators, and other inflammatory mediators (McGeer *et al.*, Tohoku J Exp Med. 174(3): 269-277 (1994)).

Recently, accumulating evidence demonstrates involvement of N-terminal modified A β peptide variants in Alzheimer's disease. Aiming biopsies display a presence of A β 1-40 and A β 1-42 not only in the brain of Alzheimer's patients but also in senile plaques of unaffected individuals. However, N-terminal truncated and pyroGlu modified A β N3pE-40/A β N3pE-42 is almost exclusively engrained within plaques of Alzheimer's disease patients, making this A β variant an eligible diagnostic marker and a potential target for drug development.

At present, several commercial manufacturers offer ELISA kits which allow a detection of A β 1-40 / 1-42 and A β N3pE-40/A β N3pE-42 in the low picogram (pg) range.

The brains of Alzheimer's disease (AD) patients are morphologically characterized by the presence of neurofibrillary tangles and by deposits of A β peptides in neocortical brain structures (Selkoe, D.J. & Schenk, D. Alzheimer's disease: molecular understanding predicts amyloid-based therapeutics. Annu. Rev. Pharmacol. Toxicol. 43, 545-584 (2003)). A β peptides are liberated from the amyloid precursor protein (APP) after sequential cleavage by β - and γ -

secretase. The γ -secretase cleavage results in the generation of A β 1-40 and A β 1-42 peptides, which differ in their C-termini and exhibit different potencies of aggregation, fibril formation and neurotoxicity (Shin, R.W. *et al.* Amyloid beta-protein (Abeta) 1-40 but not Abeta 1-42 contributes to the experimental formation of Alzheimer disease amyloid fibrils in rat brain. *J. Neurosci.* 17, 8187-8193 (1997); Iwatsubo, T. *et al.* Visualization of Abeta 42(43) and Abeta 40 in senile plaques with end-specific Abeta monoclonals: evidence that an initially deposited species is Abeta 42(43). *Neuron* 13, 45-53 (1994); Iwatsubo, T., Mann, D.M., Odaka, A., Suzuki, N. & Ihara, Y. Amyloid beta protein (Abeta) deposition: Abeta 42(43) precedes Abeta 40 in Down syndrome. *Ann. Neurol.* 37, 294-299 (1995); Hardy, J.A. & Higgins, G.A. Alzheimer's disease: the amyloid cascade hypothesis. *Science* 256, 184-185 (1992); Roßner, S., Ueberham, U., Schliebs, R., Perez-Polo, J.R. & Bigl, V. The regulation of amyloid precursor protein metabolism by cholinergic mechanisms and neurotrophin receptor signaling. *Prog. Neurobiol.* 56, 541-569 (1998)).

The majority of A β peptides deposited in diffuse plaques are N-terminal truncated or modified. Studies of Piccini and Saido have shown that the core structure of senile plaques and vascular deposits consist of 50 % pyroglutamate (pyroGlu) modified peptides (Piccini *et al.*, *J Biol Chem.* 2005 Oct 7; 280(40):34186-92; Saido *et al.*, *Neuron.* 1995 Feb; 14(2): 457-66). PyroGlu modified peptides are more strongly cytotoxic than other A β species and stable against aminopeptidases (Russo *et al.*, *J Neurochem.* 2002 Sep; 82(6):1480-9). Thus, pyroGlu A β species have a longer half-life whereby the accumulation of these species and the formation of neurotoxic oligomers as well as aggregates are beneficial (Saido, *Neurobiol Aging.* 1998 Jan-Feb; 19(1 Suppl):S69-75). Due to the cyclization of glutamate to pyroGlu, charged amino acids will be lost which strongly reduces the solubility of the peptide and causes an increased aggregation tendency. *In vitro* studies have shown that the initial oligomerisation of e.g. A β 3(pE) is much faster compared to non-modified peptides (Schilling *et al.*, *Biochemistry.* 2006 Oct 17; 45(41):12393-9). The A β N3pE-42 peptides coexist with A β 1-40/1-42 peptides (Saido, T.C. *et al.* Dominant and differential deposition of distinct beta-amyloid peptide species, Abeta N3pE, in senile plaques. *Neuron* 14, 457-466 (1995) ; Saido, T.C., Yamao, H., Iwatsubo, T. & Kawashima, S. Amino- and carboxyl-terminal heterogeneity of beta-amyloid peptides deposited in human brain. *Neurosci. Lett.* 215, 173-176 (1996)), and, based on a number of observations, could play a prominent role in the pathogenesis of AD. For example, a particular neurotoxicity of A β N3pE-42 peptides has been outlined (Russo, C. *et al.* Pyroglutamate-modified amyloid beta-peptides--AbetaN3(pE)--strongly affect cultured neuron and astrocyte survival. *J. Neurochem.* 82, 1480-1489 (2002) and the pE-modification of N-truncated A β peptides confers resistance to degradation by most aminopeptidases as well as A β -degrading

endopeptidases (Russo, C. *et al.* Pyroglutamate-modified amyloid beta-peptides--AbetaN3(pE)--strongly affect cultured neuron and astrocyte survival. *J. Neurochem.* 82, 1480-1489 (2002); Saido, T.C. Alzheimer's disease as proteolytic disorders: anabolism and catabolism of beta-amyloid. *Neurobiol. Aging* 19, S69-S75 (1998)). The cyclization of glutamic acid into pE leads to a loss of N-terminal charge resulting in accelerated aggregation of A β N3pE compared to the unmodified A β peptides (He, W. & Barrow, C.J. The Abeta 3-pyroglutamyl and 11-pyroglutamyl peptides found in senile plaque have greater beta-sheet forming and aggregation propensities in vitro than full-length A beta. *Biochemistry* 38, 10871-10877 (1999); Schilling, S. *et al.* On the seeding and oligomerization of pGlu-amyloid peptides (in vitro). *Biochemistry* 45, 12393-12399 (2006)). Thus, reduction of A β N3pE-42 formation should destabilize the peptides by making them more accessible to degradation and would, in turn, prevent the formation of higher molecular weight A β aggregates and enhance neuronal survival.

However, for a long time it was not known how the pE-modification of A β peptides occurs. Recently, it was discovered that glutaminyl cyclase (QC) is capable to catalyze A β N3pE-42 formation under mildly acidic conditions and that specific QC inhibitors prevent A β N3pE-42 generation *in vitro* (Schilling, S., Hoffmann, T., Manhart, S., Hoffmann, M. & Demuth, H.-U. Glutaminyl cyclases unfold glutamyl cyclase activity under mild acid conditions. *FEBS Lett.* 563, 191-196 (2004) ; Cynis, H. *et al.* Inhibition of glutaminyl cyclase alters pyroglutamate formation in mammalian cells. *Biochim. Biophys. Acta* 1764, 1618-1625 (2006)).

All facts suggest that pyroGlu A β is a kind of germ for the initialization of fibril formation. In a further study (Piccini *et al.*, 2005, *supra*) volunteers with plaque depositions but without AD specific pathology could be distinguished from AD patients due to the characteristic amount of A β -species. Thereby the amount of N-terminal truncated, pyroGlu modified peptides was significant higher in the brain of AD patients.

The posttranslational formation of pyroGlu at position 3 or 11 of A β -peptide implies cyclization of an N-terminal glutamate residue. Glutaminyl cyclase (QC) plays an important role in the generation of pyroGlu peptides. QC is wide-spread in the plant- and animal kingdom and *inter alia*, is involved in the maturation of peptide hormones. Both the cyclisation of glutamine by release of ammonia and of glutamate by release of water to pyroGlu is performed by QC. In contrast to the glutamine cyclization the glutamate cyclisation occurs not spontaneously. QC catalyses the efficient (unwanted) side reaction from glutamate to pyroGlu. The generated

pyroGlu residue protects the protein against proteolytic degradation. There are several references which shows that QC plays an important role in the generation of pyroGlu A β :

1. In several studies it was shown that QC catalyses the formation of pyroGlu residues from glutamate at N-terminus of A β (Cynis *et al.*, Biochim Biophys Acta. 2006 Oct; 1764(10):1618-25, Schilling *et al.*, FEBS Lett. 2004 Apr 9;563(1-3):191-6);

2. Both A β peptides and QC are expressed in large quantities in hippocampus and cortex. These brain areas are at particular risk in AD (Pohl *et al.*, Proc Natl Acad Sci U S A. 1991 Nov 15; 88(22):10059-63, Selkoe, Physiol Rev. 2001 Apr;81(2):741-66);

3. The APP is cleaved by β -secretase during the transport to the plasma membrane whereby the N-terminus of A β with the free glutamate residue can be produced (Greenfield *et al.*, Proc Natl Acad Sci U S A. 1999 Jan 19; 96(2):742-7). In the secretory vesicles a co-localisation of processed APP and the QC was determined. So in the mild acid milieu of the vesicles an accelerated modification of glutamate residue to pyroglutamate can occur.

4. Also other neurodegenerative diseases (familial Danish (FDD) or British dementia (FBD)) are related with N-terminal pyroGlu modified peptides e.g. Bri2, but in contrast they are not related to A β in terms of their primary structure (Vidal R. *et al.*, 1999 Proc. Natl. Acad. Sci. U.S.A. 97, 4920-4925).

Possibly the QC-catalysed formation of pyroGlu A β is involved in the development and progression of neurodegenerative diseases. The formation of N-terminal modified amyloid peptides certainly represents a fundamental factor in the process of A β aggregation and could be the onset of disease. The suppression of the pyroGlu A β formation by inhibition of QC, might represent a therapeutic approach. QC inhibitors would be able to prevent the formation of pyroGlu A β , reduce the concentration of pyroglutamate A β in the brain and so delay the oligomerisation of A β -peptides. Schilling *et al.* show, that QC expression was up regulated in the cortex of AD patients and correlated with the appearance of pyroGlu-modified A β -peptide. Oral application of a QC inhibitor resulted in reduced pyroglutamate modified A β pE(3-42) level in two different transgenic mouse models of AD and in a new *Drosophila* model (Schilling *et al.*, 2008 *Biol. Chem.* (389), 983-991).

Lewy body dementia (LBD) is a neurodegenerative disorder that can occur in persons older than 65 years of age, and typically causes symptoms of cognitive (thinking) impairment and abnormal behavioral changes. Symptoms can include cognitive impairment, neurological signs, sleep disorder, and autonomic failure. Cognitive impairment is the presenting feature of LBD in most cases. Patients have recurrent episodes of confusion that progressively worsen. The fluctuation in cognitive ability is often associated with shifting degrees of attention and

alertness. Cognitive impairment and fluctuations of thinking may vary over minutes, hours, or days. Lewy bodies are formed from phosphorylated and nonphosphorylated neurofilament proteins; they contain the synaptic protein alpha-synuclein as well as ubiquitin, which is involved in the elimination of damaged or abnormal proteins. In addition to Lewy Bodies, Lewy neurites, which are inclusion bodies in the cell processes of the nerve cells, may also be present. Amyloid plaques may form in the brains of patients afflicted with DLB, however they tend to be fewer in number than seen in patients with Alzheimer's disease. Neurofibrillary tangles, the other micropathological hallmark of AD, are not a main characteristic of LBD but are frequently present in addition to amyloid plaques.

Amyotrophic lateral sclerosis (ALS) is characterized by degeneration of upper and lower motor neurons. In some ALS patients, dementia or aphasia may be present (ALS-D). The dementia is most commonly a frontotemporal dementia (FTD), and many of these cases have ubiquitin-positive, tau-negative inclusions in neurons of the dentate gyrus and superficial layers of the frontal and temporal lobes.

Inclusion-body myositis (IBM) is a crippling disease usually found in people over age 50, in which muscle fibers develop inflammation and begin to atrophy — but in which the brain is spared and patients retain their full intellect. Two enzymes involved in the production of amyloid- β protein were found to be increased inside the muscle cells of patients with this most common, progressive muscle disease of older people, in which amyloid- β is also increased.

Another disease that is based on or associated with the accumulation and deposit of amyloid-like protein is macular degeneration. Macular degeneration is a common eye disease that causes deterioration of the macula, which is the central area of the retina (the paper-thin tissue at the back of the eye where light-sensitive cells send visual signals to the brain). Sharp, clear, "straight ahead" vision is processed by the macula. Damage to the macula results in the development of blind spots and blurred or distorted vision. Age-related macular degeneration (AMD) is a major cause of visual impairment in the United States and for people over age 65 it is the leading cause of legal blindness among Caucasians. Approximately 1.8 million Americans of age 40 and older have advanced AMD, and another 7.3 million people with intermediate AMD are at substantial risk for vision loss. The government estimates that by 2020 there will be 2.9 million people with advanced AMD. Victims of AMD are often surprised and frustrated to find out how little is known about the causes and treatment of this blinding condition.

There are two forms of macular degeneration: dry macular degeneration and wet macular degeneration. The dry form, in which the cells of the macula slowly begin to break down, is diagnosed in 85 percent of macular degeneration cases. Both eyes are usually affected by dry AMD, although one eye can lose vision while the other eye remains unaffected. Drusen, which are yellow deposits under the retina, are common early signs of dry AMD. The risk of developing advanced dry AMD or wet AMD increases as the number or size of the drusen increases. It is possible for dry AMD to advance and cause loss of vision without turning into the wet form of the disease; however, it is also possible for early-stage dry AMD to suddenly change into the wet form.

The wet form, although it only accounts for 15 percent of the cases, results in 90 percent of the blindness, and is considered advanced AMD (there is no early or intermediate stage of wet AMD). Wet AMD is always preceded by the dry form of the disease. As the dry form worsens, some people begin to have abnormal blood vessels growing behind the macula. These vessels are very fragile and will leak fluid and blood (hence 'wet' macular degeneration), causing rapid damage to the macula.

The dry form of AMD will initially often cause slightly blurred vision. The center of vision in particular may then become blurred and this region grows larger as the disease progresses. No symptoms may be noticed if only one eye is affected. In wet AMD, straight lines may appear wavy and central vision loss can occur rapidly.

Diagnosis of macular degeneration typically involves a dilated eye exam, visual acuity test, and a viewing of the back of the eye using a procedure called fundoscopy to help diagnose AMD, and — if wet AMD is suspected — fluorescein angiography may also be performed. If dry AMD reaches the advanced stages, there is no current treatment to prevent vision loss. However, a specific high dose formula of antioxidants and zinc may delay or prevent intermediate AMD from progressing to the advanced stage. Macugen® (pegaptanib sodium injection), laser photocoagulation and photodynamic therapy can control the abnormal blood vessel growth and bleeding in the macula, which is helpful for some people who have wet AMD; however, vision that is already lost will not be restored by these techniques. If vision is already lost, low vision aids exist that can help improve the quality of life.

One of the earliest signs of age-related macular degeneration (AMD) is the accumulation of extracellular deposits known as drusen between the basal lamina of the retinal pigmented epithelium (RPE) and Bruch's membrane (BM). Recent studies conducted by Anderson *et al.*

have confirmed that drusen contain amyloid beta. (Experimental Eye Research 78 (2004) 243 - 256).

Pyroglutamated A β peptides have been shown to play a key role in accumulation of A β peptides and in plaque formation in Alzheimer's diseases. Due to their hydrophobic potential it has been shown that these peptides promote aggregation and plaque formation. It has further been shown in a transgenic mouse model expressing A β N3pE-42 in neurons that this peptide is neurotoxic *in vivo* and leads to loss of neurons (Wirths et al. (2009) Acta Neuropathol 118, 487-496).

Antibodies with specificities against the N-terminal pyroglutamate of A β peptides are believed to be advantageous because of their specificity towards only the pathogenic species of A β , which carry a pyroglutamate at the N-terminus, but not detecting APP or other A β species w/o the N-terminal pyroglutamate. It is thus believed that the risk of potential side effects, such as uncontrollable cerebral inflammation, will be reduced by use of the antibodies of the invention compared to antibodies directed to other A β species than the pyroglutamated variants.

Antibodies targeting A β N3pE peptides are known (Acero et al (2009) J Neuroimmunol 213, 39-46; Saido et al. (1996) Neuron 14, 457-466; US 7,122,374 and WO 2012/136552).

However, there is a need for humanized antibodies with specificity for A β N3pE peptides that can be used in human treatment and that positively affect amyloidosis, in particular cognition in diseases and conditions where A β N3pE may be involved, such as clinical or pre-clinical Alzheimer's disease, Down's syndrome, and clinical or pre-clinical cerebral amyloid angiopathy.

Summary of the invention

The invention provides novel methods and compositions comprising highly specific and highly effective antibodies, including chimeric antibodies and fragments thereof, including partially or fully humanized antibodies and fragments thereof, having the ability to specifically recognize and bind to specific epitopes from a range of β -amyloid antigens, in particular A β N3pE peptides, which may be presented to the antibody in a monomeric, dimeric, trimeric, etc, or a polymeric form, in form of an aggregate, fibers, filaments or in the condensed form of a plaque.

In particular, the present invention pertains to a humanized antibody or a functional variant thereof, wherein the variable part of the light chain of said antibody comprises, consists essentially of or consists of an amino acid sequence of:

5 DVVMTQSPLSLPVTLGQPASISCKSSQSLLX₁SDGKTYLNWFQQRPGQSPRRLX₂YLVSKLD
SGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCVQGTHFPFTFGGGTKVEIK (SEQ ID NO:
7),

wherein

10 X₁ is selected from Y and H; and
X₂ is selected from A, I and T;

or of an amino acid sequence selected from

15 DIQMTQSPSSLSASVGDRVTITCRSSQSLVHSDGNTYLNHWYQQKPGKAPKLLIYKVSNRFS
GVPSRFSGSGSGTDFTLTISLQPEDFATYYCSQSTHVPPTFGQGTKVEIK (SEQ ID NO:
28); and

20 DVVMTQSPLSLPVTLGQPASISCKSSQSLLYSNGKTYLNWFQQRPGQSPRRLIYVSKLDS
GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCVQGTHFPFTFGGGTKVEIK (SEQ ID NO:
36).

and/or

25 wherein the variable part of heavy chain of said antibody comprises, consists essentially of or
consists of an amino acid sequence of:

30 QVQLVQSGAEVKKSGASVKVSCKASGYSFTGX₃TMNWVRQAPGQGLEWMGLINPX₄NX₅V
TRYNQKF₆GRVTX₇X₈RDTSTTTVX₉MELTSLTSEDTAX₁₀YYCTREAKREWDETYWGQGT
LTVSS (SEQ ID NO: 17);

wherein

X₃ is selected from Y and H;
X₄ is selected from Y and S;
35 X₅ is selected from G, T, A and E;
X₆ is selected from K and Q;

X₇ is selected from L and I;
X₈ is selected from I and T;
X₉ is selected from Y and H; and
X₁₀ is selected from V and T;

or of an amino acid sequence selected from

EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYGMWVRQAPGKGLEWVSFISNLAYSIYY
ADTVTGRFTISRDNKNSLYLQMNSLRAEDTAVYYCARYDYDNILDYVMDYWGGQGLTVTS
S (SEQ ID NO: 32), and

QVQLVESGAIEVKKPGASVKVSCKASGYIFNNYWINWVRQAPGQGLEWMGQIYPGDGDTN
YNGKFKGRVTITADESTSTAYMELSSLRSEDVAVYYCAREGYIVYWGGQGLTVTVSS (SEQ ID
NO: 40).

The invention provides humanized antibodies, or fragments thereof, that positively affect diseases and conditions of amyloidosis, where A β N3pE may be involved.

In another embodiment, the invention provides humanized antibodies and fragments thereof that bind to A β N3pE peptides in the circulation and tissue, in particular in the brain. The humanized antibodies of the invention are capable of binding free A β N3pE peptide molecules or even bound forms of A β N3pE peptides.

Thus, the present invention further provides humanized antibodies that alter clearance of soluble and bound forms of A β N3pE peptides in the central nervous system, such as the brain, and the circulation, such as plasma.

In a further embodiment, the invention provides humanized antibodies and fragments thereof, wherein the humanized antibodies specifically bind to the pyroglutamate carrying N-terminus of A β N3pE.

In yet a further embodiment, the present invention also relates to the host cells transformed with the vectors or incorporating the polynucleotides that express the humanized antibodies or fragments thereof.

Moreover, the present invention provides pharmaceutical compositions comprising the humanized antibodies of the invention and fragments thereof.

The invention further relates to the use of the humanized antibodies and fragments thereof are useful for binding to and clearing or removing of A β N3pE in humans and thereby for diagnosing, preventing and treating diseases and conditions characterized by amyloidosis or A β N3pE toxicity.

In a particular embodiment, the humanized antibodies of the invention, which are capable of binding to and clearing or removing of A β N3pE peptides in biological fluids and tissues, are useful for the prevention and/or treatment of conditions associated with the formation of A β N3pE-containing plaques, such as diffuse, neuritic, and cerebrovascular plaques in the brain.

The administration of the humanized antibodies of the invention, including immunologically reactive fragments thereof, may lead to the clearance or removal of A β N3pE from the aforementioned plaques or other biological complexes. Thus, the humanized antibody of the invention will readily be transport in the circulation, other body fluids and to sites where the aforementioned plaques and/or other biological complexes are formed or elsewhere where A β N3pE exhibits damaging effects.

In addition, removal of A β N3pE from plaques or other biological complexes by the humanized antibodies of the invention may lead to the solubilization of insoluble forms of plaques and thus lead to the removal of complete plaques from the affected tissue, such as brain tissue. This, in turn, may lead to improvement of cognition in patients diagnosed with a neurodegenerative disease, such as mild cognitive impairment (MCI), Alzheimer's disease (AD), like for instance sporadic Alzheimer's disease (SAD) or Familial Alzheimer's dementias (FAD) like Familial British Dementia (FBD) and Familial Danish Dementia (FDD) or others, neurodegeneration in Down Syndrome, Lewy body dementia, hereditary cerebral hemorrhage with amyloidosis (Dutch type); the Guam Parkinson-Dementia complex.

The binding of the humanized antibodies of the invention to A β N3pE in the circulation or other body fluids may further result to the removal of the circulating or soluble forms of A β N3pE. As discussed above, A β N3pE exhibits a high hydrophobicity and has a high affinity to other, e.g. nonpyroglutamated A β peptides, which results in the formation of oligomeric and supermolecular structures, such as amyloid plaques. It has been shown that in particular these oligomeric structures are highly neurotoxic. The formation of oligomeric structures leads to cell

damage and death of neuronal cells. Thus, the removal of circulating or soluble forms of A β N3pE or even of oligomers comprising A β N3pE leads to the prevention of cell damage and/or neurotoxicity. Thus, the invention also provides methods of preventing of neurodegenerative disease, such as mild cognitive impairment (MCI), Alzheimer's disease (AD), like for instance
5 sporadic Alzheimer's disease (SAD) or Familial Alzheimer's dementias (FAD) like Familial British Dementia (FBD) and Familial Danish Dementia (FDD) or others, neurodegeneration in Down Syndrome, Lewy body dementia, hereditary cerebral hemorrhage with amyloidosis (Dutch type), the Guam Parkinson-Dementia complex.

10 The invention further provides methods of preventing and /or treating of other diseases which are based on or associated with amyloid-like proteins, in particular A β N3pE, such as progressive supranuclear palsy, multiple sclerosis, Creutzfeld Jacob disease, Parkinson's disease, HIV-related dementia, ALS (amyotrophic lateral sclerosis), dementia related to Adult Onset Diabetes; senile cardiac amyloidosis, and others, including macular degeneration.

15 The invention further provides a highly sensitive and concomitantly robust detection technique that allows quantitative determination of A β variants, in particular A β N3pE, in biological samples, e.g. liquor or serum samples, preferably serum samples, or tissue samples. This is a tremendous challenge, taking the low abundance of these A β N3pE peptides in blood into
20 account. Having such a detection technique available is, however, a prerequisite for studying efficacy of small molecule inhibitors in drug screening and drug development programs.

The antibodies enabled by the teaching of the present invention are particularly useful for diagnosis of amyloidosis, a group of diseases and disorders associated with amyloid plaque
25 formation including secondary amyloidosis and age-related amyloidosis including, but not limited to, neurological disorders such as Alzheimer's Disease (AD), Lewy body dementia, Down's syndrome, hereditary cerebral hemorrhage with amyloidosis (Dutch type), the Guam Parkinson- Dementia complex, as well as other diseases which are based on or associated with amyloid-like proteins such as progressive supranuclear palsy, multiple sclerosis;
30 Creutzfeld Jacob disease, hereditary cerebral hemorrhage with amyloidosis Dutch type, Parkinson's disease, HIV-related dementia, ALS (amyotrophic lateral sclerosis), dementia related to Adult Onset Diabetes, senile cardiac amyloidosis, and others, including macular degeneration, to name just a few.

35 Description of the Figures

Figure 1 shows the CDR definition of mouse antibody clone#6

Amino acid sequence of variable domain of light chain (LC) and heavy chain (HC) are shown. The three CDRs of LC and HC are framed (selected by Sircar et al., 2009 (Sircar A. et al.:
5 RosettaAntibody: antibody variable region homology modeling server. Nucleic Acids Research, 2009, Vol. 37, pp. W474-W479) according to Kabat and Wu, 1971 (Kabat E. A. and WU, T.T.: An Attempt to Locate the Non-helical and Permissively Helical Sequences of Proteins: Application to the Variable Regions of Immunoglobulin Light and Heavy Chains. *Proc. Nat. Acad. Sci. USA*; Vol. 68, No. 7, pp. 1501-1506, 1971) and Kabat et al. 1991 (Kabat E. A.
10 and WU, T.T.: IDENTICAL V REGION AMINO ACID SEQUENCES AND SEGMENTS OF SEQUENCES IN ANTIBODIES OF DIFFERENT SPECIFICITIES: Relative Contributions of VH and VL Genes, Minigenes, and Complementarity-Determining Regions to Binding of Antibody-Combining Sites. *The Journal of Immunology*, Vol. 147; pp. 1709-1719, 1991); beside CDR1 of HC which is defined according to Clothia et al., 1989 (Clothia C. et al.:
15 Conformations of immunoglobulin hypervariable regions. *Nature*, Vol. 342, pp. 877-883, 1989).

Figure 2 shows the purification of humanized antibody clone#6 by Protein G chromatography. Recombinant produced humanized antibody clone#6 was purified by Protein G Chromatography. 24 µl of input fraction (lane 1), flow through fraction (lane 22) and elution
20 fraction (lane 3) are loaded onto 10% SDS PAGE under non reducing conditions. 2 µg of and humanized (lane 4) antibody were compared to mouse antibody (lane 5) in 10% coomassie stained SDS gel.

Figure 3 shows the calculation of KD of humanized antibody clone#6 HC T97 variant and LC
25 L41 variant. The binding affinities of HC T97 and LC L41 variant of humanized antibody clone#6 to Aβ(pE3-18) was measured by SPR using peptide concentrations of 1 - 100 nM (HC T97 variant) and 10-1000 nM (LC L41 variant). The KD is calculated with 5.3 nM. The KD value of LC L41 variant was determined with 162.7 nM by plotting RU_{equ} values against the peptide concentration and fitting by steady-state model as described above
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Figure 4 shows the generation of stable cell line expressing humanized antibody clone #6 variant HC T97. A) MTX Treatment of CHO DG44 cells stable expressing humanized antibody clone#6 variant HC T97. 0.5 µM MTX (lane 2) leads to increasing expression in comparison with 0.1 µM and without MTX (lane 1 and 4). More over low amount of cells dying compared with
35 treatment using 1µM MTX (lane 4). 24 µl supernatant were loaded onto a 10% SDS PAGE under non reducing conditions. B) 18 clones were yielded after clonal selection by limiting

dilution and analyzed by Western blot. 24 µl of supernatant were loaded onto a 12% SDS PAGE under non reducing conditions. C) Five of these 18 clones were scaled up and cultivated for 7 days, so expression levels in supernatant could be analyzed by SPR.

Figure 5 shows the ITC measurement of humanized antibody clone#6 with AβpE3-18 peptide. Purified humanized antibody clone#6 HC T97 variant was used for ITC measurement. Top: raw data of ITC measurement. Bottom: Integration of raw data to represent the concentration of added peptide as a function molar ratio peptide/antibody. The values of the thermodynamic parameters are shown left.

Figure 6 shows the binding of two antibodies, which either comprise the human IgG1 Fc wild-type region of SEQ ID NO: 73 (WT) or the K322A mutant variant thereof (SEQ ID NO: 74), to the Fc gamma receptor CD16A.

Figure 7 shows the binding of two antibodies, which either comprise the human IgG1 Fc wild-type region of SEQ ID NO: 73 (WT) or the K322A mutant variant thereof (SEQ ID NO: 74), to the Fc gamma receptor CD32A.

Figure 8 shows the binding of two antibodies, which either comprise the human IgG1 Fc wild-type region of SEQ ID NO: 73 (WT) or the K322A mutant variant thereof (SEQ ID NO: 74), to the Fc gamma receptor CD32B.

Figure 9 shows the binding of two antibodies, which either comprise the human IgG1 Fc wild-type region of SEQ ID NO: 73 (WT) or the K322A mutant variant thereof (SEQ ID NO: 74), to the Fc gamma receptor CD64.

Figure 10 shows the binding analysis of two antibodies, which either comprised the human IgG1 Fc wild-type region of SEQ ID NO: 73 (WT) or the K322A mutant variant thereof (SEQ ID NO: 74), to C1q.

Detailed description of the Invention

Definitions

The term "antibody" is used in the broadest sense and specifically covers intact monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g. bispecific antibodies) formed

from at least two intact antibodies, and antibody fragments so long as they exhibit the desired biological activity. The antibody may be an IgM, IgG (e.g. IgG1, IgG2, IgG3 or IgG4), IgD, IgA or IgE, for example. Preferably however, the antibody is not an IgM antibody.

5 "Antibody fragments" comprise a portion of an intact antibody, generally the antigen binding or variable region of the intact antibody. Examples of antibody fragments include Fab, Fab', F(ab')₂, and Fv fragments; diabodies; single-chain antibody molecules; and multispecific antibodies formed from antibody fragments.

10 The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e. the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to "polyclonal antibody" preparations which
15 typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies can frequently be advantageous in that they are synthesized by the hybridoma culture, uncontaminated by other immunoglobulins. The "monoclonal" indicates the character of the antibody as being obtained from a substantially
20 homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Köhler *et al.*, Nature, 256:495 (1975), or may be made by generally well known recombinant DNA methods. The "monoclonal antibodies" may also be isolated from phage antibody
25 libraries using the techniques described in Clackson *et al.*, Nature, 352:624-628 (1991) and Marks *et al.*, J. Mol. Biol., 222:581-597 (1991), for example.

The monoclonal antibodies herein specifically include chimeric antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is identical with or homologous to
30 corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity.

"Humanized" forms of non-human (e.g., murine) antibodies are immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', F(ab')₂ or other antigen-binding subsequences of antibodies) which contain a minimal sequence derived from a non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a complementarity-determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity, and capacity. In some instances, Fv framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences.

These modifications are made to further refine and optimize antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones *et al.*, Nature, 321:522-525 (1986), Reichmann *et al.*, Nature, 332:323-329 (1988); and Presta, Curr. Op. Struct. Biol., 2:593-596 (1992).

"Single-chain Fv" or "sFv" antibody fragments comprise the V_H and V_L domains of antibody, wherein these domains are present in a single polypeptide chain. Generally, the Fv polypeptide further comprises a polypeptide linker between the V_H and V_L domains which enables the sFv to form the desired structure for antigen binding. For a review of sFv see Plückthun in The Pharmacology of Monoclonal Antibodies, vol. 113, Rosenberg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994).

The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy-chain variable domain (V_H) connected to a light-chain variable domain (V_D) in the same polypeptide chain (V_H - V_D). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies are described more fully in Hollinger *et al.*, Proc. Natl. Acad. Sci. USA, 90:6444-6448 (1993).

An "isolated" antibody is one which has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials which would interfere with diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. In preferred embodiments, the antibody will be purified (1) to greater than 95% by weight of antibody as determined by the Lowry method, and most preferably more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under reducing or nonreducing conditions using Coomassie blue or, preferably, silver stain. Isolated antibody includes the antibody *in situ* within recombinant cells since at least one component of the antibody's natural environment will not be present. Ordinarily, however, isolated antibody will be prepared by at least one purification step.

As used herein, the expressions "cell", "cell line," and "cell culture" are used interchangeably and all such designations include progeny. Thus, the words "transformants" and "transformed cells" include the primary subject cell and culture derived therefrom without regard for the number of transfers. It is also understood that all progeny may not be precisely identical in DNA content, due to deliberate or inadvertent mutations. Mutant progeny that have the same function or biological activity as screened for in the originally transformed cell are included. Where distinct designations are intended, this will be clear from the context.

The terms "polypeptide", "peptide", and "protein", as used herein, are interchangeable and are defined to mean a biomolecule composed of amino acids linked by a peptide bond.

If peptide or amino acid sequences are mentioned herein, each amino acid residue is represented by a one-letter or a three-letter designation, corresponding to the trivial name of the amino acid, in accordance with the following conventional list:

<u>Amino Acid</u>	<u>One-Letter Symbol</u>	<u>Three-Letter Symbol</u>
Alanine	A	Ala
Arginine	R	Arg
Asparagine	N	Asn
Aspartic acid	D	Asp
Cysteine	C	Cys
Glutamine	Q	Gln
Glutamic acid	E	Glu

	Glycine	G	Gly
	Histidine	H	His
	Isoleucine	I	Ile
	Leucine	L	Leu
5	Lysine	K	Lys
	Methionine	M	Met
	Phenylalanine	F	Phe
	Proline	P	Pro
	Serine	S	Ser
10	Threonine	T	Thr
	Tryptophan	W	Trp
	Tyrosine	Y	Tyr
	Valine	V	Val

15 The terms "a", "an" and "the" as used herein are defined to mean "one or more" and include the plural unless the context is inappropriate.

20 The language "diseases and disorders which are caused by or associated with amyloid or amyloid-like proteins" includes, but is not limited to, diseases and disorders caused by the presence or activity of amyloid-like proteins in monomeric, fibril, or polymeric state, or any combination of the three. Such diseases and disorders include, but are not limited to, amyloidosis, endocrine tumors, and macular degeneration.

25 The term "amyloidosis" refers to a group of diseases and disorders associated with amyloid plaque formation including, but not limited to, secondary amyloidosis and age-related amyloidosis such as diseases including, but not limited to, neurological disorders such as Alzheimer's Disease (AD), including diseases or conditions characterized by a loss of cognitive memory capacity such as, for example, mild cognitive impairment (MCI), sporadic Alzheimer's disease, Lewy body dementia, Down's syndrome, hereditary cerebral hemorrhage with amyloidosis (Dutch type); the Guam Parkinson-Dementia complex, familial forms of
30 Alzheimer's disease like Familial British Dementia (FBD) and Familial Danish Dementia (FDD); as well as other diseases which are based on or associated with amyloid-like proteins such as progressive supranuclear palsy, multiple sclerosis; Creutzfeldt Jacob disease, Parkinson's disease, HIV-related dementia, ALS (amyotrophic lateral sclerosis), inclusion-body myositis
35 (IBM), Adult Onset Diabetes, and senile cardiac amyloidosis; and various eye diseases

including macular degeneration, drusen-related optic neuropathy, and cataract due to beta-amyloid deposition.

"Amyloid β , A β or β -amyloid" is an art recognized term and refers to amyloid β proteins and peptides, amyloid β precursor protein (APP), as well as modifications, fragments and any functional equivalents thereof. In particular, by amyloid β as used herein is meant any fragment produced by proteolytic cleavage of APP but especially those fragments which are involved in or associated with the amyloid pathologies including, but not limited to, A β ₁₋₃₈, A β ₁₋₄₀, A β ₁₋₄₂. The amino acid sequences of these A β peptides are as follows:

A β 1-42 (SEQ ID NO. 1):

Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys-Leu-Val-Phe-Phe-Ala-Glu-Asp-Val-Gly-Ser-Asn-Lys-Gly-Ala-Ile-Ile-Gly-Leu-Met-Val-Gly-Gly-Val-Val-Ile-Ala

A β 1-40 (SEQ ID NO. 2):

Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys-Leu-Val-Phe-Phe-Ala-Glu-Asp-Val-Gly-Ser-Asn-Lys-Gly-Ala-Ile-Ile-Gly-Leu-Met-Val-Gly-Gly-Val-Val

A β 1-38 (SEQ ID NO. 3):

Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys-Leu-Val-Phe-Phe-Ala-Glu-Asp-Val-Gly-Ser-Asn-Lys-Gly-Ala-Ile-Ile-Gly-Leu-Met-Val-Gly-Gly

"pGlu-A β " or "A β N3pE" refers to N-terminally truncated forms of A β , that start at the glutamic acid residue at position 3 in the amino acid sequence of A β , and wherein said glutamic acid residue is cyclized to form a pyroglutamic acid residue. In particular, by pGlu-A β or A β N3pE as used herein are meant those fragments which are involved in or associated with the amyloid pathologies including, but not limited to, pGlu-A β ₃₋₃₈, pGlu-A β ₃₋₄₀, pGlu-A β ₃₋₄₂.

The sequences of the N-terminally truncated forms of A β , A β ₃₋₃₈, A β ₃₋₄₀, A β ₃₋₄₂ are as follows:

A β 3-42 (SEQ ID NO. 4):

Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys-Leu-Val-Phe-Phe-Ala-Glu-Asp-Val-Gly-Ser-Asn-Lys-Gly-Ala-Ile-Ile-Gly-Leu-Met-Val-Gly-Gly-Val-Val-Ile-Ala

A β 3-40 (SEQ ID NO. 5):

Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys-Leu-Val-Phe-Phe-Ala-Glu-Asp-Val-Gly-Ser-Asn-Lys-Gly-Ala-Ile-Ile-Gly-Leu-Met-Val-Gly-Gly-Val-Val

A β 3-38 (SEQ ID NO. 6):

5 Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys-Leu-Val-Phe-Phe-Ala-Glu-Asp-Val-Gly-Ser-Asn-Lys-Gly-Ala-Ile-Ile-Gly-Leu-Met-Val-Gly-Gly

10 The present invention pertains to humanized antibodies specific for human A β peptides that are N-terminally truncated by cleaving off or losing amino acids no. 1 and 2 of the N-terminus and in which the so uncovered N-terminal amino acid no. 3 is modified by pyroglutamate formation and which thus bear a pyroglutamate residue at position 3 of the N-terminus (further referred to as A β N3pE peptides or N3pE-A β peptides or pyroglutamated A β peptides).

15 In a first aspect, the present invention pertains to a humanized antibody, wherein the variable part of the light chain of said antibody comprises, consists essentially of or consists of an amino acid sequence of:

20 DVVMTQSPLSLPVTLGQPASISCKSSQSLLX₁SDGKTYLNWFQQRPQGQSPRRLX₂YLVSCLD SGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCVQGTHFPFTFGGGTKVEIK (SEQ ID NO: 7),

wherein

X₁ is selected from Y and H; and

25 X₂ is selected from A, I and T.

In a preferred embodiment of the present invention, the antibody having the variable part of the light chain of said antibody, which comprises, consists essentially of or consists of the amino acid sequence of SEQ ID NO: 7, comprises the following CDR regions in the light chain:

30 V_L CDR1: KSSQSLLX₁SDGKTYLN (SEQ ID NO: 8),
wherein X₁ is selected from Y and H;
V_L CDR2: LVSKLDS (SEQ ID NO: 9); and
V_L CDR3: VQGTHFP (SEQ ID NO: 10).

35

More preferably, in the variable part of the light chain of the antibody of the present invention, X_1 is Y and X_2 is I, and the variable part of the light chain thus comprises, consists essentially of or consists of the amino acid sequence of:

5 DVVMTQSPLSLPVTLGQPASISCKSSQSLLYSDGKTYLNWFFQQRPGQSPRRLIYLVSKLDS
GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCVQGTHFPFTFGGGTKVEIK (SEQ ID NO:
11).

10 In a preferred embodiment of the present invention, the antibody having the variable part of
the light chain of said antibody, which comprises, consists essentially of or consists of the
amino acid sequence of SEQ ID NO: 11, comprises the following CDR regions in the light
chain:

15 V_L CDR1: KSSQSLLYSDGKTYLN (SEQ ID NO: 12),
 V_L CDR2: LVSKLDS (SEQ ID NO: 9); and
 V_L CDR3: VQGTHFP (SEQ ID NO: 10).

20 Even preferably, in the variable part of the light chain of the antibody of the present invention,
 X_1 is Y and X_2 is A and the variable part of the light chain thus comprises, consists essentially
of or consists of the amino acid sequence of:

25 DVVMTQSPLSLPVTLGQPASISCKSSQSLLYSDGKTYLNWFFQQRPGQSPRRLAYLVSKLDS
GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCVQGTHFPFTFGGGTKVEIK (SEQ ID NO:
13).

In a preferred embodiment of the present invention, the antibody having the variable part of
the light chain of said antibody, which comprises, consists essentially of or consists of the
amino acid sequence of SEQ ID NO: 13, comprises the CDR regions V_L CDR1 of SEQ ID NO:
12, V_L CDR2 SEQ ID NO: 9 and V_L CDR3 of SEQ ID NO: 10.

30 Most preferably, in the variable part of the light chain of the antibody of the present invention,
 X_1 is Y and X_2 is T and the variable part of the light chain thus comprises, consists essentially
of or consists of the amino acid sequence of:

DVVMTQSPLSLPVTLGQPASISCKSSQSLLYSDGKTYLNWFFQQRPGQSPRRLTYLVSKLDS
GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCVQGTHFPFTFGGGTKVEIK (SEQ ID NO:
14).

- 5 In a preferred embodiment of the present invention, the antibody having the variable part of the light chain of said antibody, which comprises, consists essentially of or consists of the amino acid sequence of SEQ ID NO: 14, comprises the CDR regions V_L CDR1 of SEQ ID NO: 12, V_L CDR2 SEQ ID NO: 9 and V_L CDR3 of SEQ ID NO: 10.
- 10 Even most preferably, in the variable part of the light chain of the antibody of the present invention, X₁ is H and X₂ is T and the light chain thus comprises, consists essentially of or consists of the amino acid sequence of:

DVVMTQSPLSLPVTLGQPASISCKSSQSLLHSDGKTYLNWFFQQRPGQSPRRLTYLVSKLDS
15 GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCVQGTHFPFTFGGGTKVEIK (SEQ ID NO:
15).

- In a preferred embodiment of the present invention, the antibody having the variable part of the light chain of said antibody, which comprises, consists essentially of or consists of the amino acid sequence of SEQ ID NO: 15, comprises the following CDR regions in the light chain:
- 20

V_L CDR1: KSSQSLLHSDGKTYLN (SEQ ID NO: 16),
V_L CDR2: LVSKLDS (SEQ ID NO: 9); and
25 V_L CDR3: VQGTHFP (SEQ ID NO: 10).

- Further in accordance with the first aspect, present invention pertains to a humanized antibody, wherein the variable part of heavy chain of said antibody comprises, consists essentially of or consists of an amino acid sequence of:
- 30

QVQLVQSGAEVKKSGASVKVSCKASGYSFTGX₃TMNWVRQAPGQGLEWMGLINPX₄NX₅V
TRYNQKF₆GRVTX₇X₈RDSTTTTVX₉MELTSLTSED₁₀YYCTREAKREWDETYWGQGTL
VTVSS (SEQ ID NO: 17);

- 35 wherein
X₃ is selected from Y and H;

X₄ is selected from Y and S;
 X₅ is selected from G, T, A and E;
 X₆ is selected from K and Q;
 X₇ is selected from L and I;
 5 X₈ is selected from I and T;
 X₉ is selected from Y and H; and
 X₁₀ is selected from V and T;

10 In a preferred embodiment of the present invention, the antibody having the variable part of the heavy chain of said antibody, which comprises, consists essentially of or consists of the amino acid sequence of SEQ ID NO: 17, comprises the following CDR regions in the heavy chain:

V_H CDR1: GYSFTGX₃TMN (SEQ ID NO: 18),
 15 wherein X₃ is selected from Y and H;
 V_H CDR2: LINPX₄NX₅VTRYNQKF₆G (SEQ ID NO: 19);
 wherein X₄ is selected from Y and S, X₅ is selected from G, T, A and E
 and X₆ is selected from K and Q; and
 V_H CDR3: EAKREWDETY (SEQ ID NO: 20).
 20

Preferably, in the variable part of the heavy chain of the antibody of the present invention, X₃ is Y, X₄ is Y, X₅ is G, X₆ is K, X₇ is T, X₈ is I, X₉ is Y and X₁₀ is V and the heavy chain thus comprises, consists essentially of or consists of the amino acid sequence of:

25 QVQLVQSGAEVKKSGASVKVSCASGYSFTGYTMNWVRQAPGQGLEWMGLINPYNGVTR
 YNQKF₆KGRVTLIRDTSTTTVYME₅LTSLTSED₄AVYYCTREAKREWDETYWGQGT₃LVTVSS
 (SEQ ID NO: 21).

30 In a preferred embodiment of the present invention, the antibody having the variable part of the heavy chain of said antibody, which comprises, consists essentially of or consists of the amino acid sequence of SEQ ID NO: 21, comprises the following CDR regions in the heavy chain:

V_H CDR1: GYSFTGYTMN (SEQ ID NO: 22),
 35 V_H CDR2: LINPYNGVTRYNQKF₆KG (SEQ ID NO: 23);
 V_H CDR3: EAKREWDETY (SEQ ID NO: 20).

More preferably, in the variable part of the heavy chain of the antibody of the present invention, X₃ is H, X₄ is S, X₅ is G, X₆ is Q, X₇ is I, X₈ is T, X₉ is H and X₁₀ is V and the heavy chain thus comprises, consists essentially of or consists of the amino acid sequence of:

5

QVQLVQSGAEVKKSGASVKVSCASGYSFTGHTMNWVRQAPGQGLEWMGLINPSNGVTR
YNQKFQGRVTITRDTSTTTVHMELTSLTSED TAVYYCTREAKREWDETYWGQGTLVTVSS
(SEQ ID NO: 24).

10 In a preferred embodiment of the present invention, the antibody having the variable part of the heavy chain of said antibody, which comprises, consists essentially of or consists of the amino acid sequence of SEQ ID NO: 24, comprises the following CDR regions in the heavy chain:

15 V_H CDR1: GYSFTGHTMN (SEQ ID NO: 25),
V_H CDR2: LINPSNGVTRYNQKFQG (SEQ ID NO: 26);
V_H CDR3: EAKREWDETY (SEQ ID NO: 20).

20 Even more preferably, in the variable part of the heavy chain of the antibody of the present invention, X₃ is H, X₄ is S, X₅ is G, X₆ is Q, X₇ is I, X₈ is T, X₉ is H and X₁₀ is T and the heavy chain thus comprises, consists essentially of or consists of the amino acid sequence of:

QVQLVQSGAEVKKSGASVKVSCASGYSFTGHTMNWVRQAPGQGLEWMGLINPSNGVTR
YNQKFQGRVTITRDTSTTTVHMELTSLTSED TATYYCTREAKREWDETYWGQGTLVTVSS
25 (SEQ ID NO: 27).

30 In a preferred embodiment of the present invention, the antibody having the variable part of the heavy chain of said antibody, which comprises, consists essentially of or consists of the amino acid sequence of SEQ ID NO: 27, comprises the following CDR regions in the heavy chain:

V_H CDR1: GYSFTGHTMN (SEQ ID NO: 25),
V_H CDR2: LINPSNGVTRYNQKFQG (SEQ ID NO: 26);
V_H CDR3: EAKREWDETY (SEQ ID NO: 20).

35

Most preferably, in the variable part of the heavy chain of the antibody of the present invention, X₃ is H, X₄ is S, X₅ is T, X₆ is Q, X₇ is I, X₈ is T, X₉ is H and X₁₀ is T and the heavy chain thus comprises, consists essentially of or consists of the amino acid sequence of:

5 QVQLVQSGAEVKKSGASVKVSCASGYSFTGHTMNWVRQAPGQGLEWMGLINPSNTVTR
YNQKFQGRVTITRDTSTTTVHMELTSLTSEDATYYCTREAKREWDETYWGQGTLVTVSS
(SEQ ID NO: 66).

10 In a preferred embodiment of the present invention, the antibody having the variable part of
the heavy chain of said antibody, which comprises, consists essentially of or consists of the
amino acid sequence of SEQ ID NO: 66, comprises the following CDR regions in the heavy
chain:

15 V_H CDR1: GYSFTGHTMN (SEQ ID NO: 25),
V_H CDR2: LINPSNTVTRYNQKFQG (SEQ ID NO: 67);
V_H CDR3: EAKREWDETY (SEQ ID NO: 20).

20 Even most preferably, in the variable part of the heavy chain of the antibody of the present
invention, X₃ is H, X₄ is S, X₅ is A, X₆ is Q, X₇ is I, X₈ is T, X₉ is H and X₁₀ is T and the heavy
chain thus comprises, consists essentially of or consists of the amino acid sequence of:

25 QVQLVQSGAEVKKSGASVKVSCASGYSFTGHTMNWVRQAPGQGLEWMGLINPSNAVTR
YNQKFQGRVTITRDTSTTTVHMELTSLTSEDATYYCTREAKREWDETYWGQGTLVTVSS
(SEQ ID NO: 68).

30 In a preferred embodiment of the present invention, the antibody having the variable part of
the heavy chain of said antibody, which comprises, consists essentially of or consists of the
amino acid sequence of SEQ ID NO: 68, comprises the following CDR regions in the heavy
chain:

V_H CDR1: GYSFTGHTMN (SEQ ID NO: 25),
V_H CDR2: LINPSNAVTRYNQKFQG (SEQ ID NO: 69);
V_H CDR3: EAKREWDETY (SEQ ID NO: 20).

Even most preferably, in the variable part of the heavy chain of the antibody of the present invention, X₃ is H, X₄ is S, X₅ is E, X₆ is Q, X₇ is I, X₈ is T, X₉ is H and X₁₀ is T and the heavy chain thus comprises, consists essentially of or consists of the amino acid sequence of:

5 QVQLVQSGAEVKKSGASVKVSCASGYSFTGHTMNWVRQAPGQGLEWMGLINPSNEVTR
YNQKFQGRVTITRDTSTTTVHMELTSLTSEDATYYCTREAKREWDETYWGQGTLVTVSS
(SEQ ID NO: 70).

10 In a preferred embodiment of the present invention, the antibody having the variable part of the heavy chain of said antibody, which comprises, consists essentially of or consists of the amino acid sequence of SEQ ID NO: 70, comprises the following CDR regions in the heavy chain:

15 V_H CDR1: GYSFTGHTMN (SEQ ID NO: 25),
V_H CDR2: LINPSNEVTRYNQKFQG (SEQ ID NO: 71);
V_H CDR3: EAKREWDETY (SEQ ID NO: 20).

20 Further according to first aspect of the invention, the humanized antibodies comprising, essentially consisting of or consisting of the following combinations of the variable parts of the light chain and heavy chain are preferred:

Humanized antibody variant	Light chain variable part, SEQ ID NO:	Heavy chain variable part, SEQ ID NO:
General	7	17
i)	11	21
ii)	11	24
iii)	11	27
iv)	11	66
v)	11	68
vi)	11	70
vii)	13	21
viii)	13	24
ix)	13	27
x)	13	66
xi)	13	68

xii)	13	70
xiii)	14	21
xiv)	14	24
xv)	14	27
Xvi	14	66
Xvii	14	68
Xviii	14	70
xix)	15	21
xx)	15	24
xxi)	15	27
Xxii	15	66
Xxiii	15	68
Xiv	15	70

More preferably, the variable part of the light chain of the humanized antibody according to the invention comprises, consists essentially of or consists of the amino acid sequence of SEQ ID NO: 14.

5

Even more preferably, the variable part of the heavy chain of the humanized antibody according to the invention comprises, consists essentially of or consists of the amino acid sequence of SEQ ID NO: 27.

10 Even more preferably, the variable part of the heavy chain of the humanized antibody according to the invention comprises, consists essentially of or consists of the amino acid sequence selected from SEQ ID NO: 66, 72 and 74.

15 Most preferably, the variable part of the heavy chain of the humanized antibody according to the invention comprises, consists essentially of or consists of the amino acid sequence of SEQ ID NO: 70.

20 Most preferably, the variable part of the light chain of the humanized antibody according to the invention comprises, consists essentially of or consists of the amino acid sequence of SEQ ID NO: 14 and the variable part of the heavy chain of the humanized antibody according to the invention comprises, consists essentially of or consists of the amino acid sequence of SEQ ID NO: 27.

Even most preferably,

- the variable part of the light chain of the humanized antibody according to the invention comprises, consists essentially of or consists of the amino acid sequence of SEQ ID NO: 14; and
- the variable part of the heavy chain of the humanized antibody according to the invention comprises, consists essentially of or consists of the amino acid sequence of SEQ ID NO: 27; and
- the variable part of the light chain of said antibody, which comprises, consists essentially of or consists of the amino acid sequence of SEQ ID NO: 14, comprises the CDR regions V_L CDR1 of SEQ ID NO: 12, V_L CDR2 SEQ ID NO: 9 and V_L CDR3 of SEQ ID NO: 10; and
- the variable part of the heavy chain of said antibody, which comprises, consists essentially of or consists of the amino acid sequence of SEQ ID NO: 27, comprises the CDR regions V_H CDR1 of SEQ ID NO: 25, V_H CDR2 SEQ ID NO: 26 and V_H CDR3 of SEQ ID NO: 20.

Even most preferably, the variable part of the light chain of the humanized antibody according to the invention comprises, consists essentially of or consists of the amino acid sequence of SEQ ID NO: 14 and the variable part of the heavy chain of the humanized antibody according to the invention comprises, consists essentially of or consists of the amino acid sequence of SEQ ID NO: 70.

Even most preferably,

- the variable part of the light chain of the humanized antibody according to the invention comprises, consists essentially of or consists of the amino acid sequence of SEQ ID NO: 14; and
- the variable part of the heavy chain of the humanized antibody according to the invention comprises, consists essentially of or consists of the amino acid sequence of SEQ ID NO: 70; and
- the variable part of the light chain of said antibody, which comprises, consists essentially of or consists of the amino acid sequence of SEQ ID NO: 14, comprises the CDR regions V_L CDR1 of SEQ ID NO: 12, V_L CDR2 SEQ ID NO: 9 and V_L CDR3 of SEQ ID NO: 10; and

- the variable part of the heavy chain of said antibody, which comprises, consists essentially of or consists of the amino acid sequence of SEQ ID NO: 70, comprises the CDR regions V_H CDR1 of SEQ ID NO: 25, V_H CDR2 SEQ ID NO: 71 and V_H CDR3 of SEQ ID NO: 20.

5

In a second aspect, the present invention pertains to a humanized antibody, wherein the variable part of the light chain of said antibody comprises, consists essentially of or consists of an amino acid sequence of:

10 DIQMTQSPSSLSASVGDRVTITCRSSQSLVHSDGNTYLHWYQQKPGKAPKLLIYKVSNRFS
GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCSQSTHVPPTFGQGTKVEIK (SEQ ID NO:
28).

15 In a preferred embodiment of the present invention, the antibody having the variable part of
the light chain of said antibody, which comprises, consists essentially of or consists of the
amino acid sequence of SEQ ID NO: 28, comprises the following CDR regions in the light
chain:

20 V_L CDR1: RSSQSLVHSDGNTYLH (SEQ ID NO: 29),
V_L CDR2: KVSNRFS (SEQ ID NO: 30); and
V_L CDR3: SQSTHVPPT (SEQ ID NO: 31).

25 Further in accordance with the second aspect, the present invention pertains to a humanized
antibody, wherein the variable part of the heavy chain of said antibody comprises, consists
essentially of or consists of an amino acid sequence of:

30 EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYGMAWVRQAPGKGLEWVSFISNLAYSIIY
ADTVTGRFTISRDNKNSLYLQMNSLRAEDTAVYYCARYDYDNILDYVMDYWGQGTLVTVS
S (SEQ ID NO: 32).

30

In a preferred embodiment of the present invention, the antibody having the variable part of
the heavy chain of said antibody, which comprises, consists essentially of or consists of the
amino acid sequence of SEQ ID NO: 32, comprises the following CDR regions in the heavy
chain:

35

V_H CDR1: GFTFSDYGMA (SEQ ID NO: 33),

V_H CDR2: FISNLAYSIYYADTVTG (SEQ ID NO: 34);

V_H CDR3: YDYDNILDYVMDY (SEQ ID NO: 35).

In a third aspect, the present invention pertains to a humanized antibody, wherein the variable part of the light chain of said antibody comprises, consists essentially of or consists of an amino acid sequence of:

DVVMTQSPLSLPVTLGQPASISCKSSQSLLYSNGKTYLNWFFQQRPGQSPRRLIYVVS KLDS
GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCVQGTHFPFTFGGGTKVEIK (SEQ ID NO:
36).

In a preferred embodiment of the present invention, the antibody having the variable part of the light chain of said antibody, which comprises, consists essentially of or consists of the amino acid sequence of SEQ ID NO: 36, comprises the following CDR regions in the light chain:

V_L CDR1: KSSQSLLYSNGKTYLN (SEQ ID NO: 37),

V_L CDR2: VVSKLDS (SEQ ID NO: 38); and

V_L CDR3: VQGTHFPFT (SEQ ID NO: 39).

Further in accordance with the third aspect, the present invention pertains to a humanized antibody, wherein the variable part of the heavy chain of said antibody comprises, consists essentially of or consists of an amino acid sequence of:

QVQLVESGAIEVKKPGASVKVSKASGYIFNNYWINWVRQAPGQGLEWMGQIYPGDGDTN
YNGKFKGRVTITADESTSTAYMELSSLRSEDYAVYYCAREGYIVYWGQGTLVTVSS (SEQ ID
NO: 40).

In a preferred embodiment of the present invention, the antibody having the variable part of the heavy chain of said antibody, which comprises, consists essentially of or consists of the amino acid sequence of SEQ ID NO: 40, comprises the following CDR regions in the heavy chain:

V_H CDR1: GYIFNNY (SEQ ID NO: 41),

V_H CDR2: QIYPGDGDTNYNGKFKG (SEQ ID NO: 42);

V_H CDR3: EGYIVY (SEQ ID NO: 43).

In a further particularly preferred embodiment, the present invention pertains to humanized antibodies specific for human N3pE-A β peptides, which comprise CDR regions in the light chain selected from:

5

V_L CDR 1	V_L CDR 2	V_L CDR 3
KSSQSLLHSDGKTYLN (SEQ ID NO: 16)	LVSKLDS (SEQ ID NO: 9)	VQGTHFP (SEQ ID NO: 10)
RSSQSLVHSDGNTYLH (SEQ ID NO: 29)	KVSNRFS (SEQ ID NO: 30)	SQSTHVPPT (SEQ ID NO: 31)
KSSQSLLYSNGKTYLN (SEQ ID NO: 37)	VVSKLDS (SEQ ID NO: 38)	VQGTHFPFT (SEQ ID NO: 39)

Furthermore, the present invention pertains to human antibodies specific for human N3pE-A β peptides, which comprise CDR regions in the heavy chain selected from:

10

V_H CDR 1	V_H CDR 2	V_H CDR 3
GYSFTGYTMN (SEQ ID NO: 22)	LINPYNGVTRYNQKFKG (SEQ ID NO: 23)	EAKREWDETY (SEQ ID NO: 20)
GYSFTGHTMN (SEQ ID NO: 25)	LINPSNGVTRYNQKFQG (SEQ ID NO: 26)	YDYDNILDYVMDY (SEQ ID NO: 35)
GFTFSDYGMA (SEQ ID NO: 33)	FISNLAYSIIYADTVTG (SEQ ID NO: 34)	EGYIVY (SEQ ID NO: 43)
GYIFNNY (SEQ ID NO: 41)	QIYPGDGDTNYNGKFKG (SEQ ID NO: 42)	
	LINPSNTVTRYNQKFQG (SEQ ID NO: 67)	
	LINPSNAVTRYNQKFQG (SEQ ID NO: 69)	
	LINPSNEVTRYNQKFQG (SEQ ID NO: 71)	

Preferred humanized antibodies according to the invention are humanized forms of monoclonal mouse antibodies that are produced by a hybridoma cell line selected from:

	A β 5-5-6	(Deposit No. DSM ACC 2923)
	A β 6-1-6	(Deposit No. DSM ACC 2924)
	A β 17-4-3	(Deposit No. DSM ACC 2925)
5	A β 24-2-3	(Deposit No. DSM ACC 2926)

which are described in WO 2010/009987.

10 The sequences of the light and heavy chains for the humanized antibodies of the present invention can vary. The immunoglobulins can have two pairs of light chain/heavy chain complexes, at least one chain comprising one or more mouse complementarity determining regions (CDRs) functionally joined to human framework region segments.

15 In another embodiment, the present invention is directed to recombinant polynucleotides encoding the humanized antibodies of the invention comprising the heavy and light chain CDRs as set forth herein.

20 The human framework region of the antibodies of the invention is determined by comparison of a framework or variable region amino acid sequence of a CDR-providing non-human immunoglobulin with corresponding sequences in a sequence collection comprising human immunoglobulin variable regions. A sequence having a high percentage of identical amino acids is selected.

25 Preferred polynucleotides of the present invention encode antibodies, comprising CDRs selected from those consisting of the amino acid sequences of SEQ ID NOs: 9, 10, 16, 29-31 and 37-39 in the light chain and selected from those consisting of the amino acid sequences of SEQ ID NOs: 20, 22, 23, 25, 26, 33-35, 41-43, 67, 69 and 71 in the heavy chain.

30 Further preferred are polynucleotides, which encode antibodies, wherein the variable part of the light chain comprises, essentially consists or consists of an amino acid sequence selected from SEQ ID NOs: 7, 11, 13, 14, and 28.

35 Even preferred are polynucleotides, which encode antibodies, wherein the variable part of the heavy chain comprises, essentially consists or consists of an amino acid sequence selected from SEQ ID NOs: 17, 21, 24, 27, 32, 36, 40, 66, 68 and 70.

In a further embodiment, the humanized antibodies of the present invention have a human IgG1 Fc region, which comprises, consists essentially of or consists of an amino acid sequence of SEQ ID NO: 73.

5 C1q and two serine proteases, C1r and C1s, form the complex C1, the first component of the complement dependent cytotoxicity (CDC) pathway. C1q is a hexavalent molecule with a molecular weight of approximately 460,000 and a structure likened to a bouquet of tulips in which six collagenous "stalks" are connected to six globular head regions (Burton and Woof, Advances in Immunol 51:1-84; 1992). Binding of IgG1 molecules to C1q initiates complement
10 activation and subsequently leads to complement-mediated cell lysis. The humanized antibodies of the present invention shall be used in treatment of inflammatory diseases and conditions, i.e. the humanized antibodies of the present invention shall have anti-inflammatory properties.

15 Effector functions of the humanized antibodies of the invention can also be mediated by the interaction of the Fc region of an antibody with Fc receptors (FcRs), which are specialized cell surface receptors on hematopoietic cells. Fc receptors belong to the immunoglobulin superfamily, and have been shown to mediate both the removal of antibody-coated pathogens by phagocytosis of immune complexes, and the lysing of erythrocytes and various other
20 cellular targets (e.g. tumor cells) coated with the corresponding antibody, via antibody dependent cell mediated cytotoxicity (ADCC) (Van de Winkel and Anderson, J. Leuk. Bioi. 49:511-24; 1991).

Therefore, the present invention further provides humanized antibodies that still bind to the Fc
25 receptors to fulfill their effector functions. But, preferably the humanized antibodies of the invention do not show a complement dependent cytotoxicity. More preferably, the humanized antibodies of the invention do not activate the complement system, but rather inhibit the complement-mediated cell lysis.

30 Thus, in a preferred embodiment, the humanized antibodies of the present invention have a human IgG Fc region, which comprises one or more an amino acid substitutions, preferably the substitution of 3 or 2 amino acids, most preferably the substitution of one amino acid. The amino acid substitutions can be achieved by conventional methods, such as site-directed mutagenesis of the human IgG1 Fc region of the antibodies of the present invention.

35

In a more preferred embodiment, the humanized antibodies of the present invention have a human IgG Fc region which comprises an amino acid substitution at position 322. The amino acid substitution is preferably K322A.

- 5 In a most preferred embodiment, the humanized antibodies of the present invention have a human IgG Fc region, which comprises, consists essentially of or consists of an amino acid sequence of SEQ ID NO: 74.

10 Further most preferably, the variable part of the light chain of the humanized antibody according to the invention comprises, consists essentially of or consists of the amino acid sequence of SEQ ID NO: 14; and the variable part of the heavy chain of the humanized antibody according to the invention comprises, consists essentially of or consists of the amino acid sequence of SEQ ID NO: 27; and the human IgG Fc region comprises, consists essentially of or consists of the amino acid sequence of SEQ ID NO: 74.

15

Even most preferably,

- the variable part of the light chain of the humanized antibody according to the invention comprises, consists essentially of or consists of the amino acid sequence of SEQ ID NO: 14; and
- 20 – the variable part of the heavy chain of the humanized antibody according to the invention comprises, consists essentially of or consists of the amino acid sequence of SEQ ID NO: 27; and
- the human IgG Fc region comprises, consists essentially of or consists of the amino acid sequence of SEQ ID NO: 74;
- 25 – the variable part of the light chain of said antibody, which comprises, consists essentially of or consists of the amino acid sequence of SEQ ID NO: 14, comprises the CDR regions V_L CDR1 of SEQ ID NO: 12, V_L CDR2 SEQ ID NO: 9 and V_L CDR3 of SEQ ID NO: 10; and
- the variable part of the heavy chain of said antibody, which comprises, consists
- 30 essentially of or consists of the amino acid sequence of SEQ ID NO: 27, comprises the CDR regions V_H CDR1 of SEQ ID NO: 25, V_H CDR2 SEQ ID NO: 26 and V_H CDR3 of SEQ ID NO: 20.

35 Even most preferably, the variable part of the light chain of the humanized antibody according to the invention comprises, consists essentially of or consists of the amino acid sequence of

SEQ ID NO: 14; and the variable part of the heavy chain of the humanized antibody according to the invention comprises, consists essentially of or consists of the amino acid sequence of SEQ ID NO: 70; and the human IgG Fc region comprises, consists essentially of or consists of the amino acid sequence of SEQ ID NO: 74.

5

Even most preferably,

- the variable part of the light chain of the humanized antibody according to the invention comprises, consists essentially of or consists of the amino acid sequence of SEQ ID NO: 14; and
- 10 – the variable part of the heavy chain of the humanized antibody according to the invention comprises, consists essentially of or consists of the amino acid sequence of SEQ ID NO: 70; and
- the human IgG Fc region comprises, consists essentially of or consists of the amino acid sequence of SEQ ID NO: 74;
- 15 – the variable part of the light chain of said antibody, which comprises, consists essentially of or consists of the amino acid sequence of SEQ ID NO: 14, comprises the CDR regions V_L CDR1 of SEQ ID NO: 12, V_L CDR2 SEQ ID NO: 9 and V_L CDR3 of SEQ ID NO: 10; and
- the variable part of the heavy chain of said antibody, which comprises, consists
- 20 essentially of or consists of the amino acid sequence of SEQ ID NO: 70, comprises the CDR regions V_H CDR1 of SEQ ID NO: 25, V_H CDR2 SEQ ID NO: 71 and V_H CDR3 of SEQ ID NO: 20.

25

Preferred polynucleotides of the present invention encode antibodies, comprising CDRs selected from those consisting of the amino acid sequences of SEQ ID NOs: 9, 10, 16, 29-31 and 37-39 in the light chain and selected from those consisting of the amino acid sequences of SEQ ID NOs: 20, 22, 23, 25, 26, 33-35, 41-43, 67, 69 and 71 in the heavy chain; and comprising a human IgG Fc region selected from SEQ ID NOs: 73 and 74.

30

Further preferred are polynucleotides, which encode antibodies, wherein the variable part of the light chain comprises, essentially consists or consists of an amino acid sequence selected from SEQ ID NOs: 7, 11, 13, 14, and 28; and wherein the human IgG Fc region comprises, consists essentially of or consists of an amino acid sequence selected from SEQ ID NO: 73 or 74.

35

Even preferred are polynucleotides, which encode antibodies, wherein the variable part of the heavy chain comprises, essentially consists or consists of an amino acid sequence selected from SEQ ID NOs: 17, 21, 24, 27, 32, 36, 40, 66, 68 and 70; and wherein the human IgG Fc region comprises, consists essentially of or consists of an amino acid sequence selected from
5 SEQ ID NO: 73 or 74.

The aforementioned polynucleotides can be integrated into expression vectors well known in the art. Transfection of these expression vectors in an appropriate host, the selection of the host as well as the expression collection and purification of the light chains, heavy chains,
10 light/heavy chain dimers or intact antibodies, binding fragments or other immunoglobulin forms are well-known procedures in the art.

One skilled in the art can select a vector based on desired properties, for example, for production of a vector in a particular cell such as a mammalian cell or a bacterial cell.

Any of a variety of inducible promoters or enhancers can be included in the vector for expression of an antibody of the invention or nucleic acid that can be regulated. Such inducible systems, include, for example, tetracycline inducible System (Gossen & Bizard, Proc. Natl. Acad. Sci. USA, 89:5547-5551 (1992); Gossen et al., Science, 268:1766-1769 (1995);
20 Clontech, Palo Alto, Calif.); metallothionein promoter induced by heavy metals; insect steroid hormone responsive to ecdysone or related steroids such as muristerone (No et al., Proc. Natl. Acad. Sci. USA, 93:3346-3351 (1996); Yao et al., Nature, 366:476-479 (1993); Invitrogen, Carlsbad, Calif.); mouse mammary tumor virus (MMTV) induced by steroids such as glucocorticoid and estrogen (Lee et al., Nature, 294:228-232 (1981); and heat shock promoters
25 inducible by temperature changes; the rat neuron specific enolase gene promoter (Forss-Petter, et al., Neuron 5; 197-197 (1990)); the human β -actin gene promoter (Ray, et al., Genes and Development (1991) 5:2265-2273); the human platelet derived growth factor B (PDGF-B) chain gene promoter (Sasahara, et al., Cell (1991) 64:217-227); the rat sodium channel gene promoter (Maue, et al., Neuron (1990) 4:223-231); the human copper-zinc superoxide
30 dismutase gene promoter (Ceballos-Picot, et al., Brain Res. (1991) 552:198-214); and promoters for members of the mammalian POU-domain regulatory gene family (Xi et al., (1989) Nature 340:35-42).

Regulatory elements, including promoters or enhancers, can be constitutive or regulated,
35 depending upon the nature of the regulation. The regulatory sequences or regulatory elements are operatively linked to one of the polynucleotide sequences of the invention such that the

physical and functional relationship between the polynucleotide sequence and the regulatory sequence allows transcription of the polynucleotide sequence. Vectors useful for expression in eukaryotic cells can include, for example, regulatory elements including the CAG promoter, the SV40 early promoter, the cytomegalovirus (CMV) promoter, the mouse mammary tumor virus (MMTV) steroid-inducible promoter, Pgdf, Moloney murine leukemia virus (MMLV) promoter, thy-1 promoter and the like.

If desired, the vector can contain a selectable marker. As used herein, a "selectable marker" refers to a genetic element that provides a selectable phenotype to a cell in which the selectable marker has been introduced. A selectable marker is generally a gene whose gene product provides resistance to an agent that inhibits cell growth or kills a cell. A variety of selectable markers can be used in the DNA constructs of the invention, including, for example, Neo, Hyg, hisD, Gpt and Ble genes, as described, for example in Ausubel et al. (Current Protocols in Molecular Biology (Supplement 47), John Wiley & Sons, New York (1999)) and U.S. Patent No. 5,981,830. Drugs useful for selecting for the presence of a selectable marker include, for example, G418 for Neo, hygromycin for Hyg, histidinol for hisD, xanthine for Gpt, and bleomycin for Ble (see Ausubel et al, supra, (1999); U.S. Patent No. 5,981,830). DNA constructs of the invention can incorporate a positive selectable marker, a negative selectable marker, or both (see, for example, U.S. Patent No. 5,981,830).

Various mammalian cell culture systems can also be employed to express a recombinant protein. Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblasts, described by Gluzman, Cell, 23: 175 (1981). Other cell lines capable of expressing a compatible vector include, for example, the C127, 3T3, CHO, HeLa and BHK cell lines. Mammalian expression vectors will generally comprise an origin of replication, a suitable promoter and enhancer, and also any necessary ribosome binding sites, polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking nontranscribed sequences. DNA sequences derived from the SV40 splice, and polyadenylation sites may be used to provide required nontranscribed genetic elements.

The polypeptides can be recovered and purified from recombinant cell cultures by methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Recovery can be facilitated if the polypeptide is expressed at the surface of

the cells, but such is not a prerequisite. Recovery may also be desirable of cleavage products that are cleaved following expression of a longer form of the polypeptide. Protein refolding steps as known in this art can be used, as necessary, to complete configuration of the mature protein. High performance liquid chromatography (HPLC) can be employed for final purification steps.

Human constant region DNA sequences can be isolated in accordance with well-known procedures from a variety of human cells.

The present invention pertains in particular to humanized antibodies which are characterized in that they bind to A β N3pE peptides with a high affinity. The present invention also pertains to antibodies which are characterized in that they bind to A β N3pE peptides or immunologically active fragments thereof with a high affinity. Said high affinity means in the context of the present invention an affinity of a K_D value of 10^{-5} M, 10^{-6} M or 10^{-7} M or better, preferably a K_D value of 10^{-8} M or better, and even more preferably a K_D value of 10^{-9} M – 10^{-12} M. Thereby, the inventive antibodies bind to monomeric A β N3pE with a higher affinity than previously known antibodies.

Preferably, the binding epitope of the humanized antibodies of the present invention in A β N3pE binds is an epitope, which carries a pyroglutamate at the N-terminus. More preferably, the binding epitope of the humanized antibody of the invention is selected from the group consisting of

pEFRHDSGYEVHHQKLV (SEQ ID NO: 50),
pEFRHDSGYEVHHQKL (SEQ ID NO: 54),
pEFRHDSGYEVHHQK (SEQ ID NO: 55),
pEFRHDSGYEVHHQ (SEQ ID NO: 56),
pEFRHDSGYEVHH (SEQ ID NO: 57),
pEFRHDSGYEVH (SEQ ID NO: 58),
pEFRHDSGYEV (SEQ ID NO: 59),
pEFRHDSGYE (SEQ ID NO: 60),
pEFRHDSGY (SEQ ID NO: 61),
pEFRHDSG (SEQ ID NO: 62),
pEFRHDS (SEQ ID NO: 63),
pEFRHD (SEQ ID NO: 72)

pEFRH (SEQ ID NO: 64), and
pEFR (SEQ ID NO: 65).

Most preferably, the humanized antibodies of the invention do not bind to binding epitopes that do not carry a pyroglutamate at the N-terminus.

Even most preferably, when binding to the aforementioned and subsequently mentioned binding epitopes, the humanized antibodies of the invention always bind to sequences or parts of sequences, which contain the pyroglutamate at the N-terminus. The humanized antibodies of the invention do not bind to sequences or parts of sequences, which do not contain the pyroglutamate at the N-terminus.

Further, the humanized antibody of the invention can also bind to an A β N3pE variant.

In the context of the present invention, an A β N3pE variant is in particular

pE-A β ₃₋₃₈,
pE-A β ₃₋₄₀,
pE-A β ₃₋₄₂

Further variants of A β N3pE peptides are all A β N3pE variants, which have been shown to accumulate in the brain as a consequence of Alzheimer's disease or preceding Alzheimer's disease. These are the pE-A β _{3-x} peptides, wherein x is defined as an integer between 19 and 42, e.g. in the above pE-A β ₃₋₄₂, "42" would be the integer for "x".

In the context of the present invention a "functional variant" of the inventive humanized antibody is an antibody which retains the binding capacities, in particular binding capacities with high affinity to a pE-A β _{3-x} peptide. The provision of such functional variants is known in the art and encompasses the above-mentioned possibilities, which were indicated under the definition of antibodies and fragments thereof.

In a further embodiment, the humanized antibody is an antibody fragment, as defined above.

In a further preferred embodiment, the humanized antibody of the invention is a humanized antibody which has the complementarity-determining regions (CDRs) of the above-defined antibodies. Preferably, the antibody can be labeled; possible labels are those as mentioned

above and all those known to a person skilled in the art of diagnostic uses of antibodies in particular.

In another embodiment, the humanized antibodies may be immobilized on a solid phase.

In another embodiment, the humanized antibodies according to the invention and as described herein before or a fragments thereof, exhibit a binding affinity to an A β N3pE oligomer, fiber, fibril or filament which is at least 2 times, particularly at least 4 times, particularly at least 10 times, particularly at least 15 times, more particularly at least 20 times, but especially at least 25 times higher than the binding affinity to an A β N3pE monomer.

In still another embodiment, humanized antibodies or fragments thereof are provided as described herein before, which substantially bind to aggregated A β , including A β plaques, which contain A β N3pE, in the mammalian, particularly the human brain but, preferably, do not show any significant cross-reactivity with amyloid precursor protein (APP).

In another aspect of the invention, humanized antibodies or fragments thereof are provided as described herein before, which antibodies substantially bind to oligomeric or polymeric amyloid, which contains A β N3pE, particularly amyloid β (A β) in the mammalian, particularly the human brain but, preferably, do not show any significant cross-reactivity with amyloid precursor protein (APP).

The present invention relates also to compositions comprising said humanized antibodies and the use of said compositions for the treatment of amyloidosis, especially for the treatment of neurodegenerative disease in a mammal, in particular in a human. Said neurodegenerative disease is in particular selected from the group consisting of mild cognitive impairment (MCI), Alzheimer's disease (AD), like for instance sporadic Alzheimer's disease (SAD) or Familial Alzheimer's dementias (FAD) like Familial British Dementia (FBD) and Familial Danish Dementia (FDD), neurodegeneration in Down Syndrome. Preferably, said neurodegenerative disease is Alzheimer's disease.

Thus, in a preferred embodiment, the present invention is directed to a method of treating and/or preventing conditions characterized by the formation of plaques comprising A β N3pE in mammals, preferably in humans, which method comprises administering, preferably peripherally, to a human in need of such treatment a therapeutically or prophylactically effective amount of a humanized monoclonal antibody of the invention or a immunologically

reactive fragment thereof, which antibody specifically binds to an epitope of the A β N3pE peptide that carries pyroglutamate at the N-terminus.

In another embodiment, the invention is directed to a method to inhibit the formation of amyloid plaques and to clear or remove amyloid plaques in mammals, preferably in humans, which method comprises administering to a human subject in need of such inhibition an effective amount of a humanized antibody that binds to A β N3pE in the circulation, body fluids or tissues, especially in the brain and further preferably, leads to the clearance of A β N3pE in plasma and the brain.

Accordingly, the invention also provides methods of reversing cognitive decline, improving cognition, treating cognitive decline, and preventing cognitive decline in a subject diagnosed with mild cognitive impairment (MCI), Alzheimer's disease (AD), like for instance sporadic Alzheimer's disease (SAD) or Familial Alzheimer's dementias (FAD) like Familial British Dementia (FBD) and Familial Danish Dementia (FDD), neurodegeneration in Down Syndrome and clinical or pre-clinical cerebral amyloid angiopathy, preferably Alzheimer's disease comprising administering to the subject an effective amount of a humanized antibody of the invention.

The invention also provides the use of a humanized antibody of the invention for the manufacture of a medicament, for treating, preventing, or reversing mild cognitive impairment (MCI), Alzheimer's disease (AD), like for instance sporadic Alzheimer's disease (SAD) or Familial Alzheimer's dementias (FAD) like Familial British Dementia (FBD) and Familial Danish Dementia (FDD), neurodegeneration in Down Syndrome and clinical or pre-clinical cerebral amyloid angiopathy, preferably Alzheimer's disease; or to reverse cognitive decline, improve cognition, treat cognitive decline, and prevent cognitive decline in a subject diagnosed with mild cognitive impairment (MCI), Alzheimer's disease (AD), like for instance sporadic Alzheimer's disease (SAD) or Familial Alzheimer's dementias (FAD) like Familial British Dementia (FBD) and Familial Danish Dementia (FDD), neurodegeneration in Down Syndrome and clinical or pre-clinical cerebral amyloid angiopathy, preferably Alzheimer's disease.

The invention further provides the humanized antibodies disclosed herein for use in the prevention, treatment, or the reversion of mild cognitive impairment (MCI), Alzheimer's disease (AD), like for instance sporadic Alzheimer's disease (SAD) or Familial Alzheimer's dementias (FAD) like Familial British Dementia (FBD) and Familial Danish Dementia (FDD), neurodegeneration in Down Syndrome and clinical or pre-clinical cerebral amyloid angiopathy,

preferably Alzheimer's disease; for treating, preventing, or the reversion of cognitive decline, improvement of cognition, treatment of cognitive decline, and prevention of cognitive decline in a subject diagnosed with mild cognitive impairment (MCI), Alzheimer's disease (AD), like for instance sporadic Alzheimer's disease (SAD) or Familial Alzheimer's dementias (FAD) like
5 Familial British Dementia (FBD) and Familial Danish Dementia (FDD), neurodegeneration in Down Syndrome and clinical or pre-clinical cerebral amyloid angiopathy, preferably Alzheimer's disease; or the inhibition of the formation of amyloid plaques or the effects of A β N3pE in mammals, preferably in humans.

10 In a specific embodiment the invention provides a method for retaining or increasing cognitive memory capacity but, particularly, for restoring the cognitive memory capacity of a mammal, particularly a human, suffering from memory impairment by administering to an animal, particularly a mammal or a human, a humanized antibody, or a pharmaceutical composition comprising a humanized antibody according to the invention and as described herein before.

15 The invention further provides methods to assess the response of a human subject to treatment with a humanized antibody that binds A β N3pE or a variant thereof, comprising:

a) administering a humanized antibody of the invention or a fragment thereof to the subject; and

20 b) measuring the concentration of A β N3pE in a biological sample taken from the subject.

The invention also provides a method of treating a human subject with an antibody that binds A β N3pE or a variant thereof, comprising:

a) administering a first amount of the antibody or fragment thereof to the subject;

25 b) within 3 hours to two weeks after administering the first dose, measuring the concentration of A β N3pE in a biological sample taken from the subject;

c) if necessary, calculating a second amount of antibody or fragment thereof based on the result of step b), which second amount is the same as or different than the first amount; and

d) administering the second amount of the antibody or fragment.

30 The invention also includes a method of assessing in a mammalian, preferably a human subject the efficacy of an antibody that binds to A β N3pE, or a fragment thereof, for inhibiting or preventing A β N3pE related amyloid plaque formation, for reducing the load of A β N3pE containing plaques, for reducing the effects of toxic A β N3pE and variants thereof, or for
35 treating a condition or a disease associated with plaques containing A β N3pE, comprising:

a) obtaining a first biological sample from the subject;

- b) measuring a baseline concentration of A β N3pE in the first sample;
- c) administering a humanized antibody of the invention or fragment thereof to the subject;
- d) within 3 hours to two weeks after administering the antibody or fragment thereof, obtaining a second biological sample from the subject; and
- 5 e) measuring the concentration of A β N3pE in the second biological sample; wherein, efficacy is related to the quantity of A β N3pE bound to the antibody in the blood and the concentration of A β N3pE, in particular the reduction of the concentration thereof, in the second biological sample compared to the first biological sample.

10 The biological sample may be any sample, for example from a human. In one specific example, the sample is a tissue sample, a body fluid sample or a cell sample. In one embodiment, the biological sample is selected from the group consisting of blood, serum, urine, cerebrospinal fluid (CSF), plasma, lymph, saliva, sweat, pleural fluid, synovial fluid, tear fluid, bile and pancreas secretion. In a further embodiment, the biological sample is plasma. In a preferred
15 embodiment, the biological sample is CSF.

The biological sample can be obtained from a subject in a manner well-known to a person skilled in the art. In particular, a blood sample can be obtained from a subject and the blood sample can be separated into serum and plasma by conventional methods. The subject, from
20 which the biological sample is obtained is preferably a subject suspected of being afflicted with a disease or condition of amyloidosis, preferably Alzheimer's disease, at risk of developing Alzheimer's disease and/or being at risk of or having any other kind of dementia. In particular, the sample is obtained from a subject suspected of having Mild Cognitive Impairment (MCI) and/or being in the early stages of Alzheimer's disease.

25 The efficacy of the humanized antibodies of the invention in the diagnosis, prevention and/or treatment of amyloidosis, such as mild cognitive impairment, Alzheimer's Disease, Familial British Dementia or Familial Danish Dementia and, e.g. neurodegeneration in Down Syndrome can be tested in existing animal models of Alzheimer's disease.

30 Suitable animal models of Alzheimer's Disease are reviewed in McGowan et al. TRENDS in Genetics, Vol. 22, No. May 2006, pp 281-289, and are selected from PDAPP, Tg2576, APP23, TgCRND8, PSEN_{1M146V} or PSEN_{1M146L}, PSAPP, APP_{Dutch}, BRI-A β 40 and BRI-A β 42, JNPL3, Tau_{P301S}, Tau_{V337M}, Tau_{R406W}, rTg4510, H_{tau}, TAPP, 3 x TgAD, as described below.

35

PDAPP: First mutant APP transgenic model with robust plaque pathology. Mice express a human *APP* cDNA with the Indiana mutation (*APP*_{V717F}). Plaque pathology begins between 6-9 months in hemizygous PDAPP mice. There is synapse loss but no overt cell loss and not NFT pathology is observed. This model has been used widely in vaccination therapy strategies.

Tg2576: Mice express mutant *APP*_{SWE} under control of the hamster prion promoter. Plaque pathology is observed from 9 months of age. These mice have cognitive deficits but no cell loss or NFT pathology. This model is one of the most widely used transgenic models in the field of Alzheimer's disease.

APP23: Mice express mutant *APP*_{SWE} under control of the Thy1 promoter. Prominent cerebrovascular amyloid, amyloid deposits are observed from 6 months of age and some hippocampal neuronal loss is associated with amyloid plaque formation.

TgCRND8: Mice express multiple APP mutations (Swedish plus Indiana). Cognitive deficits coincide with rapid extracellular plaque development at ~ 3 months of age. The cognitive deficits can be reversed by A β vaccination therapy.

PSEN_{1M146V} or PSEN_{1M146L} (lines 6.2 and 8.9, respectively): These models where the first demonstration *in vivo* that mutant *PSEN1* selectively elevates A β 42. No overt plaque pathology is observed.

PSAPP (Tg2576 x PSEN_{1M146L}, PSEN1-A246E + *APP*_{SWE}): Bigenic transgenic mice, with the addition of the mutant *PSEN1* transgene which markedly accelerated amyloid pathology compared with singly transgenic mutant *APP* mice, demonstrating that the PSEN1-driven elevation of A β 42 enhances plaque pathology.

APP_{Dutch}: Mice express *APP* with the Dutch mutation that causes hereditary cerebral hemorrhage with amyloidosis-Dutch type in humans. *APP*_{Dutch} mice develop severe congophilic amyloid angiopathy. The addition of a mutant *PSEN1* transgene redistributes the amyloid pathology to the parenchyma indicating differing roles for A β 40 and A β 42 in vascular and parenchymal amyloid pathology.

BRI-A β 40 and BRI-A β 42: Mice express individual A β isoforms without *APP* over-expression. Only mice expressing A β 42 develop senile plaques and CAA, whereas BRI-A β 40 mice do not develop plaques, suggesting that A β 42 is essential for plaque formation.

- 5 **JNPL3:** Mice express 4R0N *MAPT* with the P301L mutation. This is the first transgenic model, with marked tangle pathology and cell loss, demonstrating that MAPT alone can cause cellular damage and loss. JNPL3 mice develop motor impairments with age owing to severe pathology and motor neuron loss in the spinal cord.
- 10 **Tau_{P301S}:** Transgenic mice expressing the shortest isoform of 4R *MAPT* with the P301S mutation. Homozygous mice develop severe paraparesis at 5-6 months of age with widespread neurofibrillary pathology in the brain and spinal cord and neuronal loss in the spinal cord.
- 15 **Tau_{V337M}:** Low level synthesis of 4R MAPT with the V337M mutation (1/10 endogenous MAPT) driven by the promoter of platelet-derived growth factor (PDGF). The development of neurofibrillary pathology in these mice suggests the nature of the MAPT rather than absolute MAPT intracellular concentration drives pathology.
- 20 **Tau_{R406W}:** Mice expressing 4R human MAPT with the R406W mutation under control of the CAMKII promoter. Mice develop MAPT inclusions in the forebrain from 18 months of age and have impaired associative memory.
- 25 **rTg4510:** Inducible *MAPT* transgenic mice using the TET-off system. Abnormal MAPT pathology occurs from one month of age. Mice have progressive NFT pathology and severe cell loss. Cognitive deficits are evident from 2.5 months of age. Turning off the transgene improves cognitive performance but NT pathology worsens.
- 30 **H_{tau}:** Transgenic mice expressing human genomic MAPT only (mouse MAPT knocked-out). Htau mice accumulate hyperphosphorylated MAPT from 6 months and develop Thio-S-positive NFT by the time they are 15 months old.
- TAPP (Tg2576 x JNPL3):** Increased MAPT forebrain pathology in TAPP mice compared with JNPL3 suggesting mutant APP and/or A β can affect downstream MAPT pathology.

3xTgAD: Triple transgenic model expressing mutant APP_{SWE}, MAPT_{P301L} on a PSEN1_{M146V} 'knock-in' background (PSNE1-KI). Mice develop plaques from 6 months and MAPT pathology from the time they are 12 months old, strengthening the hypothesis that APP or A β can directly influence neurofibrillary pathology.

Moreover, WO 2009/034158 discloses non-human transgenic animal models, wherein the transgene encodes at least one amyloid beta (A β) peptide selected from the group consisting of A β N3E-42, A β N3Q-42, A β N3E-40 and A β N3Q-40. These A β peptides are substrates of QC and QPCTL, resulting in the cyclization of the N-terminal glutamine (Q) or glutamate (N) to pyroglutamate (pGlu). Thus, these transgenic animal models provide a model system for the investigation of the effect of pGlu- A β peptides on the course of the development of neurodegeneration.

Anti-A β pN3pE antibodies may also be useful in diagnostic assays for A β pN3pE, e.g. detecting its occurrence in specific cells, tissues, or serum. Thus, The humanized antibodies according to the present invention are especially useful in a diagnostic method to detect amyloidosis, in particular a neurodegenerative disease selected from the group consisting of mild cognitive impairment (MCI), Alzheimer's disease (AD), like for instance sporadic Alzheimer's disease (SAD) or Familial Alzheimer's dementias (FAD) like Familial British Dementia (FBD) and Familial Danish Dementia (FDD), neurodegeneration in Down Syndrome; preferably Alzheimer's disease.

For diagnostic applications, the antibody typically will be labelled with a detectable moiety. Numerous labels are available which can be generally grouped into the following categories:

(a) Radioisotopes, such as ³⁵S, ¹⁴C, ¹²⁵I, ³H, and ¹³¹I. The antibody can be labeled with the radioisotope using the techniques described in *Current Protocols in Immunology*, Volumes 1 and 2, Gütigen *et al.*, Ed., Wiley-Interscience. New York, New York. Pubs., (1991) for example and radioactivity can be measured using scintillation counting.

(b) Fluorescent labels such as rare earth chelates (europium chelates) or fluorescein and its derivatives, rhodamine and its derivatives, dansyl, Lissamine, p hycoerythrin and Texas Red are available. The fluorescent labels can be conjugated to the antibody using the techniques disclosed in *Current Protocols in Immunology*, *supra* for example. Fluorescence can be quantified using a fluorimeter.

(c) Various enzyme-substrate labels are available. The enzyme generally catalyses a chemical alteration of the chromogenic substrate which can be measured using various techniques. For example, the enzyme may catalyze a color change in a substrate, which can be measured spectrophotometrically. Alternatively, the enzyme may alter the fluorescence or chemiluminescence of the substrate. Techniques for quantifying a change in fluorescence are described above. The chemiluminescent substrate becomes electronically excited by a chemical reaction and may then emit light which can be measured (using a chemiluminometer, for example) or donates energy to a fluorescent acceptor. Examples of enzymatic labels include luciferases (e.g. firefly luciferase and bacterial luciferase; U.S. Patent No. 4,737,456), luciferin, 2,3-dihydrophthalazinediones, malate dehydrogenase, urease, peroxidase such as horseradish peroxidase (HRPO), alkaline phosphatase, β -galactosidase, glucoamylase, lysozyme, saccharide oxidases (e.g., glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase), heterocyclic oxidases (such as uricase and xanthine oxidase), lactoperoxidase, microperoxidase, and the like. Techniques for conjugating enzymes to antibodies are described in O'Sullivan *et al.*, Methods for the Preparation of Enzyme-Antibody Conjugates for use in Enzyme Immunoassay, in Methods in Enzym (ed Langone & H. Van Vunakis), Academic Press, New York, 73: 147-166 (1981).

Examples of enzyme-substrate combinations include, for example:

- (i) Horseradish peroxidase (HRPO) with hydrogen peroxide as a substrate, wherein the hydrogen peroxide oxidizes a dye precursor (e.g. orthophenylene diamine (OPD) or 3,3',5,5'-tetramethyl benzidine hydrochloride (TMB));
- (ii) alkaline phosphatase (AP) with para-Nitrophenyl phosphate as chromogenic substrate; and
- (iii) β -D-galactosidase (β -D-Gal) with a chromogenic substrate (e.g. p-nitrophenyl- β -D-galactosidase) or the fluorogenic substrate 4-methylumbelliferyl- β -D-galactosidase.

Numerous other enzyme-substrate combinations are available to those skilled in the art.

Sometimes, the label is indirectly conjugated with the antibody. The skilled artisan will be aware of various techniques for achieving this. For example, the antibody can be conjugated with biotin and any of the three broad categories of labels mentioned above can be conjugated with avidin, or *vice versa*. Biotin binds selectively to avidin and thus, the label can be conjugated with the antibody in this indirect manner. Alternatively, to achieve indirect conjugation of the label with the antibody, the antibody is conjugated with a small hapten (e.g. digoxin) and one

of the different types of labels mentioned above is conjugated with an anti-hapten antibody (e.g. anti-digoxin antibody). Thus, indirect conjugation of the label with the antibody can be achieved.

5 The humanized antibodies of the present invention may be employed in any known assay method, such as competitive binding assays, direct and indirect sandwich assays, and immunoprecipitation assays. Zola, *Monoclonal Antibodies A Manual of Techniques*, pp.147-158 (CRC Press. Inc., 1987)

10 Competitive binding assays rely on the ability of a labeled standard to compete with the test sample analyte for binding with a limited amount of antibody. The amount of A β N3pE in the test sample is inversely proportional to the amount of standard that becomes bound to the antibodies. To facilitate determining the amount of standard that becomes bound, the antibodies generally are insolubilized before or after the competition, so that the standard and
15 analyte that are bound to the antibodies may conveniently be separated from the standard and analyte which remain unbound.

Sandwich assays involve the use of two antibodies, each capable of binding to a different immunogenic portion, or epitope, of the protein to be detected. In a sandwich assay, the test
20 sample analyte is bound by a first antibody which is immobilized on a solid support, and thereafter a second antibody binds to the analyte, thus forming an insoluble three-part complex. The second antibody may itself be labeled with a detectable moiety (direct sandwich assays) or may be measured using an anti-immunoglobulin antibody that is labeled with a detectable moiety (indirect sandwich assay). For example, one preferable type of sandwich
25 assay is an ELISA assay, in which case the detectable moiety is an enzyme.

For immunohistochemistry, the tissue sample may be fresh or frozen or may be embedded in paraffin and fixed with a preservative such as formalin, for example.

30 The present invention also relates to a composition which comprises the humanized antibodies as defined above, wherein said composition is a composition for a diagnostic use, especially for the diagnosis of a neurodegenerative disease selected from the group consisting of mild cognitive impairment (MCI), Alzheimer's disease (AD), like for instance sporadic Alzheimer's disease (SAD) or Familial Alzheimer's dementias (FAD) like Familial British Dementia (FBD)
35 and Familial Danish Dementia (FDD), neurodegeneration in Down Syndrome; preferably

Alzheimer's disease; in particular by detection of A β N3pE or variants thereof in a biological sample.

Diagnostic Kits

5 As a matter of convenience, the antibody of the present invention can be provided in a kit, i.e., a packaged combination of reagents in predetermined amounts with instructions for performing the diagnostic assay. Where the antibody is labelled with an enzyme, the kit will include substrates and cofactors required by the enzyme (e.g. a substrate precursor which provides the detectable chromophore or fluorophore). In addition, other additives may be included such
10 as stabilizers, buffers (e.g. a block buffer or lysis buffer) and the like. The relative amounts of the various reagents may be varied widely to provide for concentrations in solution of the reagents which substantially optimize the sensitivity of the assay. Particularly, the reagents may be provided as dry powders, usually lyophilized, including excipients which on dissolution will provide a reagent solution having the appropriate concentration.

15 The diagnostic kit according to the invention may contain a further biologically active substance as described below. Especially preferred for the use in the diagnostic kit as said further further biologically active substance is an inhibitor of glutaminy cyclase.

20 The diagnostic kit of the invention is especially useful for the detection and diagnosis of amyloid- associated diseases and conditions, in particular neurodegenerative diseases selected from the group consisting of mild cognitive impairment (MCI), Alzheimer's disease (AD), like for instance sporadic Alzheimer's disease (SAD) or Familial Alzheimer's dementias (FAD) like Familial British Dementia (FBD) and Familial Danish Dementia (FDD),
25 neurodegeneration in Down Syndrome; preferably Alzheimer's disease.

The present invention also pertains to the humanized antibody of the invention or the composition comprising the humanized antibody, both as defined above, for use in an *in vitro* diagnostic method. In particular, this diagnostic method is directed to diagnosis of a
30 neurodegenerative disease selected from the group consisting of mild cognitive impairment (MCI), Alzheimer's disease (AD), like for instance sporadic Alzheimer's disease (SAD) or Familial Alzheimer's dementias (FAD) like Familial British Dementia (FBD) and Familial Danish Dementia (FDD), neurodegeneration in Down Syndrome; preferably Alzheimer's disease; especially by detecting an A β N3pE or variants thereof in a biological sample.

35 In a particularly preferred embodiment, the present invention pertains to the following method:

In vitro or *in situ* diagnostic method for the diagnosis of an amyloid-associated disease or condition, preferably Alzheimer's disease, comprising the following steps:
contacting a humanized antibody according to the invention with a sample, preferably selected
5 from a serum, liquor or CSF sample, most preferably a serum sample; or a specific body part or body area of a subject suspected to be afflicted with said condition or disease, and detecting binding of the antibody to A β N3pE, from the sample.

More particularly, the invention relates to a method of diagnosis of an amyloid-associated
10 disease or condition, preferably Alzheimer's disease, comprising detecting the immunospecific binding of a humanized antibody of the invention or an immunologically active fragment thereof to A β N3pE, in a sample or *in situ* which includes the steps of

- (a) bringing the sample or a specific body part or body area suspected to contain the amyloid protein into contact with a humanized antibody of the invention, or a fragment thereof;
- 15 (b) allowing the antibody and/or a functional part thereof, to bind to A β N3pE to form an immunological complex;
- (c) detecting the formation of the immunological complex; and
- (d) correlating the presence or absence of the immunological complex with the presence or absence of A β N3pE in the sample or specific body part or area.

Also comprised is a method of determining the extent of amyloidogenic plaque burden in a
20 tissue and/or body fluids comprising

- (a) obtaining a sample representative of the tissue and/or body fluids under investigation;
- (b) testing said sample for the presence of amyloid protein with a humanized antibody
25 according to the invention, or a chimeric antibody or a fragment thereof;
- (c) determining the amount of humanized antibody bound to the protein; and
- (d) calculating the plaque burden in the tissue and/or body fluids.

In particular, the invention relates to a method of determining the extent of amyloidogenic
30 plaque burden in a tissue and/or body fluids, wherein the formation of the immunological complex in step c) is determined such that presence or absence of the immunological complex correlates with presence or absence of amyloid protein, in particular A β N3pE.

In still another embodiment, the invention relates to a composition comprising the humanized
35 antibody according to the invention, or a chimeric antibody or a fragment thereof, and as described herein before including any functionally equivalent antibody or any derivative or

functional parts thereof, in particular a composition which is a pharmaceutical composition optionally further comprising a pharmaceutically acceptable carrier.

In another embodiment of the invention, said composition comprises the humanized antibody
5 in a therapeutically effective amount.

Further comprised by the invention is a mixture comprising a humanized antibody of the invention, or a chimeric antibody or a fragment thereof, and as described herein before including any functionally equivalent antibody or any derivative or functional parts thereof, in a
10 therapeutically effective amount and, optionally, a further biologically active substance and/or a pharmaceutically acceptable carrier and/or a diluent and/or an excipient.

In particular, the invention relates to a mixture, wherein the further biologically active substance is a compound used in the medication of amyloidosis, a group of diseases and disorders
15 associated with amyloid or amyloid-like protein such as A β N3pE involved in neurodegenerative diseases selected from the group consisting of mild cognitive impairment (MCI), Alzheimer's disease (AD), like for instance sporadic Alzheimer's disease (SAD) or Familial Alzheimer's dementias (FAD) like Familial British Dementia (FBD) and Familial Danish Dementia (FDD), neurodegeneration in Down Syndrome; preferably Alzheimer's disease.

In another embodiment of the invention, the other biologically active substance or compound may also be a therapeutic agent that may be used in the treatment of amyloidosis caused by A β N3pE or may be used in the medication of other neurological disorders.

The other biologically active substance or compound may exert its biological effect by the same or a similar mechanism as the antibody according to the invention or by an unrelated mechanism of action or by a multiplicity of related and/or unrelated mechanisms of action.

Generally, the other biologically active compound may include neutron-transmission
30 enhancers, psychotherapeutic drugs, acetylcholine esterase inhibitors, calcium-channel blockers, biogenic amines, benzodiazepine tranquillizers, acetylcholine synthesis, storage or release enhancers, acetylcholine postsynaptic receptor agonists, monoamine oxidase-A or -B inhibitors, N-methyl- D-aspartate glutamate receptor antagonists, non-steroidal anti-inflammatory drugs, antioxidants, and serotonergic receptor antagonists.

More particularly, the invention relates to a mixture comprising at least one compound selected from the group consisting of compounds effective against oxidative stress, anti-apoptotic compounds, metal chelators, inhibitors of DNA repair such as pirenzepin and metabolites, 3-amino-1-propanesulfonic acid (3 APS), 1,3-propanedisulfonate (1,3PDS), α -secretase
5 activators, β - and γ -secretase inhibitors, tau proteins, neurotransmitter, β -sheet breakers, attractants for amyloid beta clearing / depleting cellular components, inhibitors of N-terminal truncated amyloid beta including pyroglutamated amyloid beta 3-42, such as inhibitors of glutaminy cyclase, anti-inflammatory molecules, or cholinesterase inhibitors (ChEIs) such as tacrine, rivastigmine, donepezil, and/or galantamine, MI agonists and other drugs including any
10 amyloid or tau modifying drug and nutritive supplements, and nutritive supplements, together with an antibody according to the present invention and, optionally, a pharmaceutically acceptable carrier and/or a diluent and/or an excipient.

The invention further relates to a mixture, wherein the compound is a cholinesterase inhibitor (ChEIs), particularly a mixture, wherein the compound is one selected from the group
15 consisting of tacrine, rivastigmine, donepezil, galantamine, niacin and memantine.

In a further embodiment, the mixtures according to the invention may comprise niacin or memantine together with an antibody according to the present invention and, optionally, a
20 pharmaceutically acceptable carrier and/or a diluent and/or an excipient.

In a further embodiment, the mixtures according to the invention may comprise a glutaminy cyclase inhibitor together with an antibody according to the present invention and, optionally,
25 a pharmaceutically acceptable carrier and/or a diluent and/or an excipient.

Preferred inhibitors of glutaminy cyclase are described in WO 2005/075436, WO 2008/055945, WO 2008/055947, WO 2008/055950, WO 2008/065141, WO 2008/110523, WO 2008/128981, WO 2008/128982, WO 2008/128983, WO 2008/128984, WO 2008/128985, WO 2008/128986, WO 2008/128987, WO 2010/026212, WO 2011/131748, WO 2011/029920, WO
30 2011/107530, WO 2011/110613, WO 2012/123563 and WO 2014/140279, the disclosure of which is incorporated herein by reference.

In still another embodiment of the invention mixtures are provided that comprise "atypical antipsychotics" such as, for example clozapine, ziprasidone, risperidone, aripiprazole or
35 olanzapine for the treatment of positive and negative psychotic symptoms including hallucinations, delusions, thought disorders (manifested by marked incoherence, derailment,

tangentiality), and bizarre or disorganized behavior, as well as anhedonia, flattened affect, apathy, and social withdrawal, together with an antibody, particularly a monoclonal antibody according to the invention, but particularly a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to the invention and as described herein and, optionally, a pharmaceutically acceptable carrier and/or a diluent and/or an excipient.

In a specific embodiment of the invention, the compositions and mixtures according to the invention and as described herein before comprise the humanized antibody of the invention and the biologically active substance, respectively, in a therapeutically effective amount.

Other compounds that can be suitably used in mixtures in combination with the humanized antibody according to the present invention are described in WO2008/065141 (see especially pages 37/38), including PEP-inhibitors (pp. 43/44), LiCl, inhibitors of dipeptidyl aminopeptidases, preferably inhibitors of DP IV or DP IV-like enzymes (see pp. 48/49); acetylcholinesterase (ACE) inhibitors (see p. 47), PIMT enhancers, inhibitors of beta secretases (see p. 41), inhibitors of gamma secretases (see pp. 41/42), inhibitors of neutral endopeptidase, inhibitors of phosphodiesterase-4 (PDE-4) (see pp. 42/43), TNFalpha inhibitors, muscarinic M1 receptor antagonists (see p. 46), NMDA receptor antagonists (see pp. 47/48), sigma-1 receptor inhibitors, histamine H3 antagonists (see p. 43), immunomodulatory agents, immunosuppressive agents or an agent selected from the group consisting of antegren (natalizumab), Neurelan (fampridine-SR), campath (alemtuzumab), IR 208, NBI 5788/MSP 771 (tiplimotide), paclitaxel, Anergix.MS (AG 284), SH636, Differin (CD 271, adapalene), BAY 361677 (interleukin-4), matrix-metalloproteinase-inhibitors (e.g. BB 76163), interferon-tau (trophoblastin) and SAIK-MS; beta-amyloid antibodies (see p.44), cysteine protease inhibitors (see p. 44); MCP-1 antagonists (see pp. 44/45), amyloid protein deposition inhibitors (see 42) and beta amyloid synthesis inhibitors (see p. 42), which document is incorporated herein by reference.

In another embodiment, the invention relates to a mixture comprising the humanized antibody according to the invention, or a chimeric antibody or a fragment thereof and as described herein before and/or the biologically active substance in a therapeutically effective amount.

The pharmaceutical compositions may be formulated with pharmaceutically acceptable carriers or diluents as well as any other known adjuvants and excipients in accordance with conventional techniques such as those disclosed in Remington : The Science and Practice of Pharmacy, 21th Edition, Gennaro, Ed., Mack Publishing Co., Easton, PA, 2005.

The pharmaceutically acceptable carriers or diluents as well as any other known adjuvants and excipients should be suitable for the chosen humanized antibody of the present invention and the chosen mode of administration. Suitability for carriers and other components of pharmaceutical compositions is determined based on the lack of significant negative impact on the desired biological properties of the chosen humanized antibody or pharmaceutical composition of the present invention (e.g., less than a substantial impact (10% or less relative inhibition, 5% or less relative inhibition, etc.)) on antigen binding.

A pharmaceutical composition of the present invention may also include diluents, fillers, salts, buffers, detergents (e.g., a nonionic detergent, such as Tween-20 or Tween- 80), stabilizers (e.g., sugars or protein-free amino acids), preservatives, tissue fixatives, solubilizers, and/or other materials suitable for inclusion in a pharmaceutical composition.

The actual dosage levels of the active ingredients in the pharmaceutical compositions of the present invention may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration. The selected dosage level will depend upon a variety of pharmacokinetic factors including the activity of the particular compositions of the present invention employed, or the amide thereof, the route of administration, the time of administration, the rate of excretion of the particular humanized antibody being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compositions employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

The pharmaceutical composition may be administered by any suitable route and mode. Suitable routes of administering a humanized antibody of the present invention in vivo and in vitro are well known in the art and may be selected by those of ordinary skill in the art.

In one embodiment, a pharmaceutical composition of the present invention is administered parenterally.

The phrases "parenteral administration" and "administered parenterally" as used herein means modes of administration other than enteral and topical administration, usually by injection, and include epidermal, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, intratendinous, transtracheal,

subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, intracranial, intrathoracic, epidural and intrasternal injection and infusion.

In one embodiment that pharmaceutical composition is administered by intravenous or subcutaneous injection or infusion.

Pharmaceutically acceptable carriers include any and all suitable solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonicity agents, antioxidants and absorption delaying agents, and the like that are physiologically compatible with a humanized antibody of the present invention.

Examples of suitable aqueous and nonaqueous carriers which may be employed in the pharmaceutical compositions of the present invention include water, saline, phosphate buffered saline, ethanol, dextrose, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, corn oil, peanut oil, cottonseed oil, and sesame oil, carboxymethyl cellulose colloidal solutions, tragacanth gum and injectable organic esters, such as ethyl oleate, and/or various buffers. Other carriers are well known in the pharmaceutical arts.

Pharmaceutically acceptable carriers include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. The use of such media and agents for pharmaceutically active substances is known in the art. Except insofar as any conventional media or agent is incompatible with the active humanized antibody, use thereof in the pharmaceutical compositions of the present invention is contemplated.

Proper fluidity may 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.

Pharmaceutical compositions of the present invention may also comprise pharmaceutically acceptable antioxidants for instance (1) water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; (2) oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha- tocopherol, and the like; and (3) metal

chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

Pharmaceutical compositions of the present invention may also comprise isotonicity agents, such as sugars, polyalcohols, such as mannitol, sorbitol, glycerol or sodium chloride in the compositions.

The pharmaceutical compositions of the present invention may also contain one or more adjuvants appropriate for the chosen route of administration such as preservatives, wetting agents, emulsifying agents, dispersing agents, preservatives or buffers, which may enhance the shelf life or effectiveness of the pharmaceutical composition. The humanized antibodies of the present invention may be prepared with carriers that will protect the humanized antibody against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Such carriers may include gelatin, glyceryl monostearate, glyceryl distearate, biodegradable, biocompatible polymers such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid alone or with a wax, or other materials well known in the art. Methods for the preparation of such formulations are generally known to those skilled in the art. See, e.g., Sustained and Controlled Release Drug Delivery Systems, J. R. Robinson, ed., Marcel Dekker, Inc., New York, 1978.

In one embodiment, the antibodies of the present invention may be formulated to ensure proper distribution in vivo. Pharmaceutically acceptable carriers for parenteral administration include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. The use of such media and agents for pharmaceutically active substances is known in the art. Except insofar as any conventional media or agent is incompatible with the humanized antibody, use thereof in the pharmaceutical compositions of the present invention is contemplated.

Pharmaceutical compositions for injection must typically be sterile and stable under the conditions of manufacture and storage. The composition may be formulated as a solution, microemulsion, liposome, or other ordered structure suitable to high drug concentration. The carrier may be a aqueous or non-aqueous solvent or dispersion medium containing for instance water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. The proper fluidity may be maintained, for example, by the use of

a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as glycerol, mannitol, sorbitol, or sodium chloride in the composition. Prolonged absorption of the injectable compositions may be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin. Sterile injectable solutions may be prepared by incorporating the humanized antibody in the required amount in an appropriate solvent with one or a combination of ingredients e.g. as enumerated above, as required, followed by sterilization microfiltration. Generally, dispersions are prepared by incorporating the humanized antibody into a sterile vehicle that contains a basic dispersion medium and the required other ingredients e.g. from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, examples of methods of preparation are vacuum drying and freeze-drying (lyophilization) that yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Sterile injectable solutions may be prepared by incorporating the humanized antibody in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by sterilization microfiltration. Generally, dispersions are prepared by incorporating the humanized antibody into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, examples of methods of preparation are vacuum drying and freeze-drying (lyophilization) that yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Dosage regimens in the above methods of treatment and uses are adjusted to provide the optimum desired response (e.g., a therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. Parenteral compositions may be formulated in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit contains a predetermined quantity of humanized antibody calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the present invention are dictated by and directly dependent on (a) the unique characteristics of the humanized antibody and the particular therapeutic effect to be achieved, and (b) the limitations

inherent in the art of compounding such an humanized antibody for the treatment of sensitivity in individuals.

The effective dosages and the dosage regimens for the humanized antibodies of the invention depend on the disease or condition to be treated and may be determined by the persons skilled in the art. An exemplary, non-limiting range for a therapeutically effective amount of an antibody of the present invention is about 0.1 -10 mg/kg/body weight, such as about 0.1-5 mg/kg/body weight, for example about 0.1-2 mg/kg/body weight, such as about 0.1-1 mg/kg/body weight, for instance about 0.15, about 0.2, about 0.5, about 1 , about 1.5 or about 2 mg/kg/body weight.

A physician or veterinarian having ordinary skill in the art may readily determine and prescribe the effective amount of the pharmaceutical composition required. For example, the physician or veterinarian could start doses of the 8ηii-AβpE3 antibody employed in the pharmaceutical composition at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved. In general, a suitable daily dose of a composition of the present invention will be that amount of the humanized antibody which is the lowest dose effective to produce a therapeutic effect. Such an effective dose will generally depend upon the factors described above. Administration may e.g. be intravenous, intramuscular, intraperitoneal, or subcutaneous, and for instance administered proximal to the site of the target. If desired, the effective daily dose of a pharmaceutical composition may be administered as two, three, four, five, six or more sub-doses administered separately at appropriate intervals throughout the day, optionally, in unit dosage forms. While it is possible for a humanized antibody of the present invention to be administered alone, it is preferable to administer the humanized antibody as a pharmaceutical composition as described above.

EXAMPLES

1. Humanization approach to generate N3pE-Aβ specific humanized antibodies

N3pE-Aβ specific mouse monoclonal antibody clones #6, #17 and #24 were obtained from hybridoma cell lines 6-1-6, 17-4-3, and 24-2-3, which have been deposited in accordance with the Budapest Treaty and are available at the Deutsche Sammlung für Mikroorganismen und Zellkulturen (DSMZ) in Braunschweig, DE, with a deposit date of June 17, 2008, and with the respective deposit numbers

(clone 6-1-6): DSM ACC2924
 (clone 17-4-3): DSM ACC2925
 (clone 24-2-3): DSM ACC2926.

The first step in humanization process of antibody clones #6, #17 and #24 was the definition of the CDRs in the variable domains of light and heavy chains. By Rosetta Antibody Modeling Server (<http://antibody.graylab.jhu.edu>) the CDRs were predicted. Figure 1 shows exemplary the CDRs predicted for clone #6.

To select the appropriate framework for CDR-Grafting human sequences with the highest similarity to the non-human antibody need to be identified. By Blast analysis the variable domain of the light chain (Lv) and the heavy chain (Hv) were separately fitted with the pool of published human sequences. For the light chain a human antibody sequence with 82% identity was found, which belongs to the class of kappa LC. The highest homology for the heavy chain has a human amino acid sequence with 62% identity.

To group the selected human antibody framework sequence to germline gene sequences, a Blast search was performed in the germline library IMGT. For light chain clone#6 and clone#24 a sequence with the sequence code IGKV2-30*01 was found. The light chain variable region of clone#17 was most similar to IGKV2-30*02. The heavy chain variable region codes could be identified with IGHV1-3*01, IGHV1-69*13 and IGHV3-48*01 corresponding to clone#6, clone#17 and clone#24, respectively. Table 1 shows the framework parameters of variable regions of the humanized antibodies.

Table 1: Framework parameters of variable regions of the humanized antibodies.

clone#	Lv			Hv		
	Aa	acc nr.	family	aa	acc nr.	family
6	BAC01730.1	AB064102.1	IGKV2-30*01	AAS85817.1	AY392875.1.	IGHV1-3*01
24	BAC01730.1	AB064102.1	IGKV2-30*01	AAD30405.1	AF115119.1	IGHV1-69*13
17	BAC01734.1	AB064106.1	IGKV2-30*02	AAS858165	AY392925.1	IGHV3-48*01

aa =amino acid sequence, acc nr. = accession number, family = gene family

By CDR grafting the CDRs of the mouse antibody clones #6, #17 and #24 were combined with the respective human antibody framework to create a humanized antibody. The heavy chain constant region of human IgG1 was used for reconstitution the entire antibodies. The light chain variable domains were fused to the human kappa chain constant region.

2. RNA isolation and cDNA synthesis

As source for constant sequences, RNA of human B cells was isolated by lysis of 500 µl whole blood with 5ml 1x FACS Lysis Solution (Becton Dickinson) for 10 minutes at room temperature. The lysate was centrifuged at 300g for 5 min; the pellet was washed two times with PBS and was then resolved in 350 µl RA1 Buffer of Nucleo Spin® RNA II (Macherey-Nagel) and added with 3.5µl 0,5M TCEP (SIGMA). The RNA was isolated by manufacturers' instructions. 10 µl of RNA was first incubated with 1µl 0.5µg/µl OligodT Primer (Invitrogen) and 1µl 10mM dNTPs for 5 min at 65 °C. Then 4µl of 5x First Strand Buffer (Invitrogen), 2µl of 100mM DTT and 0.5 µl SuperScript III Reverse Transcriptase (Invitrogen) was added to 20µl and mix was incubated for 5 minutes at 25 °C, 50min at 50 °C and 15 min at 70 °C. By PCR with primer pairs shown in Table 2, synthesized cDNA of constant region of light and heavy chain could be amplified.

Table 2: Primer for cloning of constant region

SEQ ID NO	Name	Sequence
44	hkappa5'	ACTGTGGCTGCACCATCTGTCTTC
45	hkappa3'	CTAACACTCTCCCCTGTTGAAGCTC
46	hlgG1Hc5'1	AGGGAACCCTGGTCACCGTCTCC
47	hlgG1Hc3'	TCATTTACCCGGAGACAGGGAGAGG

For amplification of the PCR product of humanized light chain clone#6 following forward and reverse primers were used:

RT_chim_humKI6f: CAAGTCAGAGCCTCTTATATAGTG (SEQ ID NO: 48);

RT_chim_humKI6r: GTACCTTGCACGCAGTAATAAAC (SEQ ID NO: 49).

For amplification of the reference gene mouse HPRT Primer were used.

To perform the amplification 7.5 µl Sybergreen (Firma), 1 µl Primer forward (25 pmol/µl), 1µl Primer reverse (25 pmol/µl), 5.5 µl ddH₂O and 1 µl cDNA were used in cyclor.

3. Expression of recombinant antibody in CHO cells by separately cloning LC and HC into two different expression plasmids

The sequences of the light and heavy chain of the humanized antibodies were separately cloned into two different mammalian expression vectors, pCDNA3.1 and HC-pOptiVEC respectively. To identify the optimal combination of vectors to express the recombinant antibody in CHO cell culture, different plasmid combinations were used to perform transient expressions in adherent CHO cells. In a second step, it was investigated whether different DNA ratios between LC and HC plasmid influences the expression level. With transfection of 3 µg LC-pCDNA3.1 and 1 µg HC-pOptiVEC, an increased expression level was found.

For further adherent CHO cells expression of humanized antibody, plasmid combination of LC-pCDNA3.1 and 1 µg HC-pOptiVEC and a plasmid DNA ratio of LC 3:1 HC was used.

Freestyle™ CHO suspension cells were used in the following transfections to cultivate a higher amount of transient expressing cells which to generate recombinant antibodies. First was tested whether an excess of LC plasmid could improve the expression of antibody like in case of the adherent cells. Like in adherent CHO cells a LC to HC plasmid DNA ratio of 1:1 and 3.1 was used. Western blot analysis revealed that an excess of LC plasmid increases the expression of humanized antibody as in the case of adherent CHO cells. By measurement of cell viability it become obvious, that the cell viability decreases to about 50% of transfected cells after 6 days. After day six, no further increase of antibody level in supernatant was detectable. Consequently, culture supernatants were harvest at day six in the case of following transfections.

To investigate if the produced antibodies are efficient transported into the cell supernatant, a cell lysate sample was applied to SDS PAGE, analyzed by Western blot. GAPDH, a housekeeping cytoplasmic protein was used for reference loading comparable amounts of cell lysate protein to the SDS gel. In cell lysate of humanized antibody expressing CHO cells a strong band of 120 kDa occurs migrating at the same size as detected in the cell supernatant.

4. Purification of recombinant antibody by Protein G chromatography

The humanized antibody clone#6 was purified to investigate the antigen binding property of the protein in comparison with the original murine antibody. Therefore 300 ml supernatant with expressed chimeric and humanized antibody was produced and purified by Protein G chromatography (Fig. 2). Because the amount of expressed antibody was very low, the yield

was less than 0.1 µg/ml, in total 25 µg purified protein. The eluted antibody was concentrated to about 200 µg/ml and 2 µg protein was applied to SDS-PAGE following coomassie staining (Fig. 2).

5. Surface Plasmon Resonance measurement to compare antigen binding of murine and humanized antibody

Surface Plasmon Resonance measurement was used to investigate the binding efficacy of humanized antibody to AβpE3-18 (Fig. 3). To prevent mass transfer and avidity effects during measurement, the following procedure was used.

First a polyclonal α-human antibody was coupled to an SPR-Chip subsequent loaded with the humanized antibody until the Response Unit was more than 1000.

Kinetic measurement was performed at different concentrations (of 5 to 1000 nM) of AβpE3-18 –peptide. The graphs of the measured series are shown as an overlay plot with the sensorgrams, corrected by the sensogram measuring the running buffer, aligned at the time of injection and the baseline adjusted to zero before injection. The results are evaluated according to a simple 1:1 interaction model (Langmuir fit), which promote the k_{off} and k_{on} rate constants. In figure 3 the SPR-binding curves are shown, consisting of association and dissociation curve.

Apparent kinetic constants according to the 1:1 Langmuir fitting are listed in Table 3. Due to the fact that the humanized antibody is non-covalently bound to the chip surface, small amounts of antibody molecules were washed out during the measurement. Therefore the R_{max} values were fitted locally for every single sensogram.

Table 3: Statistics of Langmuir fit in kinetics of humanized antibody clone#6

AβpE3- 18 in nM	k_{on} (1/Ms)	k_{off} (1/s)	R_{max} (RU)	RI (RU)	KA (1/M)	KD (M)	Req (RU)	Chi2
	8,09E+05	0,0222		0,303	3,65E+07	2,74E- 08		0,803
0			1,19				0	
5			18,1				2,79	
10			15,5				4,15	
20			16,7				7,05	
50			16,9				10,9	1.measurement

100	18,7	14,7	
200	20,2	17,7	
500	21,7	20,5	
1000	22,7	22,1	
0	0	0	2.measurement
5	11,4	1,76	
10	15,5	4,14	
20	17,5	7,39	
50	16,9	10,9	
100	18,3	14,4	
200	20,2	17,7	
500	21	19,9	
1000	22,3	21,7	

The plot of the curves during association of peptide (Fig.3A, upper inset) fits very well, whereas the plot of peptide dissociation curves (Fig.3A, lower inset) don't localize accurate on experimental curves. With the fit the kinetic parameter k_{off} of $22.2 \cdot 10^{-3} \text{ 1/s}$ was computed (Table 2). This parameter describes the time which is necessary for removing the half of bounded peptide. This means that during $1/0,0222 \text{ s} = 45 \text{ s}$ the half of injected peptide is removed (see Fig.3A, lower inset). Because Langmuir fit doesn't match very well during dissociation phase, K_D determination was performed by fitting R_{equ} values over peptide concentration (Fig. 3B) using following equation: $R_{equ} = R_{max} \cdot K_A \cdot c / (1 + K_A \cdot c)$, at which R_{equ} the signal in equilibrium with corresponding concentration c are variables and R_{max} and K_A the constants to fit. K_D can be calculated by $1/K_A$.

By structural analysis of mouse Fab fragment antibody clone#6 was concluded that Thr97 in HC could have an important effect on binding affinity to A β pE3. After replacement of Ala97 by Thr97 in the humanized antibody clone#6 an improved binding affinity was yielded with nearly the same K_D value compared to the mouse antibody clone#6. Higher amount of expressed antibody was required to prove these findings by additional ITC measurement. Therefore the expression level was increased by a replacement of LC and HC signal sequences of humanized antibody T97 variant by the murine sequences. In a second step generation of a stable cell line was performed to increase antibody production.

Table 4 shows the apparent kinetic constants according to the 1:1 Langmuir fitting of further sequence variants of humanized antibody clone #6:

Variant	K _D (nM)	kon (s ⁻¹ M ⁻¹)	koff (s ⁻¹)
VH: SEQ ID NO: 24 VL: SEQ ID NO: 14	9.36	6.16 × 10 ⁵	5.76 × 10 ⁻³
VH: SEQ ID NO: 27 VL: SEQ ID NO: 14	5.09	6.35 × 10 ⁵	3.23 × 10 ⁻³
VH: SEQ ID NO: 27 VL: SEQ ID NO: 15	15.5	2.67 × 10 ⁵	4.15 × 10 ⁻³
VH: SEQ ID NO: 27 VL: SEQ ID NO: 13	7.35	5.62 × 10 ⁵	4.13 × 10 ⁻³
VH: SEQ ID NO: 70 VL: SEQ ID NO: 14	8.43	4.23 × 10 ⁵	3.57 × 10 ⁻³

5 6. Stable cell line generation of humanized antibody variant HC T97

For stable cell line development first a pool of stable CHO-DG44 cell lines were generated by treatment with different concentrations of methotrexate (MTX). The best expression was detected accompanied by a low amount of dying cells at a concentration of 0.5 μM MTX (Fig.14A, lane2). Thus, cells with pretreatment of 0.5 μM MTX were used for clonal selection by limiting dilution.

One hundred potential antibody expressing clones were found after clonal selection. The supernatant of these clones were diluted 1:20 in HBS-EP buffer and analyzed by SPR using an AβpE3-18 coupled chip. A calibration curve was used to determine the antibody concentration in supernatant. 18 clones were separated which show a started antibody concentration over 0.15μg/ml after 3 days of expression. These clones were scaled up in 24 well format and supernatants were collected and analyzed by Western blot (Fig. 4B). Five of these clones revealed good expression and were further scaled up until 30 ml shaking culture. After 7 day expression time the expression level was measured by SPR (Fig. 4C). Clone 9A has shown the highest antibody concentration after 7 day cultivation. Therefore this clone was

used for expression higher amounts of supernatant. The cultivation was performed without further selection pressure to get higher cell viability. Three liters of antibody containing supernatant were collected.

7. Purification of humanized antibody clone#6 by Protein G Chromatography

One liter of supernatant was diluted with one liter 40 mM Na₂HPO₄ pH 7 and applied overnight to 5 ml Protein G column at 4°C. After that column was washed with binding buffer (20 mM Na₂HPO₄, pH 7, fraction A1-A3) and then with high-salt buffer (2 M NaCl, 40 mM Na₂HPO₄ pH 7, fraction A4-A7) to remove unspecific bound proteins. The antibody was eluted with 0.1M Glycin-HCl, pH 2.7 from column and immediately neutralized by 1 M Tris pH 9. Fractions were collected and 24 µl were loaded onto 12% SDS PAGE, respectively. The fractions A12+B1 were pooled and used for KD determination by ITC. Overall, 2.5 mg antibody was purified from one liter culture supernatant.

8. ITC measurement of humanized antibody clone#6 with AβpE3-18

For determination of the K_D-value, the humanized antibody clone#6 was diluted to a concentration of 1 µM. The ligand AβpE3-18 with a concentration of 20 µM was titrated at 293.15K. After integration of the raw data of ITC-measurement (Fig. 5B, bottom) the stoichiometry of 1.5 was calculated. The calculated K_D-value was 2.7 nM according quite well with value of 5.3 nM calculated from the SPR data characterizing the humanized clone#6 HC T97 ligand binding in solvent as a high affine interaction. The interaction is driven mainly by enthalpic contributions (ΔH=-23.45 kcal/mol) and opposed by entropic penalties (TΔS=-11.96 kcal/mol), which is typical for structural rearrangement at the binding site by formation of hydrogen bonds yielding in significant loss of degrees of freedom.

9. Humanization, expression and purification of the two further AβpE3-specific antibodies clone#17 and clone#24

The humanization, expression and purification of the two further AβpE3-specific antibodies clone#17 and clone#24 was performed using the same protocols, materials and methods as well as experimental conditions as used for clone#6.

10. SPR measurement with humanized antibody clone#24 and clone#17

SPR measurements using the pE3-Aβ18 ligand revealed that both humanized antibodies are capable to bind the pE3-Aβ peptide. The rate constants and the K_D value could be calculated very well by Langmuir 1:1 model fit. The origin KD values of mouse antibodies to pE3-Aβ are shown in table 5. It became obvious that the k_{off} value of humanized antibody clone#24 is 20

fold higher than the k_{off} value of mouse antibody clone#24. This means that after humanization the pE3-A β peptide dissociates faster from the antigen binding pocket of the antibody.

Table 5: Determination of k_{on} , k_{off} and K_{D} value of different A β peptides

Abeta	sequence	Ab clone#	KD in nM	k_{on} in 1/Ms	k_{off} in 1/s
human pE3-18	pEFRHDSGYEVHHQKLV (SEQ ID NO: 50)	mouse 6	6.7	6.89E+05	4.58E-03
		mouse 24	2.2	1.76E+05	0.38E-03
		mouse 17	1.8	19.5E+05	3.46E-03
		human 6	5.3	6.89E+05	4.58E-03
		human 24	16	4.91E+05	7.84E-03
		human 17	1.5	19.9E+05	2.90E-03
human 1-18	DAEFRHDSGYEVHHQKLV (SEQ ID NO: 51)	mouse 6	-	-	-
		mouse 24	-	-	-
		mouse 17	-	-	-
		human 6	-	-	-
		human 17	-	-	-
human 2-18	AEFRHDSGYEVHHQKLV (SEQ ID NO: 52)	mouse 6	n.d.	-	-
		mouse 24	n.d.	-	-
		mouse 17	490	0.0741E+05	3.63E-03
		human 6	n.d.	-	-
		human 17	190	0.149E+05	2.84E-03
human 4-18	FRHDSGYEVHHQKLV (SEQ ID NO: 53)	mouse 6	-	-	-
		mouse 24	-	-	-
		mouse 17	-	-	-
		human 6	-	-	-
		human 17	-	-	-

11. Binding to Fc gamma receptors

The binding of two antibodies, which either comprised the human IgG1 Fc wild-type region of SEQ ID NO: 73 or the K322A mutant variant thereof (SEQ ID NO: 74), to different Fc gamma receptors (CD16A, CD32A, CD32B, and CD64) was compared.

The K322A mutant was produced by site-directed mutagenesis. The binding was measured in a FACS based bioassay to Chinese Hamster Ovary (CHO) cells stably expressing full length human CD16A, CD32A, CD32B, or CD64. Both antibodies were incubated with each cell line

at 7 different concentrations for one hour followed by washing. Receptor-bound H6 or H67 was detected with fluorochrome conjugated-anti-Fab'. Binding capacity was measured by FACS and the Kd and Bmax were calculated by non-linear regression.

- 5 Results: Both antibodies showed comparable binding to all receptors. See figures 6, 7, 8 and 9.

12. Binding to C1q

- 10 The binding of two antibodies, which either comprised the human IgG1 Fc wild-type region of SEQ ID NO: 73 or the K322A mutant variant thereof (SEQ ID NO: 74), to C1q was compared in order to better characterize the effector functions of the antibodies.

A number of assay formats of binding of the antibodies to C1q was tested, including

- 15 a) direct binding of the two antibodies to the plate and then binding to C1q in solution; and
b) streptavidin coated plates first incubated with biotinylated pE-A β peptide, binding to antibodies and then C1q.

In summary, format a) produced best results. The procedure is summarized below:

- 20 The ELISA plate was coated with the antibody, comprising the human IgG1 Fc wild-type region of SEQ ID NO: 73, the K322A mutant variant thereof (SEQ ID NO: 74), and a K322A control (hu14.18K322A) that does not bind C1q at 10, 8, 6, 4, 3, 2, 1 and 0 μ g/ml in triplicate and incubated at 4 °C overnight. Next day, the plate was washed three times with 1x PBS and then blocked with 1% BSA in 1x PBS at 50 μ l/well. C1q (Sigma, Cat.# C1740) was added to each
25 well at 2 μ g/ml in blocking buffer and incubated for 1 hour at room temperature. The plate was then washed three times with 200 μ l of 1x PBS. Anti-C1q-HRP (Thermo, Cat. # PA1-84324) was added to the plate to detect the binding at a 1:250 dilution in blocking buffer (50 μ l/well) for 1 hour. The plate was washed again three times with 200 μ l of 1x PBS. 50 μ l of TMB (Invitrogen, Cat.# 002023) was added to each well to visualize the interaction (Invitrogen, Cat.# 002023)
30 for 2 min. 50 μ l of stop solution ((1M Sulfuric Acid) was added to each well before reading the absorbance at 450nm.

- Results: The antibody, which comprised the human IgG1 Fc wild-type region of SEQ ID NO: 73 did bind to C1q. The K322A mutant variant thereof (comprising the IgG1 Fc region of SEQ
35 ID NO: 74), did not bind to C1q. See also Figure 10.

13. Immunohistochemistry

With IHC the antigen A β N3pE can be localized in cerebral tissue sections. Therefore the humanized antibodies of the invention were used for detection of A β N3pE.

For the IHC human cerebral tissue sections of the hippocampus and the frontal cortex from AD patients and furthermore cerebral tissue sections of hippocampus from existing animal models for Alzheimer's disease as described herein can be used. These mouse models show increased brain A β levels followed by development of neuritic plaques. The tissue sections were paraffin-embedded and serial cut. The sections were stained with hematoxylin to colored nuclei of cells and then immunostained with the anti A β N3pE antibodies of the invention. The tissue section preparation and staining were performed in accordance with standard methodology.

14. Treatment of Alzheimer mice in vivo

A total of 62 male mice were utilized in this study. Prior to the start of immunization, four mice of an existing mouse model for Alzheimer's disease (avg. 5.6 mo $\pm$ 0.45) mice were sacrificed as baseline controls to assess cerebral A β plaque burden at the commencement of treatment. The remaining mice were divided into four groups and received the following treatment: 250 μ l sterile PBS (n= 12; avg. 5.89 mo $\pm$ 0.13), 200 μ g of a humanized antibody of the invention. A group of age- and gender-matched Wt littermates were injected with 250 μ l PBS (n=12; avg. 5.80 mo $\pm$ 0.12) and served as behavioral controls. Mice were treated with a total volume of 250 μ l (antibody or PBS) via intraperitoneal injection for 28 weeks.

Euthanasia and tissue preparation

Mice were euthanized, perfused and plasma harvested at 6 months (baseline) or 13 months of age. The brain was extracted and divided sagittally. The hippocampus, cortex and cerebellum were dissected from one hemisphere and snap frozen for biochemical analyses. The other hemisphere was drop-fixed in 4% paraformaldehyde (Electron Microscopy Sciences) for 24 h at 4°C, cryoprotected in graded sucrose solutions at 4°C and embedded in OCT compound (Tissue Tek).

SEQUENCE LISTING

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 1 5 10 15

Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu Leu Tyr Ser
 20 25 30

Asp Gly Lys Thr Tyr Leu Asn Trp Phe Gln Gln Arg Pro Gly Gln Ser
 35 40 45

Pro Arg Arg Leu Ala Tyr Leu Val Ser Lys Leu Asp Ser Gly Val Pro
 50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
 65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Val Gln Gly

85

90

95

Thr His Phe Pro Phe Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
 100 105 110

<210> 14

<211> 112

<212> PRT

<213> Artificial

<220>

<223> Synthetic peptide

<400> 14

Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Leu Gly
 1 5 10 15

Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu Leu Tyr Ser
 20 25 30

Asp Gly Lys Thr Tyr Leu Asn Trp Phe Gln Gln Arg Pro Gly Gln Ser
 35 40 45

Pro Arg Arg Leu Thr Tyr Leu Val Ser Lys Leu Asp Ser Gly Val Pro
 50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
 65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Val Gln Gly
 85 90 95

Thr His Phe Pro Phe Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
 100 105 110

<210> 15

<211> 112

<212> PRT

<213> Artificial

<220>

<223> Synthetic peptide

<400> 15

Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Leu Gly
 1 5 10 15

Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu Leu His Ser
 20 25 30

Asp Gly Lys Thr Tyr Leu Asn Trp Phe Gln Gln Arg Pro Gly Gln Ser
 35 40 45

Pro Arg Arg Leu Thr Tyr Leu Val Ser Lys Leu Asp Ser Gly Val Pro
 50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
 65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Val Gln Gly
 85 90 95

Thr His Phe Pro Phe Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
 100 105 110

<210> 16

<211> 16

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic peptide

<400> 16

Lys Ser Ser Gln Ser Leu Leu His Ser Asp Gly Lys Thr Tyr Leu Asn
 1 5 10 15

<210> 17

<211> 119

<212> PRT

<213> artificial sequence

<220>

<223> Synthetic peptide

<220>

<221> Variant

<222> (32)..(32)

<223> Xaa is His or Tyr

<220>

<221> Variant

<222> (54)..(54)

<223> Xaa is Ser or Tyr

<220>

<221> Variant

<222> (56)..(56)

<223> Xaa is Gly, Thr, Ala or Glu

<220>

<221> Variant

<222> (65)..(65)

<223> Xaa is Lys or Gln

<220>

<221> Variant

<222> (70)..(70)

<223> Xaa is Leu or Ile

<220>

<221> Variant

<222> (71)..(71)

<223> Xaa is Ile or Thr

<220>

<221> Variant

<222> (80)..(80)

<223> Xaa is Tyr or His

<220>

<221> Variant

<222> (93)..(93)

<223> Xaa is Val or Thr

<400> 17

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Ser Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr Gly Xaa
 20 25 30

Thr Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
 35 40 45

Gly Leu Ile Asn Pro Xaa Asn Xaa Val Thr Arg Tyr Asn Gln Lys Phe
 50 55 60

Xaa Gly Arg Val Thr Xaa Xaa Arg Asp Thr Ser Thr Thr Thr Val Xaa
 65 70 75 80

Met Glu Leu Thr Ser Leu Thr Ser Glu Asp Thr Ala Xaa Tyr Tyr Cys
 85 90 95

Thr Arg Glu Ala Lys Arg Glu Trp Asp Glu Thr Tyr Trp Gly Gln Gly
 100 105 110

Thr Leu Val Thr Val Ser Ser
 115

<210> 18
 <211> 10
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Synthetic peptide

<220>
 <221> Variant
 <222> (7)..(7)
 <223> Xaa is Tyr or His

<400> 18

Gly Tyr Ser Phe Thr Gly Xaa Thr Met Asn
 1 5 10

<210> 19
 <211> 17
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Synthetic peptide

<220>
 <221> Variant
 <222> (5)..(5)
 <223> Xaa is Tyr or Ser

<220>
 <221> Variant
 <222> (7)..(7)
 <223> Xaa is Gly, Thr, Ala or Glu

<220>
 <221> Variant
 <222> (16)..(16)
 <223> Xaa is Lys and Gln

<400> 19

Leu	Ile	Asn	Pro	Xaa	Asn	Xaa	Val	Thr	Arg	Tyr	Asn	Gln	Lys	Phe	Xaa
1				5					10					15	

Gly

<210> 20
 <211> 10
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Synthetic peptide

<400> 20

Glu	Ala	Lys	Arg	Glu	Trp	Asp	Glu	Thr	Tyr
1				5				10	

<210> 21
 <211> 119

<212> PRT
 <213> Artificial sequence

<220>
 <223> Synthetic peptide

<400> 21

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Ser Gly Ala
 1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr Gly Tyr
 20 25 30

Thr Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
 35 40 45

Gly Leu Ile Asn Pro Tyr Asn Gly Val Thr Arg Tyr Asn Gln Lys Phe
 50 55 60

Lys Gly Arg Val Thr Leu Ile Arg Asp Thr Ser Thr Thr Thr Val Tyr
 65 70 75 80

Met Glu Leu Thr Ser Leu Thr Ser Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Thr Arg Glu Ala Lys Arg Glu Trp Asp Glu Thr Tyr Trp Gly Gln Gly
 100 105 110

Thr Leu Val Thr Val Ser Ser
 115

<210> 22
 <211> 10
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Synthetic peptide

<400> 22

Gly Tyr Ser Phe Thr Gly Tyr Thr Met Asn
 1 5 10

<210> 23
 <211> 17
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Synthetic peptide

<400> 23

Leu Ile Asn Pro Tyr Asn Gly Val Thr Arg Tyr Asn Gln Lys Phe Lys
 1 5 10 15

Gly

<210> 24
 <211> 119
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Synthetic peptide

<400> 24

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Ser Gly Ala
 1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr Gly His
 20 25 30

Thr Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
 35 40 45

Gly Leu Ile Asn Pro Ser Asn Gly Val Thr Arg Tyr Asn Gln Lys Phe
 50 55 60

Gln Gly Arg Val Thr Ile Thr Arg Asp Thr Ser Thr Thr Thr Val His
 65 70 75 80

Met Glu Leu Thr Ser Leu Thr Ser Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Thr Arg Glu Ala Lys Arg Glu Trp Asp Glu Thr Tyr Trp Gly Gln Gly
 100 105 110

Thr Leu Val Thr Val Ser Ser
 115

<210> 25
 <211> 10
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Synthetic peptide

<400> 25

Gly Tyr Ser Phe Thr Gly His Thr Met Asn
 1 5 10

<210> 26
 <211> 17
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Synthetic peptide

<400> 26

Leu Ile Asn Pro Ser Asn Gly Val Thr Arg Tyr Asn Gln Lys Phe Gln
 1 5 10 15

Gly

<210> 27
 <211> 119
 <212> PRT
 <213> Artificial sequence

<220>

<223> Synthetic peptide

<400> 27

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Ser Gly Ala
 1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr Gly His
 20 25 30

Thr Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
 35 40 45

Gly Leu Ile Asn Pro Ser Asn Gly Val Thr Arg Tyr Asn Gln Lys Phe
 50 55 60

Gln Gly Arg Val Thr Ile Thr Arg Asp Thr Ser Thr Thr Thr Val His
 65 70 75 80

Met Glu Leu Thr Ser Leu Thr Ser Glu Asp Thr Ala Thr Tyr Tyr Cys
 85 90 95

Thr Arg Glu Ala Lys Arg Glu Trp Asp Glu Thr Tyr Trp Gly Gln Gly
 100 105 110

Thr Leu Val Thr Val Ser Ser
 115

<210> 28

<211> 112

<212> PRT

<213> Artificial sequence

<220>

<223> Synthetic peptide

<400> 28

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
 1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ser Ser Gln Ser Leu Val His Ser
 20 25 30

Asp Gly Asn Thr Tyr Leu His Trp Tyr Gln Gln Lys Pro Gly Lys Ala
 35 40 45

Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
 50 55 60

Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile
 65 70 75 80

Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Ser Gln Ser
 85 90 95

Thr His Val Pro Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
 100 105 110

<210> 29
 <211> 16
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Synthetic peptide

<400> 29

Arg Ser Ser Gln Ser Leu Val His Ser Asp Gly Asn Thr Tyr Leu His
 1 5 10 15

<210> 30
 <211> 7
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Synthetic peptide

<400> 30

Lys Val Ser Asn Arg Phe Ser
1 5

<210> 31
<211> 9
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic peptide

<400> 31

Ser Gln Ser Thr His Val Pro Pro Thr
1 5

<210> 32
<211> 122
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic peptide

<400> 32

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30

Gly Met Ala Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Phe Ile Ser Asn Leu Ala Tyr Ser Ile Tyr Tyr Ala Asp Thr Val
50 55 60

Thr Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Tyr Asp Tyr Asp Asn Ile Leu Asp Tyr Val Met Asp Tyr Trp
 100 105 110

Gly Gln Gly Thr Leu Val Thr Val Ser Ser
 115 120

<210> 33
 <211> 10
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Synthetic peptide

<400> 33

Gly Phe Thr Phe Ser Asp Tyr Gly Met Ala
 1 5 10

<210> 34
 <211> 17
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Synthetic peptide

<400> 34

Phe Ile Ser Asn Leu Ala Tyr Ser Ile Tyr Tyr Ala Asp Thr Val Thr
 1 5 10 15

Gly

<210> 35
 <211> 13
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Synthetic peptide

<400> 35

Tyr Asp Tyr Asp Asn Ile Leu Asp Tyr Val Met Asp Tyr
1 5 10

<210> 36

<211> 112

<212> PRT

<213> Artificial sequence

<220>

<223> Synthetic peptide

<400> 36

Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Leu Gly
1 5 10 15

Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu Leu Tyr Ser
20 25 30

Asn Gly Lys Thr Tyr Leu Asn Trp Phe Gln Gln Arg Pro Gly Gln Ser
35 40 45

Pro Arg Arg Leu Ile Tyr Val Val Ser Lys Leu Asp Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Val Gln Gly
85 90 95

Thr His Phe Pro Phe Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

<210> 37

<211> 16

<212> PRT

<213> Artificial sequence

<220>

<223> Synthetic peptide

<400> 37

Lys Ser Ser Gln Ser Leu Leu Tyr Ser Asn Gly Lys Thr Tyr Leu Asn
1 5 10 15

<210> 38

<211> 7

<212> PRT

<213> Artificial sequence

<220>

<223> Synthetic peptide

<400> 38

Val Val Ser Lys Leu Asp Ser
1 5

<210> 39

<211> 9

<212> PRT

<213> Artificial sequence

<220>

<223> Synthetic peptide

<400> 39

Val Gln Gly Thr His Phe Pro Phe Thr
1 5

<210> 40

<211> 115

<212> PRT

<213> Artificial sequence

<220>

<223> Synthetic peptide

<400> 40

Gln Val Gln Leu Val Glu Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Ile Phe Asn Asn Tyr
 20 25 30

Trp Ile Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
 35 40 45

Gly Gln Ile Tyr Pro Gly Asp Gly Asp Thr Asn Tyr Asn Gly Lys Phe
 50 55 60

Lys Gly Arg Val Thr Ile Thr Ala Asp Glu Ser Thr Ser Thr Ala Tyr
 65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Arg Glu Gly Tyr Ile Val Tyr Trp Gly Gln Gly Thr Leu Val Thr
 100 105 110

Val Ser Ser
 115

<210> 41
 <211> 7
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Synthetic peptide

<400> 41

Gly Tyr Ile Phe Asn Asn Tyr
 1 5

<210> 42
 <211> 17
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Synthetic peptide

<400> 42

Gln Ile Tyr Pro Gly Asp Gly Asp Thr Asn Tyr Asn Gly Lys Phe Lys
 1 5 10 15

Gly

<210> 43

<211> 6

<212> PRT

<213> Artificial sequence

<220>

<223> Synthetic peptide

<400> 43

Glu Gly Tyr Ile Val Tyr
 1 5

<210> 44

<211> 24

<212> DNA

<213> Artificial sequence

<220>

<223> PCR primer

<400> 44

actgtggctg caccatctgt cttc

24

<210> 45

<211> 25

<212> DNA

<213> Artificial sequence

<220>

<223> PCR primer

<400> 45

ctaacactct cccctgttga agctc

25

<210> 46

<211> 23

<212> DNA
 <213> Artificial sequence

<220>
 <223> PCR primer

<400> 46
 agggaaccct ggtcaccgtc tcc 23

<210> 47
 <211> 25
 <212> DNA
 <213> Artificial sequence

<220>
 <223> PCR primer

<400> 47
 tcatttaccc ggagacaggg agagg 25

<210> 48
 <211> 24
 <212> DNA
 <213> Artificial sequence

<220>
 <223> PCR primer

<400> 48
 caagtcagag cctcttatat agtg 24

<210> 49
 <211> 23
 <212> DNA
 <213> Artificial sequence

<220>
 <223> PCR primer

<400> 49
 gtaccttgca cgcagtaata aac 23

<210> 50
 <211> 16
 <212> PRT
 <213> Artificial sequence

<220>

<223> Synthetic peptide

<220>

<221> MOD_RES

<222> (1)..(1)

<223> PYRROLIDONE CARBOXYLIC ACID

<400> 50

Glu	Phe	Arg	His	Asp	Ser	Gly	Tyr	Glu	Val	His	His	Gln	Lys	Leu	Val
1				5					10					15	

<210> 51

<211> 18

<212> PRT

<213> Artificial sequence

<220>

<223> Synthetic peptide

<400> 51

Asp	Ala	Glu	Phe	Arg	His	Asp	Ser	Gly	Tyr	Glu	Val	His	His	Gln	Lys
1				5					10					15	

Leu Val

<210> 52

<211> 17

<212> PRT

<213> Artificial sequence

<220>

<223> Synthetic peptide

<400> 52

Ala	Glu	Phe	Arg	His	Asp	Ser	Gly	Tyr	Glu	Val	His	His	Gln	Lys	Leu
1				5					10					15	

Val

<210> 53
 <211> 15
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Synthetic peptide

<400> 53

Phe	Arg	His	Asp	Ser	Gly	Tyr	Glu	Val	His	His	Gln	Lys	Leu	Val
1				5					10				15	

<210> 54
 <211> 15
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Synthetic peptide

<220>
 <221> MOD_RES
 <222> (1)..(1)
 <223> PYRROLIDONE CARBOXYLIC ACID

<400> 54

Glu	Phe	Arg	His	Asp	Ser	Gly	Tyr	Glu	Val	His	His	Gln	Lys	Leu
1				5					10				15	

<210> 55
 <211> 14
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Synthetic peptide

<220>
 <221> MOD_RES
 <222> (1)..(1)
 <223> PYRROLIDONE CARBOXYLIC ACID

<400> 55

Glu Phe Arg His Asp Ser Gly Tyr Glu Val His His Gln Lys
1 5 10

<210> 56

<211> 13

<212> PRT

<213> Artificial sequence

<220>

<223> Synthetic peptide

<220>

<221> MOD_RES

<222> (1)..(1)

<223> PYRROLIDONE CARBOXYLIC ACID

<400> 56

Glu Phe Arg His Asp Ser Gly Tyr Glu Val His His Gln
1 5 10

<210> 57

<211> 12

<212> PRT

<213> Artificial sequence

<220>

<223> Synthetic peptide

<220>

<221> MOD_RES

<222> (1)..(1)

<223> PYRROLIDONE CARBOXYLIC ACID

<400> 57

Glu Phe Arg His Asp Ser Gly Tyr Glu Val His His
1 5 10

<210> 58

<211> 11

<212> PRT

<213> Artificial sequence

<220>
 <223> Synthetic peptide

<220>
 <221> MOD_RES
 <222> (1)..(1)
 <223> PYRROLIDONE CARBOXYLIC ACID

<400> 58

Glu Phe Arg His Asp Ser Gly Tyr Glu Val His
 1 5 10

<210> 59
 <211> 10
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Synthetic peptide

<220>
 <221> MOD_RES
 <222> (1)..(1)
 <223> PYRROLIDONE CARBOXYLIC ACID

<400> 59

Glu Phe Arg His Asp Ser Gly Tyr Glu Val
 1 5 10

<210> 60
 <211> 9
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Synthetic peptide

<220>
 <221> MOD_RES
 <222> (1)..(1)
 <223> PYRROLIDONE CARBOXYLIC ACID

<400> 60

Glu Phe Arg His Asp Ser Gly Tyr Glu
1 5

<210> 61

<211> 8

<212> PRT

<213> Artificial sequence

<220>

<223> Synthetic peptide

<220>

<221> MOD_RES

<222> (1)..(1)

<223> PYRROLIDONE CARBOXYLIC ACID

<400> 61

Glu Phe Arg His Asp Ser Gly Tyr
1 5

<210> 62

<211> 7

<212> PRT

<213> Artificial sequence

<220>

<223> Synthetic peptide

<220>

<221> MOD_RES

<222> (1)..(1)

<223> PYRROLIDONE CARBOXYLIC ACID

<400> 62

Glu Phe Arg His Asp Ser Gly
1 5

<210> 63

<211> 6

<212> PRT

<213> Artificial sequence

<220>
 <223> Synthetic peptide

<220>
 <221> MOD_RES
 <222> (1)..(1)
 <223> PYRROLIDONE CARBOXYLIC ACID

<400> 63

Glu Phe Arg His Asp Ser
 1 5

<210> 64
 <211> 4
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Synthetic peptide

<220>
 <221> MOD_RES
 <222> (1)..(1)
 <223> PYRROLIDONE CARBOXYLIC ACID

<400> 64

Glu Phe Arg His
 1

<210> 65
 <211> 3
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Synthetic peptide

<220>
 <221> MOD_RES
 <222> (1)..(1)
 <223> PYRROLIDONE CARBOXYLIC ACID

<400> 65

Glu Phe Arg
1

<210> 66

<211> 119

<212> PRT

<213> Artificial sequence

<220>

<223> Synthetic peptide

<400> 66

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Ser Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr Gly His
20 25 30

Thr Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Leu Ile Asn Pro Ser Asn Thr Val Thr Arg Tyr Asn Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Arg Asp Thr Ser Thr Thr Thr Val His
65 70 75 80

Met Glu Leu Thr Ser Leu Thr Ser Glu Asp Thr Ala Thr Tyr Tyr Cys
85 90 95

Thr Arg Glu Ala Lys Arg Glu Trp Asp Glu Thr Tyr Trp Gly Gln Gly
100 105 110

Thr Leu Val Thr Val Ser Ser
115

<210> 67

<211> 17

<212> PRT
 <213> Artificial sequence

<220>
 <223> Synthetic peptide

<400> 67

Leu	Ile	Asn	Pro	Ser	Asn	Thr	Val	Thr	Arg	Tyr	Asn	Gln	Lys	Phe	Gln
1				5					10					15	

Gly

<210> 68
 <211> 119
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Synthetic peptide

<400> 68

Gln	Val	Gln	Leu	Val	Gln	Ser	Gly	Ala	Glu	Val	Lys	Lys	Ser	Gly	Ala
1				5					10					15	

Ser	Val	Lys	Val	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Ser	Phe	Thr	Gly	His
			20					25					30		

Thr	Met	Asn	Trp	Val	Arg	Gln	Ala	Pro	Gly	Gln	Gly	Leu	Glu	Trp	Met
		35					40					45			

Gly	Leu	Ile	Asn	Pro	Ser	Asn	Ala	Val	Thr	Arg	Tyr	Asn	Gln	Lys	Phe
	50					55					60				

Gln	Gly	Arg	Val	Thr	Ile	Thr	Arg	Asp	Thr	Ser	Thr	Thr	Thr	Val	His
65					70					75				80	

Met	Glu	Leu	Thr	Ser	Leu	Thr	Ser	Glu	Asp	Thr	Ala	Thr	Tyr	Tyr	Cys
				85					90					95	

Thr Arg Glu Ala Lys Arg Glu Trp Asp Glu Thr Tyr Trp Gly Gln Gly
 100 105 110

Thr Leu Val Thr Val Ser Ser
 115

<210> 69
 <211> 17
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Synthetic peptide

<400> 69

Leu Ile Asn Pro Ser Asn Ala Val Thr Arg Tyr Asn Gln Lys Phe Gln
 1 5 10 15

Gly

<210> 70
 <211> 119
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Synthetic peptide

<400> 70

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Ser Gly Ala
 1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr Gly His
 20 25 30

Thr Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
 35 40 45

Gly Leu Ile Asn Pro Ser Asn Glu Val Thr Arg Tyr Asn Gln Lys Phe
 50 55 60

Gln Gly Arg Val Thr Ile Thr Arg Asp Thr Ser Thr Thr Thr Val His
65 70 75 80

Met Glu Leu Thr Ser Leu Thr Ser Glu Asp Thr Ala Thr Tyr Tyr Cys
85 90 95

Thr Arg Glu Ala Lys Arg Glu Trp Asp Glu Thr Tyr Trp Gly Gln Gly
100 105 110

Thr Leu Val Thr Val Ser Ser
115

<210> 71
<211> 17
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic peptide

<400> 71

Leu Ile Asn Pro Ser Asn Glu Val Thr Arg Tyr Asn Gln Lys Phe Gln
1 5 10 15

Gly

<210> 72
<211> 5
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic peptide

<220>
<221> MOD_RES
<222> (1)..(1)
<223> PYRROLIDONE CARBOXYLIC ACID

<400> 72

Glu Phe Arg His Asp
1 5

<210> 73

<211> 449

<212> PRT

<213> Homo sapiens

<400> 73

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Ser Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr Gly His
20 25 30

Thr Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Leu Ile Asn Pro Ser Asn Glu Val Thr Arg Tyr Asn Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Arg Asp Thr Ser Thr Thr Thr Val His
65 70 75 80

Met Glu Leu Thr Ser Leu Thr Ser Glu Asp Thr Ala Thr Tyr Tyr Cys
85 90 95

Thr Arg Glu Ala Lys Arg Glu Trp Asp Glu Thr Tyr Trp Gly Gln Gly
100 105 110

Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
115 120 125

Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
130 135 140

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp

Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr
 355 360 365

Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
 370 375 380

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
 385 390 395 400

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
 405 410 415

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
 420 425 430

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
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Lys

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<213> Artificial sequence

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<223> Synthetic polypeptide

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Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr Gly His
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Thr Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
 35 40 45

Gly Leu Ile Asn Pro Ser Asn Glu Val Thr Arg Tyr Asn Gln Lys Phe
 50 55 60

Gln Gly Arg Val Thr Ile Thr Arg Asp Thr Ser Thr Thr Thr Val His
 65 70 75 80

Met Glu Leu Thr Ser Leu Thr Ser Glu Asp Thr Ala Thr Tyr Tyr Cys
 85 90 95

Thr Arg Glu Ala Lys Arg Glu Trp Asp Glu Thr Tyr Trp Gly Gln Gly
 100 105 110

Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
 115 120 125

Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
 130 135 140

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
 145 150 155 160

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
 165 170 175

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
 180 185 190

Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
 195 200 205

Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys
 210 215 220

Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
 225 230 235 240

Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser

245

250

255

Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
 260 265 270

Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
 275 280 285

Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
 290 295 300

Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
 305 310 315 320

Tyr Lys Cys Ala Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
 325 330 335

Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
 340 345 350

Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr
 355 360 365

Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
 370 375 380

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
 385 390 395 400

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
 405 410 415

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
 420 425 430

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
 435 440 445

Lys

CLAIMS

1. A humanized antibody or a functional variant thereof, wherein the variable part of the light chain of said antibody comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 11, SEQ ID NO: 13 and SEQ ID NO: 14 comprising the CDR regions V_L CDR1 of SEQ ID NO: 12, V_L CDR2 SEQ ID NO: 9 and V_L CDR3 of SEQ ID NO: 10 in the light chain; and wherein the variable part of the heavy chain of said antibody comprises the CDR regions V_H CDR1: SEQ ID NO: 25, V_H CDR2: SEQ ID NO: 26; and V_H CDR3: SEQ ID NO: 20.
2. The humanized antibody of claim 1, wherein the variable part of the light chain comprises the amino acid sequence of SEQ ID NO: 11.
3. The humanized antibody of claim 1, wherein the variable part of the light chain comprises the amino acid sequence of SEQ ID NO: 13.
4. The humanized antibody of claim 1, wherein the variable part of the light chain comprises the amino acid sequence of SEQ ID NO: 14.
5. The humanized antibody of claim 1, wherein the variable part of the heavy chain comprises the amino acid sequence of SEQ ID NO: 24.
6. The humanized antibody of claim 1, wherein the variable part of the heavy chain comprises the amino acid sequence of SEQ ID NO: 27.
7. The humanized antibody according to claim 1, having a human IgG1 Fc region which comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 73 and SEQ ID NO: 74.
8. The humanized antibody according to claim 1, having a human IgG1 Fc region which comprises the amino acid sequence of SEQ ID NO: 74.

9. The humanized antibody according to claim 1, wherein the variable part of the light chain comprises the amino acid sequence of SEQ ID NO: 14; and having a variable part of a heavy chain which comprises the amino acid sequence of SEQ ID NO: 27; and having a human IgG1 Fc region which comprises the amino acid sequence of SEQ ID NO: 74.

10. The humanized antibody according to claim 1, having a variable part of a light chain, a variable part of a heavy chain, and a human IgG1 Fc region, wherein:

- the variable part of the light chain comprises the amino acid sequence of SEQ ID NO: 14; and
- the variable part of the heavy chain comprises the amino acid sequence of SEQ ID NO: 27; and
- the human IgG1 Fc region comprises the amino acid sequence of SEQ ID NO: 74;
- the variable part of the light chain comprises the CDR regions V_L CDR1 of SEQ ID NO: 12, V_L CDR2 of SEQ ID NO: 9 and V_L CDR3 of SEQ ID NO: 10; and
- the variable part of the heavy chain comprises the CDR regions V_H CDR1 of SEQ ID NO: 25, V_H CDR2 of SEQ ID NO: 26 and V_H CDR3 of SEQ ID NO: 20.

11. A pharmaceutical composition comprising the humanized antibody according to claim 1.

12. The pharmaceutical composition of claim 11, further comprising a further biologically active substance and/or a pharmaceutically acceptable carrier and/or a diluent and/or an excipient.

13. The pharmaceutical composition of claim 12, wherein said further biologically active substance is selected from a neutron-transmission enhancer, psychotherapeutic drug, an acetylcholine esterase inhibitor, a calcium-channel blocker, a biogenic amine, a benzodiazepine tranquillizer, an acetylcholine synthesis, storage or release enhancer, an acetylcholine postsynaptic receptor agonist, a monoamine oxidase-A or -B inhibitor, a N-methyl- D-aspartate

glutamate receptor antagonist, a non-steroidal anti-inflammatory drug, an antioxidant, and a serotonergic receptor antagonist.

14. The pharmaceutical composition of claim 12, wherein said further biologically active substance is selected from the group consisting of a compound effective against oxidative stress, an anti-apoptotic compound, a metal chelator, an inhibitor of DNA repair, an α -secretase activator, a β - and γ -secretase inhibitor, a tau protein, a neurotransmitter, a β -sheet breaker, an anti-inflammatory molecule, a cholinesterase inhibitor, a M1 agonist, a amyloid- or tau-burden modifying drug, a nutritive supplement, memantine, and a glutaminy cyclase inhibitor.

15. The pharmaceutical composition of claim 13 wherein the further biologically active substance is acetylcholine esterase inhibitor.

16. The pharmaceutical composition of claim 14 wherein the further biologically active substance is a glutaminy cyclase inhibitor.