(72) Inventors; and
(75) Inventors/Applicants (for US only): CHRISTENSEN,
Søren [DK/DK]; Lystbådevej 32, DK-4040 Jyllinge (DK).
HELBOE, Lone [DK/DK]; Roarsvej 17, 4.tv., DK-2000
Frederiksberg (DK).

(54) Title: ANTIBODIES SPECIFIC TO PYROGLUTAMATED Aβ

A

Clone	Isotype Type (mouse)	Ka$_1$ (1/M)	Kd$_1$ (1/s)	KD$_1$ (M)	Ka$_2$ (1/RUs)	Kd$_2$ (1/s)	KD$_2$ (RU)	KD (M)
LuAb1h	IgG1, κ	3,64e4	4,22e-6	1,16e-10	1,32e-4	9,43e-3	71,4	8,2e-9
LUAb2h	IgG2a, κ	1,35e5	4,64e-4	3,46e-9	5,94e-2	1,1	18,52	64e-9

B

Clone	Isotype Type (mouse)	Ka$_1$ (1/M)	Kd$_1$ (1/s)	KD$_1$ (M)	Ka$_2$ (1/RUs)	Kd$_2$ (1/s)	KD$_2$ (RU)	KD (M)
LuAb1m	IgG1, κ	3,04e5	5,03e-3	1,65e-8	1,57	5,6	3,57	58,9e-9
LuAb2m	IgG1, κ	5,06e5	7,5e-3	1,48e-8	0,0155	0,0676	4,36	64,6e-9

Fig. 1

(57) Abstract: The present disclosure
relates to antibodies specific for pyro-
glutamated A β, as well as their use in
the treatment of Alzheimer's disease
and as use in diagnostic methods or as
diagnostic imaging ligands. Further, is
provided pyroglutamated N- terminal
fragments of murine or human A β to
generate antibodies and for use in
therapeutic purposes.

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TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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Antibodies specific to pyroglutamated Aβ

FIELD OF THE INVENTION

The present invention relates to antibodies specific for pyroglutamated Aβ, as
well as their use in the treatment of Alzheimer's disease and as use in diagnostic
methods or as diagnostic imaging ligands. Further, is provided pyroglutamated N-
terminal fragments of murine or human Aβ to generate antibodies and for use in
therapeutic purposes.

BACKGROUND OF THE INVENTION

Alzheimer disease (AD) is characterised by a progressive dementia occurring in
mid or late life. The first events leading to AD are believed to occur some 10 to 20
years before the cognitive symptoms appear, and evidence suggests that a key
event in the pathology of AD is the deposit of extracellular aggregated amyloid β
(Aβ) plaques in the brain. Aβ is generated by proteolytic cleavage from the amy-
loid precursor protein (APP) which is a large transmembrane protein with a sug-
gested neutrophic function. The main Aβ variants observed in the human brain
are Aβ 1-40 and Aβ 1-42, but also truncated N-terminal variants and other modi-
fied species thereof are observed in the plaques.

Immunotherapy against the Aβ plaques has been shown to disrupt the Aβ aggre-
gates and promote the clearance of plaques in the brain. Both active immuno-
genic approaches with Aβ or fragments thereof as well as passive immunisation
using anti-Aβ antibodies have proven effective in different animal models. Vacci-
nation of humans with Aβ was shown to prevent the development of Aβ plaques
and reduce the Aβ burden in patients in a clinical study, however the study was
stopped due to inflammation in the brain of some of the patients. An immuno-
genic strategy to reduce the amyloid plaques thus relies on a delicate balance of
activating the immune system but still avoiding an uncontrollable inflammation to
occur (Monsonego et al (2003) *J clin invest* 112, 415–422). This requires a pre-
cise knowledge of the chemical nature of *inter alia* the nature of the deposit. In an
attempt to understand the exact composition of the amyloid plaques one of the
most abundant N-truncated Aβ peptides identified are carrying a pyroglutamate
at position 3 (Aβp3E, N-terminally truncated Aβ starting with pyroglutamate)

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(Saido et al. (1996) *Neuron* 14, 457-466). These pyroglutamated peptides have been shown to accumulate in plaques as well as around the blood vessels in AD, and due to its hydrophobic potential it has been shown that these peptides increase the aggregation. A recent transgenic mouse model expressing AβpE3-42

5   in neurons demonstrates 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 are believed to be advantageous because of their specificity towards only the pathogenic pyro-

10   glutamate N-terminal species of Aβ, thereby leaving APP or other cross reacting species untouched. It is thus envisaged that the risk of uncontrollable cerebral inflammation will be reduced compared to antibodies directed to non-pyroglutamated Aβ or fragments thereof.

15   Antibodies targeting AβpE3 peptides and aggregates have been made (Acero et al (2009) *J Neuroimmunol* 213, 39-46; Saido et al. (1996) *Neuron* 14, 457-466; and US 7,122,374) and identification of various AβpE fragments have been hypothesized as epitopes (WO2004/013172; WO2010/009987; WO2010/129276; and Wirths et al. (2010) *J Biol Chem* 285(53), 41517-24, Epub 2010 Oct 22).

20

The present invention provides two highly specific monoclonal antibodies directed against human AβpE3 (5C9 and 2E83, respectively) as well as two highly specific monoclonal antibodies directed against murine AβpE3 (2E4 and 1G11, respectively).

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SUMMARY OF THE INVENTION

The invention relates to an antibody, in particular an isolated antibody, or a fragment thereof, specific for human AβpE3 which antibody comprises, or consists,

30   of:

a heavy chain variable region (V_H) CDR1 comprising GYTFTDYYX$_1$N
a heavy chain variable region (V_H) CDR2 comprising
WX$_2$X$_3$PGSGNX$_4$KYNEKFKG

a heavy chain variable region (V_H) CDR3 comprising $EGX_5X_6X_7Y$

a light chain variable region (V_L) CDR1 comprising $KSSQSLLX_8SNGX_9X_{10}YLN$

a light chain variable region (V_L) CDR2 comprising $X_{11}VSKLDS$, and

a light chain variable region (V_L) CDR3 comprising $VQGTHX_{12}PFT$

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wherein X_1 to X_{12} symbolizes a natural amino acid.

The invention further relates to an antibody in particular an isolated antibody, or a fragment thereof, specific for murine AβpE3 which antibody comprises, or con-

10   sists, of:

a heavy chain variable region (V_H) CDR1 comprising $GX_1TLX_2DAWMX_3$

a heavy chain variable region (V_H) CDR2 comprising $EIRX_4KAX_5X_6HATX_7YAESVKG$

15   a heavy chain variable region (V_H) CDR3 comprising HX_8X_9

a light chain variable region (V_L) CDR1 comprising $X_{10}ASQGIX_{11}X_{12}X_{13}X_{14}G$

a light chain variable region (V_L) CDR2 comprising $HGTKLED$, and

a light chain variable region (V_L) CDR3 comprising $VQYX_{15}QFPYT$

20   wherein X_1 to X_{15} symbolizes a natural amino acid.

In further embodiments the invention provides four specific monoclonal antibodies as set forth in the claims, as well as their use in the treatment of Alzheimer's disease and as use in diagnostic methods or as diagnostic imaging ligands. The

25   pyroglutamated N-terminal 3-11 amino acids of murine or human Aβ is also provided for the generation of antibodies or for use in pharmaceutical compositions.

BRIEF DESCRIPTION OF DRAWINGS

Fig 1. Biacore binding of pE3-specific monoclonal antibodies (MABs) to human

30   AβpE3 (A) and murine AβpE3 (B). Data for two monoclonal antibodies highly specific for human AβpE3, LuAb1h and LuAb2h (which are the same as 5C9 and 2E83, respectively), and two monoclonal antibodies highly specific for murine AβpE3, exemplified by LuAb1m and LuAb2m is shown.

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Fig 2A and B. Shows the Biacore binding curves to human AβpE3 (A) and murine AβpE3 (B) peptides for the antibodies in Fig 1.

Fig 3. Micrographs showing immunoreactivity of Abeta and pE3-Abeta antibodies in the frontal cortex from an Alzheimer brain (A) and in the hippocampal region of an APP/PS1 transgenic mouse (B). The antibodies used are 6E10, which is specific for Aβ, 2E8 and 5C9 which are specific for human AβpE.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

The terms "pyroglutamated Aβ", and "AβpE" are used interchangeably herein, and, unless specified otherwise, include any variants, isoforms and species homologs of pyroglutamated Aβ. For example, AβpE3 refers to different Aβ variants, including, but not limited to the fragments Aβ 3-42, Aβ 3-40 or Aβ 3-11, which are pyroglutamated at the N-terminal end specifically at the position 3 glutamic acid.

The term "immunoglobulin" refers to a class of structurally related glycoproteins consisting of two pairs of polypeptide chains, one pair of light (L) low molecular weight chains and one pair of heavy (H) chains, which may all four be interconnected by disulfide bonds. The structure of immunoglobulins has been well characterized. See for instance Fundamental Immunology Ch. 7 (Paul, W., ed., 2nd ed. Raven Press, N. Y. (1989)). Briefly, each heavy chain typically is comprised of a heavy chain variable region (abbreviated herein as V_H or VH) and a heavy chain constant region. The heavy chain constant region typically is comprised of three domains, C_H1, C_H2, and C_H3. Each light chain typically is comprised of a light chain variable region (abbreviated herein as V_L or VL) and a light chain constant region. The light chain constant region typically is comprised of one domain, C_L. The V_H and V_L regions may be further subdivided into regions of hypervariability (or hypervariable regions which may be hypervariable in sequence and/or form of structurally defined loops), also termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FRs). Each V_H and V_L is typically composed of three CDRs and four FRs, arranged from amino-terminus to carboxyterminus in the

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following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4 (see also Chothia
and Lesk *J. Mol. Biol.* 196, 901-917 (1987)). Typically, the numbering of amino
acid residues in this region is according to IMGT, Sequences of Proteins of
Immunological Interest, 5th Ed. Public Health Service, National Institutes of
5   Health, Bethesda, MD. (1991).

The term "antibody" (Ab) in the context of the present invention refers to an
immunoglobulin molecule or according to some embodiments of the invention
may be a fragment of an immunoglobulin molecule, or a derivative of either
10   thereof, which has the ability to specifically bind to an antigen. The variable
regions of the heavy and light chains of the immunoglobulin molecule contain a
binding domain that interacts with an antigen as outlined above under
"immunoglobulin". An anti-AβpE3antibody may also be a bispecific antibody,
diabody, or similar molecule (see for instance *PNAS USA* 90(14), 6444-8 (1993)
15   for a description of diabodies). Further, it has been shown that the antigen-
binding function of an antibody may be performed by fragments of a full-length
antibody. Examples of binding fragments encompassed within the term
"antibody" include (i) a Fab' or Fab fragment, a monovalent fragment consisting
of the V_L, V_N, C_L and C_H1 domains, or a monovalent antibody as described in
20   WO2007059782; (ii) F(ab')$_2$ fragments, bivalent fragments comprising two Fab
fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment
consisting essentially of the V_H and C_H1 domains; (iv) a Fv fragment consisting
essentially of a V_L and V_H domains, (v) a dAb fragment (Ward et al., *Nature* 341,
544-546 (1989)), which consists essentially of a V_H domain and also called
25   domain antibodies (Holt et al; *Trends Biotechnol.*
2003 Nov;2i(ll) :484-90); (vi) camelid or nanobodies (Revets et al; *Expert Opin
Biol Ther.* 2005 Jan;5_(I) : I ll-24) and (vii) an isolated complementarity
determining region (CDR). Furthermore, although the two domains of the Fv
fragment, V_L and V_H, are coded for by separate genes, they may be joined, using
30   recombinant methods, by a synthetic linker that enables them to be made as a
single protein chain in which the V_L and V_H regions pair to form monovalent
molecules (known as single chain antibodies or single chain Fv (scFv), see for
instance Bird et al., *Science* 242, 423-426 (1988) and Huston et al., *PNAS USA*
85, 5879-5883 (1988)). Such single chain antibodies are encompassed within the

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term antibody unless otherwise noted or clearly indicated by context. Although such fragments are generally included within the meaning of antibody, they collectively, and each independently, are unique features of the present invention, exhibiting different biological properties and utility. These and other

5   useful antibody fragments in the context of the present invention are discussed further herein. It also should be understood that the term antibody, unless specified otherwise, also includes polyclonal antibodies, monoclonal antibodies (MAbs), antibody-like polypeptides, such as chimeric antibodies and humanized antibodies, and antibody fragments retaining the ability to specifically bind to the

10   antigen (antigen-binding fragments) provided by any known technique, such as enzymatic cleavage, peptide synthesis, and recombinant techniques. An antibody as generated can possess any isotype.

An "anti-AβpE3 antibody" is an antibody as described above, which binds

15   specifically to variant fragments of Aβ, including, but not limited to, pyrogluta-mated N-terminal truncated forms such as the fragments Aβ 3-42, 3-40 or 3-11, which are pyroglutamated in the N-terminal end at position 3.

The term "human antibody", as used herein, is intended to include antibodies

20   having variable and constant regions derived from human germline immunoglobulin sequences. The human antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (e.g., mutations introduced by random or site- specific mutagenesis in vitro or during gene rearrangement or by somatic mutation *in vivo*).

25
The term "humanized antibody", as used herein, is intended to include antibodies in which CDR sequences are derived from the germline of another mammalian species, such as a mouse, and have been grafted onto human framework sequences. Humanized monoclonal antibodies may be generated by a

30   hybridoma which includes a B lymphocyte cell obtained from a transgenic or transchromosomal nonhuman animal, such as a transgenic mouse, having a genome comprising a human heavy chain transgene and a light chain transgene, fused to an immortalized cell. For example, when non-human antibodies are prepared with respect to a particular antigen, the variable regions can be

"reshaped" or humanized" by grafting CDRs derived from nonhuman antibody on the FRs present in the human antibody to be modified. Application of this approach has been reported by Sato, K. et al. *Cancer Research* 53:851-856 (1993); Riechmann, L., et al., *Nature* 332:323-327 (1988); Verhoeyen, M., et al.,

5   *Science* 239:1534-1536 (1988); Kettleborough, C. A., et al., *Protein Engineering* 4:773-3783 (1991); Maeda, H., et al., *Human Antibodies Hybridoma* 2:124-134 (1991); Gorman, S. D., et al., *Proc Natl Acad Sci USA* 88:4181-4185 (1991); Tempest, P. R., et al., *Bio/Technology* 9:266-271 (1991); Co, M. S., et al., *Proc Natl Acad Sci USA* 88:2869-2873 (1991); Carter, P., et al., *Proc Natl Acad Sci*

10   *USA* 89:4285-4289 (1992); and Co, M. S. et al., *J Immunol* 148:1149-1154 (1992). In some embodiments, humanized antibodies preserve all CDR sequences (for example, a humanized mouse antibody which contains all six CDRs from the mouse antibodies). In other embodiments, humanized antibodies have one or more CDRs (one, two, three, four, five, or six) which are altered with re-

15   spect to the original antibody, which are also termed one or more CDRs "derived from" one or more CDRs from the original antibody. Humanized antibodies may refer to chimeric molecules prepared using recombinant techniques.

The terms "monoclonal antibody" or "monoclonal antibody composition" as used

20   herein refer to a preparation of antibody molecules of single molecular composition. A monoclonal antibody composition displays a single binding specificity and affinity for a particular epitope. Accordingly, the term "mouse or murine monoclonal antibody" refers to antibodies displaying a single binding specificity which have variable and constant regions derived from murine or

25   mouse germline immunoglobulin sequences.

As used herein, the term "binding" in the context of the binding of an antibody to a predetermined antigen typically refers to binding with an affinity corresponding to a K_D of about 10^{-7} M or less, such as about 10^{-8} M or less, such as about 10^{-9}

30   M or less when determined by for instance surface plasmon resonance (SPR) technology in a BIAcore 3000 instrument using the antigen as the ligand and the antibody as the analyte, and binds to the predetermined antigen with an affinity corresponding to a K_D that is at least ten-fold lower, such as at least 100 fold lower, for instance at least 1,000 fold lower, such as at least 10,000 fold lower, for

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instance at least 100,000 fold lower than its affinity for binding to a non-specific antigen (e.g., BSA, casein) other than the predetermined antigen or a closely-related antigen. The amount with which the affinity is lower is dependent on the K_D of the antibody, so that when the K_D of the antibody is very low (that is, the antibody is highly specific), then the amount with which the affinity for the antigen is lower than the affinity for a non-specific antigen may be at least 10,000 fold.

The term "k_d" (sec^{-1} or 1/s), as used herein, refers to the dissociation rate constant of a particular antibody-antigen interaction. Said value is also referred to as the k_{off} value.

The term "k_a" (M^{-1} x sec^{-1} or 1/M), as used herein, refers to the association rate constant of a particular antibody-antigen interaction.

The term "K_D" (M), as used herein, refers to the dissociation equilibrium constant of a particular antibody-antigen interaction.

The term "K_A" (M^{-1} or 1/M), as used herein, refers to the association equilibrium constant of a particular antibody-antigen interaction and is obtained by dividing the k_a by the k_d.

The present invention also provides antibodies comprising functional variants of the V_L region, V_H region, or one or more CDRs of the antibodies of the examples. A functional variant of a V_L, V_H, or CDR used in the context of an anti-AβpE3antibody still allows the antibody to retain at least a substantial proportion (at least about 80%, 90%, 95% or more) of the affinity and/or the specificity/selectivity of the parent antibody and in some cases such an anti-AβpE3antibody may be associated with greater affinity, selectivity and/or specificity than the parent antibody. Such functional variants typically retain significant amino acid sequence identity to the parent antibody. The percent identity between two amino acid sequences is a function of the number of identical positions shared by the sequences (i.e., % homology = # of identical positions/total # of positions x 100), taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the

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two sequences. The comparison of sequences and determination of percent identity between two sequences may be accomplished using a mathematical algorithm, as described in the non-limiting examples below.

5   The sequence of CDR variants may differ from the sequence of the CDR of the parent antibody sequences through substitutions; for instance substituted at least 10, such as at least 9, 8, 7, 6, 5, 4, 3, 2 or 1 of the amino acid residues. According to an embodiment of the invention it is furthermore envisaged that the amino acids in the CDR regions may be substituted with conservative 10   substitutions, as defined in the below 3 tables. For example, the acidic residue Asp can be substituted with Glu without substantially affecting the binding characteristic of the antibody.

In the context of the present invention, conservative substitutions may be defined 15   by substitutions within the classes of amino acids reflected in one or more of the following three tables:

Amino acid residue classes for conservative substitutions:

Acidic Residues	Asp (D) and Glu (E)
Basic Residues	Lys (K), Arg (R), and His (H)
Hydrophilic Uncharged Residues	Ser (S), Thr (T), Asn (N), and Gln (Q)
Aliphatic Uncharged Residues	Gly (G), Ala (A), Val (V), Leu (L), and Ile (I)
Non-polar Uncharged Residues	Cys (C), Met (M), and Pro (P)
Aromatic Residues	Phe (F), Tyr (Y), and Trp (W)

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Alternative conservative amino acid residue substitution classes:

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1	A	S	T
2	D	E	
3	N	Q	
4	R	K	
5	I	L	M
6	F	Y	W

Alternative Physical and Functional Classifications of Amino Acid Residues:

Alcohol group-containing residues	S and T
Aliphatic residues	I, L, V, and M
Cycloalkenyl-associated residues	F, H, W, and Y
Hydrophobic residues	A, C, F, G, H, I, L, M, R, T, V, W, and Y
Negatively charged residues	D and E
Polar residues	C, D, E, H, K, N, Q, R, S, and T
Positively charged residues	H, K, and R
Small residues	A, C, D, G, N, P, S, T, and V
Very small residues	A, G, and S
Residues involved in turn formation	A, C, D, E, G, H, K, N, Q, R, S, P, and T
Flexible residues	Q, T, K, S, G, P, D, E, and R

5　　　More conservative substitutions groupings include: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, and asparagine-glutamine.

　　　Additional groups of amino acids may also be formulated using the principles

10　　described in, *e.g.*, Creighton (1984) Proteins: Structure and Molecular Properties (2d Ed. 1993), W. H. Freeman and Company.

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In one embodiment of the present invention, conservation in terms of hydropathic/hydrophilic properties and residue weight/size also is substantially retained in a variant CDR as compared to a CDR of an antibody of the examples (e.g., the weight class, hydropathic score, or both of the sequences are at least

5   about 80%, at least about 85%, at least about 90%, at least about 95%, or more retained). For example, conservative residue substitutions may also or alternatively be based on the replacement of strong or weak based weight based conservation groups, which are known in the art.

10   The retention of similar residues may also or alternatively be measured by a similarity score, as determined by use of a BLAST program (e.g., BLAST 2.2.8 available through the NCBI using standard settings BLOSUM62, Open Gap= I I and Extended Gap= I). Suitable variants typically exhibit at least about 80%, at least about 90%, at least about 95%, or more similarity to the parent peptide.

15
       As used herein, "isotype" refers to the immunoglobulin class (for instance IgGl, IgG2, IgG3, IgG4, IgD, IgA, IgE, or IgM) that is encoded by heavy chain constant region genes.

20   The term "epitope" means an antigenic determinant capable of specific binding to an antibody. Epitopes usually consist of surface groupings of molecules such as amino acids or sugar side chains and usually have specific three dimensional structural characteristics, as well as specific charge characteristics. Conformational and nonconformational epitopes are distinguished in that the

25   binding to the former, but not the latter, is lost in the presence of denaturing solvents. The epitope may comprise amino acid residues directly involved in the binding (also called immunodominant component of the epitope) and other amino acid residues, which are not directly involved in the binding, such as amino acid residues which are effectively blocked by the specifically antigen binding peptide

30   (in other words, the amino acid residue is within the footprint of the specifically antigen binding peptide).

       As used herein, a humanized antibody is "derived from" a particular germline sequence if the antibody is obtained from a system using human immunoglobulin

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sequences, for instance by immunizing a transgenic mouse carrying human immunoglobulin genes or by screening a human immunoglobulin gene library, and wherein the selected human antibody V domain sequence is at least 90%, such as at least 95%, for instance at least 96%, such as at least 97%, for

5   instance at least 98%, or such as at least 99% identical in amino acid V domain sequence to the amino acid sequence encoded by the germline immunoglobulin gene.

Typically, outside the heavy chain, a human antibody derived from a particular

10   human germline sequence will display no more than 20 amino acid differences, e.g. no more than 10 amino acid differences, such as no more than 9, 8, 7, 6 or 5, for instance no more than 4, 3, 2, or 1 amino acid difference from the amino acid sequence encoded by the germline immunoglobulin gene.

15   The term "transgenic non-human animal" refers to a non-human animal having a genome comprising one or more human heavy and/or light chain transgenes or transchromosomes (either integrated or non-integrated into the animal's natural genomic DNA) and which is capable of expressing fully human antibodies. For example, a transgenic mouse can have a human light chain transgene and either

20   a human heavy chain transgene or human heavy chain transchromosome, such that the mouse produces human anti-AβpE3 antibody when immunized with AβpE3 antigen and/or cells expressing AβpE3. The human heavy chain transgene may be integrated into the chromosomal DNA of the mouse, as is the case for transgenic mice, for instance HuMAb mice, such as HCo7 or HCo12

25   mice, or the human heavy chain transgene may be maintained extrachromosomally, as is the case for transchromosomal KM mice as described in WO02/43478. Such transgenic and transchromosomal mice (collectively referred to herein as "transgenic mice") are capable of producing multiple isotypes of human monoclonal antibodies to a given antigen (such as IgG, IgA,

30   IgM, IgD and/or IgE) by undergoing V-D-J recombination and isotype switching.

Transgenic, nonhuman animal can also be used for production of antibodies against a specific antigen by introducing genes encoding such specific antibody,

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for example by operatively linking the genes to a gene which is expressed in the milk of the animal.

The term "treatment" or "treating" as used herein means ameliorating, slowing or reversing the progress or severity of a disease or disorder, or ameliorating, slowing or reversing one or more symptoms or side effects of such disease or disorder. For purposes of this invention, "treatment" or "treating" further means an approach for obtaining beneficial or desired clinical results, where "beneficial or desired clinical results" include, without limitation, alleviation of a symptom, diminishment of the extent of a disorder or disease, stabilized (i.e., not worsening) disease or disorder state, delay or slowing of the progression a disease or disorder state, amelioration or palliation of a disease or disorder state, and remission of a disease or disorder, whether partial or total, detectable or undetectable.

An "effective amount", when applied to an antibody of the invention, refers to an amount sufficient, at dosages and for periods of time necessary, to achieve an intended biological effect or a desired therapeutic result including, without limitation, clinical results. The phrase "therapeutically effective amount" when applied to an antibody of the invention is intended to denote an amount of the antibody that is sufficient to ameliorate, palliate, stabilize, reverse, slow or delay the progression of a disorder or disease state, or of a symptom of the disorder or disease. In an embodiment, the method of the present invention provides for administration of the antibody in combinations with other compounds. In such instances, the "effective amount" is the amount of the combination sufficient to cause the intended biological effect.

A therapeutically effective amount of an anti-AβpE3antibody may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the anti-AβpE3antibody to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the antibody or antibody portion are outweighed by the therapeutically beneficial effects.

Further aspects of the invention

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The present invention encompasses the production of novel monoclonal antibodies with specificities for human AβpE3, particularly human AβpE3-11 peptide, relative to murine AβpE3-11 peptide. The invention provides a method for producing anti-AβpE3 monoclonal antibodies that specifically bind AβpE3 peptides, particularly human AβpE3-11, with a greater affinity than said monoclonal antibodies bind murine AβpE3-11, said method comprising: (a) immunizing one or more mice with purified human AβpE3-11-C peptide; (b) producing hybridoma cell lines from spleen cells of said one or more mice; (c) screening said hybridoma cell lines for one or more hybridoma cell lines that produce antibodies that specifically bind human AβpE3-11, with a greater affinity than said monoclonal antibodies bind murine AβpE3-11.

Comparison between antibodies specific for human and murine AβpE3, particularly the AβpE3-11 peptide, revealed substantial conservation among several of the amino acids in the CDR regions as well as positions that allows different types of amino acid replacements.

Clone	Specific for	V_H CDR1	V_H CDR2	V_H CDR3
5C9	Human AβpE3-11	GYTFTDYYLN	WIYPGSGNVKYNEKFKG	EGLIVY
2E8	Human AβpE3-11	GYTFTDYYIN	WLNPGSGNTKYNEKFKG	EGIPDY
Difference		GYTFTDYYXN	WXXPGSGNXKYNEKFKG	EGXXXY

Clone	Specific for	V_L CDR1	V_L CDR2	V_L CDR3
5C9	Human AβpE3-11	KSSQSLLHSNGESYLN	AVSKLDS	VQGTHFPFT
2E8	Human AβpE3-11	KSSQSLLYSNGKTYLN	VVSKLDS	VQGTHYPFT
Difference		KSSQSLLXSNGXXYLN	XVSKLDS	VQGTHXPFT

Clone	Specific for	V_L CDR1	V_L CDR2	V_L CDR3
1G11	Murine AβpE3-11	GLTLSDAWMN	EIRSKAYKHATYYAESVKG	HGS
2E4	Murine AβpE3-11	GITLNDAWMT	EIRNKANNHATNYAESVKG	HSY
Difference		GXTLXDAWMX	EIRXKAXXHATXYAESVKG	HXX

Clone	Specific for	V_L CDR1	V_L CDR2	V_L CDR3
1G11	Murine AβpE3-11	RASQGISSKMG	HGTKLED	VQYAQFPYT
2E4	Murine AβpE3-11	HASQGIRNNIG	HGTKLED	VQYDQFPYT
Difference		XASQGIXXXG	HGTKLED	VQYXQFPYT

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These conserved amino acids which are to be found between the antibodies generated against AβpE3 must account for particular amino acids that are important for recognising the AβpE3.

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The invention thus relates to an antibody, in particular an isolated antibody, or a fragment thereof, specific for human AβpE3 which antibody comprises, or consists, of:

15 a heavy chain variable region (V_H) CDR1 comprising GYTFTDYYX$_1$N
a heavy chain variable region (V_H) CDR2 comprising
WX$_2$X$_3$PGSGNX$_4$KYNEKFKG
a heavy chain variable region (V_H) CDR3 comprising EGX$_5$X$_6$X$_7$Y
a light chain variable region (V_L) CDR1 comprising KSSQSLLX$_8$SNGX$_9$X$_{10}$YLN

a light chain variable region (V_L) CDR2 comprising X_{11}VSKLDS, and

a light chain variable region (V_L) CDR3 comprising VQGTHX_{12}PFT

wherein X_1 to X_{12} symbolizes a natural amino acid.

5   In a further embodiment X_1 to X_{12} may be selected as follows

X_1 is I or L,

X_2 is I or L, X_3 is Y or N and/or X_4 is V or T,

X_5 is I or L, X_6 is I or P and/or X_7 is V or D,

X_8 is H or Y, X_9 is E or K and/or X_{10} is T or S,

10   X_{11} is A or V, and/or

X_{12} is Y or F.

The invention thus also relates to an antibody, in particular an isolated antibody,

or a fragment thereof, specific for murine AβpE3 which antibody comprises, or

15   consists, of:

a heavy chain variable region (V_H) CDR1 comprising GX_1TLX_2DAWMX_3

a heavy chain variable region (V_H) CDR2 comprising

EIRX_4KA$X_5$$X_6HATX_7$YAESVKG

20   a heavy chain variable region (V_H) CDR3 comprising H$X_8$$X_9$

a light chain variable region (V_L) CDR1 comprising X_{10}ASQGI$X_{11}$$X_{12}$$X_{13}$$X_{14}$G

a light chain variable region (V_L) CDR2 comprising HGTKLED, and

a light chain variable region (V_L) CDR3 comprising VQYX_{15}QFPYT

wherein X_1 to X_{15} symbolizes a natural amino acid.

25

In a further embodiment X_1 to X_{15} may be selected as follows

X_1 is I or L, X_2 is S or N, and/or X_3 is N or T,

X_4 is S or N, X_5 is Y or N, X_6 is K or N, and/or X_7 is Y or N,

X_8 is G or S and/or S X_9 is S or Y,

30   X_{10} is R or H, X_{11} is S or R, X_{12} is S or N, X_{13} is K or N, and/or X_{14} is M or I,

and/or

X_{15} is A or D.

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The antibody may be isolated and preferably be specific for human AβpE3 with a binding affinity (K_D) of about 100 nM to about 50 nM, or about 50 nM to about 10 nM, or about 10 nM to about 5 nM for the human pyroglutamated Aβ fragment pEFRHDSGYE or the murine fragment pEFGHDSGFE.

The invention further relates to an in vitro method for making an antibody, or a fragment thereof, specific for human pyroglutamated Aβ fragment pEFRHDSGYE or the murine fragment pEFGHDSGFE, which method comprises the step varying one or more of the amino acids X. X may be selected as outlined above. Any suitable method for constructing these fragments can be used, such as PCR, and sequencing followed by a screening and selection for antibodies with the above mentioned desired properties with regards to K_D and affinity for AβpE3-11 as shown in the examples.

The present invention provides the antibodies of the invention produced by a host cell. In one embodiment, the invention provides a monoclonal antibody produced by clone 5C9 or 2E8. In another embodiment, the invention provides a mono-clonal antibody produced by clone 2E4 or 1G11. In other embodiments, the invention provides an isolated antibody, or fragment thereof, that competes with binding with the monoclonal antibody produced by clone 5C9, 2E8, 2E4 or 1G11. Furthermore, the invention provides hybridoma cell line 5C9, 2E8, 2E4 or 1G11, or progeny thereof.

Accordingly the invention relates to an antibody, or a fragment thereof, compris-ing of:

        a heavy chain variable region (V_H) CDR1 comprising SEQ ID NO: 12;
        a heavy chain variable region (V_H) CDR2 comprising SEQ ID NO: 13;
        a heavy chain variable region (V_H) CDR3 comprising SEQ ID NO: 14;
        a light chain variable region (V_L) CDR1 comprising SEQ ID NO: 15;
        a light chain variable region (V_L) CDR2 comprising SEQ ID NO: 16; and
        a light chain variable region (V_L) CDR3 comprising SEQ ID NO: 17.

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The antibody, or fragment thereof, may also comprising or consisting of a heavy chain variable region (V$_H$) having the nucleotide sequence of SEQ NO: 4 or having the amino acid sequence encoded by SEQ ID NO:5. The antibody, or fragment thereof, may further comprise or consist of a light chain

5   variable region (V$_L$) having the nucleotide sequence of SEQ NO: 6 or having the amino acid sequence encoded by SEQ ID NO:7. Alternatively, the antibody, or fragment thereof, comprising or consisting of the heavy chain variable region (V$_H$) and the light chain variable region (V$_L$).

10   In another embodiment the antibody, or a fragment thereof, comprises or consist of:

a heavy chain variable region (V$_H$) CDR1 comprising SEQ ID NO: 18;
a heavy chain variable region (V$_H$) CDR2 comprising SEQ ID NO: 19;
15   a heavy chain variable region (V$_H$) CDR3 comprising SEQ ID NO: 20;
a light chain variable region (V$_L$) CDR1 comprising SEQ ID NO: 21;
a light chain variable region (V$_L$) CDR2 comprising SEQ ID NO: 22; and
a light chain variable region (V$_L$) CDR3 comprising SEQ ID NO: 23.

20   The antibody, or a fragment thereof, may comprise or consist of a heavy chain variable region (V$_H$) having the nucleotide sequence of SEQ NO: 8 or having the amino acid sequence encoded by SEQ ID NO:9. The antibody, or fragment thereof, may further comprise or consist of a light chain variable region (V$_L$) having the nucleotide sequence of SEQ NO: 10 or having the amino acid sequence

25   encoded by SEQ ID NO:11. Alternatively, the antibody, or fragment thereof, comprising or consisting of the heavy chain variable region (V$_H$) and the light chain variable region (V$_L$).

In a further embodiment the antibody, or a fragment thereof, may comprise or
30   consist of:

a heavy chain variable region (V$_H$) CDR1 comprising SEQ ID NO: 32;
a heavy chain variable region (V$_H$) CDR2 comprising SEQ ID NO: 33;
a heavy chain variable region (V$_H$) CDR3 comprising SEQ ID NO: 34;

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a light chain variable region (V_L) CDR1 comprising SEQ ID NO: 35;

a light chain variable region (V_L) CDR2 comprising SEQ ID NO: 36; and

a light chain variable region (V_L) CDR3 comprising SEQ ID NO: 37.

5   The antibody, or a fragment thereof, may comprise consist of a heavy chain
       variable region (V_H) having the nucleotide sequence of SEQ NO: 24 or having
       the amino acid sequence encoded by SEQ ID NO:25. The antibody, or fragment
       thereof, may further comprise or consist of a light chain variable region (V_L) hav-
       ing the nucleotide sequence of SEQ NO: 26 or having the amino acid sequence
10   encoded by SEQ ID NO:27. Alternatively, the antibody, or fragment thereof,
       comprising or consisting of the heavy chain variable region (V_H) and the light
       chain variable region (V_L).

       In a still further embodiment the invention relates to an antibody, or a fragment
15   thereof, comprising or consisting of:

              a heavy chain variable region (V_H) CDR1 comprising SEQ ID NO: 38;

              a heavy chain variable region (V_H) CDR2 comprising SEQ ID NO: 39;

              a heavy chain variable region (V_H) CDR3 comprising SEQ ID NO: 40;

20   a light chain variable region (V_L) CDR1 comprising SEQ ID NO: 41;

              a light chain variable region (V_L) CDR2 comprising SEQ ID NO: 42; and

              a light chain variable region (V_L) CDR3 comprising SEQ ID NO: 43.

       The antibody, or a fragment thereof, may comprise consist of a heavy chain
25   variable region (V_H) having the nucleotide sequence of SEQ NO: 28 or having
       the amino acid sequence encoded by SEQ ID NO:29. The antibody, or fragment
       thereof, may further comprise or consist of a light chain variable region (V_L) hav-
       ing the nucleotide sequence off SEQ NO: 30 or having the amino acid se-
       quence encoded by SEQ ID NO:31. Alternatively, the antibody, or fragment
30   thereof, comprising or consisting of the heavy chain variable region (V_H) and the
       light chain variable region (V_L).

       The antibodies mentioned above may, according to one embodiment, further
       have no more than 4 amino acid differences, or no more than 3 amino acid

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differences, or no more than 2 amino acid differences, or no more than 1 amino acid difference from said CDR1-3 (V_H and/or V_L) sequences.

It is envisaged that the antibody, or fragment thereof, may be humanized, chi-
5   meric, or single-chain antibody.

Further the antibodies may be in a composition together with a pharmaceutically acceptable carrier, e.g. for use in the treatment of Alzheimer disease.

10   The invention also relates to a method of treating Alzheimer's Disease in a pa-
tient, comprising administering to the patient in need of such treatment, a thera-
peutically effective amount of an antibody of the invention or a composition as mentioned above

15   The antibodies may further be used in a diagnostic method or as a diagnostic imagining ligand.

The fragments, or immunogenic fragments, consisting of the AβpE3-11
(pEFRHDSGYE) fragment and/or AβpE3-11-C (pE-FRHDSGYEC) fragment may
20   be used for generating antibodies having a binding affinity (K_D) to such fragment
of with a binding affinity (K_D) of about 100 nM to about 50 nM, or about 50 nM to about 10 nM, or about 10 nM to about 5 nM.

The invention further relates to a therapeutic composition consisting or compris-
25   ing of an AβpE3-11 (pEFRHDSGYE) fragment and/or the AβpE3-11-C (pE-
FRHDSGYEC) fragment in an amount sufficient to elicit the production of anti-
bodies, or an antibody that specifically binds to such fragment, and a pharmaceu-
tically acceptable adjuvant or carrier.

30   The therapeutic composition may comprise one or more antibodies that binds to
AβpE3-11 (pEFRHDSGYE) fragment and/or AβpE3-11-C (pE-FRHDSGYEC)
fragment with a binding affinity (K_D) to such fragment of about with a binding af-
finity (K_D) of about 100 nM to about 50 nM, or about 50 nM to about 10 nM, or about 10 nM to about 5 nM.

The present invention provides the peptide AβpE3-11-C (pEFRHDSGYEC). In one embodiment, the present invention provides the peptide AβpE3-11-C dimer (pEFRHDSGYEC-CEYGSDHRFEp). In another embodiment, the present invention provides polypeptide AβpE3-11-C-keyhole limpet hemocyanin: pEFRHDSGYEC-[KLH]. The peptide AβpE3-11-C (pEFRHDSGYEC) conjugated to a carboxy-terminal carrier.

The present invention provides a method of immunizing an animal comprising administering AβpE3-11-C (pEFRHDSGYEC) as an immunogen.

The present invention provides a therapeutic composition comprising the AβpE3-11 (pEFRHDSGYE) and/or the AβpE3-11-C (pEFRHDSGYEC) fragment in an amount sufficient to elicit the production of antibodies, or an antibody that immunospecifically binds to such fragment.

The invention also relates to an in vitro method for making an antibody of, or a fragment thereof, specific for human pyroglutamated Aβ fragment pEFRHDSGYE and/or pE-FRHDSGYEC or specific for murine pyroglutamated Aβ fragment pEFGHDSGFE and/or pEFGHDSGFEC, which method comprises the step varying one or more of the amino acids "X" given above. In particular the in vitro method the in vitro method may be suitable for making antibodies which binds with an affinity (KD) to said fragment of about 100 nM to about 50 nM, or about 50 nM to about 10 nM, or about 10 nM to about 5 nM.

The present invention relates to improvements in therapy and prevention of Alzheimer's disease (AD) and other diseases characterized by deposition of amyloid, e. g. characterized by amyloid deposits in the central nervous system (CNS).

The present invention also provides a method of reducing Aβ plaque formation in a patient, comprising administering to the patient in need of such treatment, a therapeutically effective amount of an antibody of the invention. In one embodiment, the Aβ plaques comprise pyroglutamated Aβ.

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In one embodiment, the present invention provides a composition comprising the antibody described herein and a pharmaceutically acceptable carrier. In some embodiments, the composition of the invention is provided for use in the treatment of Alzheimer disease, or in the treatment of a neurodegenerative or cognitive disease or disorder.

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The present invention also provides a method of treating Alzheimer's Disease in a patient, comprising administering to the patient in need of such treatment, a therapeutically effective amount of an antibody of the invention. The present invention also provides a method of treating neurodegenerative or cognitive disease or disorder in a patient, comprising administering to the patient in need of such treatment, a therapeutically effective amount of an antibody of the invention.

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There are important parallels between AD and other neurological diseases, including prion diseases (such as kuru, Creutzfeld-Jacob disease and bovine spongiform encephalitis), Parkinson's disease, Huntington's disease, and frontotemporal dementia. All involve deposits of abnormal proteins in the brain. AD and prion diseases cause dementia and death, and both are associated with the formation of insoluble amyloid fibrils, but from membrane proteins that are different from each other.

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Monoclonal antibodies of the present invention may *e.g.* be produced by the hybridoma method first described by Kohler et al., *Nature* 256, 495 (1975), or may be produced by recombinant DNA methods. Monoclonal antibodies may also be isolated from phage antibody libraries using the techniques described in, for example, Clackson et al., *Nature* 352, 624-628 (1991) and Marks et al., J. Mol. Biol. 222, 581-597 (1991). Monoclonal antibodies may be obtained from any suitable source. Thus, for example, monoclonal antibodies may be obtained from hybridomas prepared from murine splenic B lymphocyte cells obtained from mice immunized with an antigen of interest, for instance, in the form of cells expressing the antigen on the surface, or a nucleic acid encoding an antigen of interest. Monoclonal antibodies may also be obtained from hybridomas derived from antibody- expressing cells of immunized humans or non-human mammals such as rats, rabbits, dogs, primates, etc.

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In one embodiment, the antibody of the invention is a human antibody. Human monoclonal antibodies directed against Aβ may be generated using transgenic or transchromosomal mice carrying parts of the human immune system rather than

5   the mouse system. Such transgenic and transchromosomic mice include mice referred to herein as HuMAb mice and KM mice, respectively, and are collectively referred to herein as "transgenic mice".

The HuMAb mouse contains a human immunoglobulin gene miniloci that

10   encodes unrearranged human heavy variable and constant (μ and Y) and light variable and constant (K) chain immunoglobulin sequences, together with targeted mutations that inactivate the endogenous μ and K chain loci (Lonberg, N. et al., *Nature* 368, 856-859 (1994)). Accordingly, the mice exhibit reduced expression of mouse IgM or K and in response to immunization, the introduced

15   human heavy and light chain transgenes, undergo class switching and somatic mutation to generate high affinity human IgG, κ monoclonal antibodies (Lonberg, N. et al. (1994), supra; reviewed in Lonberg, N., Handbook of Experimental Pharmacology 113, 49-101 (1994) , Lonberg, N. and Huszar, D., *Intern. Rev. Immunol*. Vol. 13 65-93 (1995) and Harding, F. and Lonberg, N., *Ann. N. Y. Acad.*

20   *Sci* 764 536-546 (1995)). The preparation of HuMAb mice is described in detail in Taylor, L. et al., *Nucleic Acids Research* 20, 6287-6295 (1992), Chen, J. et al., *International Immunology* 5, 647-656 (1993), Tuaillon et al., *J. Immunol.* 152, 2912-2920 (1994), Taylor, L. et al., *International Immunology* 6, 579-591 (1994), Fishwild, D. et al., *Nature Biotechnology* 14, 845-851 (1996). See also US

25   5,545,806, US 5,569,825, US 5,625,126, US 5,633,425, US 5,789,650, US 5,877,397, US 5,661,016, US 5,814,318, US 5,874,299, US 5,770,429, US 5,545,807, WO 98/24884, WO 94/25585, WO 93/1227, WO 92/22645, WO 92/03918 and WO 01/09187.

30   The HCo7 mice have a JKD disruption in their endogenous light chain (kappa) genes (as described in Chen et al., *EMBO J.* 12, 821-830 (1993)), a CMD disruption in their endogenous heavy chain genes (as described in Example 1 of WO 01/14424), a KCo5 human kappa light chain transgene (as described in

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Fishwild et al., *Nature Biotechnology* 14, 845-851 (1996)), and a HCo7 human heavy chain transgene (as described in US 5,770,429).

The HCol2 mice have a JKD disruption in their endogenous light chain (kappa) genes (as described in Chen et al., *EMBO* J. 12, 821-830 (1993)), a CMD

5   disruption in their endogenous heavy chain genes (as described in Example 1 of WO 01/14424), a KCo5 human kappa light chain transgene (as described in Fishwild et al., *Nature Biotechnology* 14, 845-851 (1996)), and a HCol2 human heavy chain transgene (as described in Example 2 of WO 01/14424).

10   In the KM mouse strain, the endogenous mouse kappa light chain gene has been homozygously disrupted as described in Chen et al., *EMBO* J. 12, 811-820 (1993) and the endogenous mouse heavy chain gene has been homozygously disrupted as described in Example 1 of WO 01/09187. This mouse strain carries a human kappa light chain transgene, KCo5, as described in Fishwild et al.,

15   *Nature Biotechnology* 14, 845-851 (1996). This mouse strain also carries a human heavy chain transchromosome composed of chromosome 14 fragment hCF (SC20) as described in WO 02/43478.

Splenocytes from these transgenic mice may be used to generate hybridomas

20   that secrete human monoclonal antibodies according to well known techniques. Human monoclonal or polyclonal antibodies of the present invention, or antibodies of the present invention originating from other species may also be generated transgenically through the generation of another non-human mammal or plant that is transgenic for the immunoglobulin heavy and light chain

25   sequences of interest and production of the antibody in a recoverable form therefrom. In connection with the transgenic production in mammals, antibodies may be produced in, and recovered from, the milk of goats, cows, or other mammals. See for instance US 5,827,690, US 5,756,687, US 5,750,172 and US 5,741,957.

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Further, human antibodies of the present invention or antibodies of the present invention from other species may be generated through display-type technologies, including, without limitation, phage display, retroviral display, ribosomal display, and other techniques, using techniques well known in the art

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and the resulting molecules may be subjected to additional maturation, such as affinity maturation, as such techniques are well known in the art (see for instance Hoogenboom et al., *J. Mol. Biol.* 227, 381 (1991) (phage display), Vaughan et al., *Nature Biotech* 14, 309 (1996) (phage display), Hanes and Plucthau, *PNAS USA*

5   94, 4937-4942 (1997) (ribosomal display), Parmley and Smith, *Gene* 73, 305-318 (1988) (phage display), Scott *TIBS* 17, 241-245 (1992), Cwirla et al., *PNAS USA* 87, 6378-6382 (1990), Russel et al., *Nucl. Acids Research* 21, 1081-1085 (1993), Hogenboom et al., *Immunol. Reviews* 130, 43-68 (1992), Chiswell and McCafferty *TIBTECH* 10, 80-84 (1992), and US 5,733,743). If display

10   technologies are utilized to produce antibodies that are not human, such antibodies may be humanized.

The antibody of the invention may be of any isotype. The choice of isotype typically will be guided by the desired effector functions, such as ADCC induction.

15   Exemplary isotypes are IgGl, IgG2, IgG3, and IgG4. Either of the human light chain constant regions, kappa or lambda, may be used. If desired, the class of an anti-AβpE3antibody of the present invention may be switched by known methods. For example, an antibody of the present invention that was originally IgM may be class switched to an IgG antibody of the present invention. Further, class

20   switching techniques may be used to convert one IgG subclass to another, for instance from IgGl to IgG2. Thus, the effector function of the antibodies of the present invention may be changed by isotype switching to, e.g., an IgG1, IgG2, IgG3, IgG4, IgD, IgA, IgE, or IgM antibody for various therapeutic uses. In one embodiment an antibody of the present invention is an IgG1 antibody, for

25   instance an IgG1, κ.

In one embodiment, the antibody of the invention is a full-length antibody, preferably an IgG antibody, in particular an IgG1, κ antibody. In another embodiment, the antibody of the invention is an antibody fragment or a single-

30   chain antibody.

Antibodies fragments may *e.g.* be obtained by fragmentation using conventional techniques, and the fragments screened for utility in the same manner as described herein for whole antibodies. For example, F(ab')$_2$ fragments may be

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generated by treating antibody with pepsin. The resulting F(ab')$_2$ fragment may
be treated to reduce disulfide bridges to produce Fab' fragments. Fab fragments
may be obtained by treating an IgG antibody with papain; Fab' fragments may be
obtained with pepsin digestion of IgG antibody. An F(ab') fragment may also be

5   produced by binding Fab' described below via a thioether bond or a disulfide
bond. A Fab' fragment is an antibody fragment obtained by cutting a disulfide
bond of the hinge region of the F(ab')$_2$. A Fab' fragment may be obtained by
treating an F(ab')$_2$ fragment with a reducing agent, such as dithiothreitol. Antibody
fragment may also be generated by expression of nucleic acids encoding such

10   fragments in recombinant cells (see for instance Evans et al., *J. Immunol. Meth.*
184, 123-38 (1995)). For example, a chimeric gene encoding a portion of an
F(ab')$_2$ fragment could include DNA sequences encoding the C_H1 domain and
hinge region of the H chain, followed by a translational stop codon to yield such a
truncated antibody fragment molecule.

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In one embodiment, anti-AβpE3 antibody is a monovalent antibody, preferably a
monovalent antibody as described in WO2007059782 (which is incorporated
herein by reference in its entirety) having a deletion of the hinge region.
Accordingly, in one embodiment, the antibody is a monovalent antibody, wherein

20   said anti-pE3Aβ antibody is constructed by a method comprising : i) providing a
nucleic acid construct encoding the light chain of said monovalent antibody, said
construct comprising a nucleotide sequence encoding the V_L region of a selected
antigen specific anti-AβpE3 antibody and a nucleotide sequence encoding the
constant C_L region of an Ig, wherein said nucleotide sequence encoding the V_L

25   region of a selected antigen specific antibody and said nucleotide sequence
encoding the C_L region of an Ig are operably linked together, and wherein, in
case of an IgG1 subtype, the nucleotide sequence encoding the C_L region has
been modified such that the C_L region does not contain any amino acids capable
of forming disulfide bonds or covalent bonds with other peptides comprising an

30   identical amino acid sequence of the C_L region in the presence of polyclonal
human IgG or when administered to an animal or human being; ii) providing a
nucleic acid construct encoding the heavy chain of said monovalent antibody,
said construct comprising a nucleotide sequence encoding the V_H region of a
selected antigen specific antibody and a nucleotide sequence encoding a

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constant C_H region of a human Ig, wherein the nucleotide sequence encoding the C_H region has been modified such that the region corresponding to the hinge region and, as required by the Ig subtype, other regions of the C_H region, such as the C_H3 region, does not comprise any amino acid residues which participate in

5   the formation of disulphide bonds or covalent or stable non- covalent inter-heavy chain bonds with other peptides comprising an identical amino acid sequence of the C_H region of the human Ig in the presence of polyclonal human IgG or when administered to an animal human being, wherein said nucleotide sequence encoding the V_H region of a selected antigen specific antibody and said

10   nucleotide sequence encoding the C_H region of said Ig are operably linked together; iii) providing a cell expression system for producing said monovalent antibody; iv) producing said monovalent antibody by co-expressing the nucleic acid constructs of (i) and (ii) in cells of the cell expression system of (iii).

15   Similarly, in one embodiment, the anti-AβpE3 antibody is a monovalent antibody, which comprises:
      (i) a variable region of an antibody of the invention as described herein or an antigen binding part of the said region, and
      (ii) a C_H region of an immunoglobulin or a fragment thereof comprising the C_H2

20   and C_H3 regions, wherein the C_H region or fragment thereof has been modified such that the region corresponding to the hinge region and, if the immunoglobulin is not an IgG4 subtype, other regions of the C_H region, such as the C_H3 region, do not comprise any amino acid residues, which are capable of forming disulfide bonds with an identical C_H region or other covalent or stable non-covalent inter-

25   heavy chain bonds with an identical C_H region in the presence of polyclonal human IgG.

      In a further embodiment, the heavy chain of the monovalent anti-AβpE3 antibody has been modified such that the entire hinge has been deleted.

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      In another further embodiment, the sequence of said monovalent antibody has been modified so that it does not comprise any acceptor sites for N-linked glycosylation.

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Anti-AβpE3 antibodies of the invention also include single chain antibodies. Single chain antibodies are peptides in which the heavy and light chain Fv regions are connected. In one embodiment, the present invention provides a single-chain Fv (scFv) wherein the heavy and light chains in the Fv of an anti-

5   AβpE3 antibody of the present invention are joined with a flexible peptide linker (typically of about 10, 12, 15 or more amino acid residues) in a single peptide chain. Methods of producing such antibodies are described in for instance US 4,946,778, Pluckthun in The Pharmacology of Monoclonal Antibodies, vol. 113, Rosenburg and Moore eds. Springer-Verlag, New York, pp. 269-315 (1994), Bird

10   et al., *Science* 242, 423-426 (1988), Huston et al., *PNAS USA* 85, 5879-5883 (1988) and McCafferty et al., *Nature* 348, 552-554 (1990). The single chain antibody may be monovalent, if only a single V_H and V_L are used, bivalent, if two V_H and V_L are used, or polyvalent, if more than two V_H and V_L are used.

15   In general, anti-AβpE3 antibodies described herein may be modified by inclusion of any suitable number of such modified amino acids and/or associations with such conjugated substituents. Suitability in this context is generally determined by the ability to at least substantially retain AβpE3 selectivity and/or specificity associated with the non-derivatized parent anti-AβpE3 antibody. The inclusion of

20   one or more modified amino acids may be advantageous in, for example, increasing polypeptide serum half-life, reducing polypeptide antigenicity, or increasing polypeptide storage stability. Amino acid(s) are modified, for example, co-translationally or post-translationally during recombinant production (*e.g.*, N-linked glycosylation at N-X-S/T motifs during expression in mammalian cells) or

25   modified by synthetic means. Non-limiting examples of a modified amino acid include a glycosylated amino acid, a sulfated amino acid, a prenylated (e. g., farnesylated, geranylgeranylated) amino acid, an acetylated amino acid, an acylated amino acid, a PEGylated amino acid, a biotinylated amino acid, a carboxylated amino acid, a phosphorylated amino acid, and the like. References

30   adequate to guide one of skill in the modification of amino acids are replete throughout the literature. Example protocols are found in Walker (1998) Protein Protocols On CD-Rom, Humana Press, Totowa, NJ. The modified amino acid may, for instance, be selected from a glycosylated amino acid, a PEGylated amino acid, a farnesylated amino acid, an acetylated amino acid, a biotinylated

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amino acid, an amino acid conjugated to a lipid moiety, or an amino acid conjugated to an organic derivatizing agent.

Anti-AβpE3 antibodies may also be chemically modified by covalent conjugation
5   to a polymer to for instance increase their circulating half-life. Exemplary polymers, and methods to attach them to peptides, are illustrated in for instance US 4,766,106, US 4,179,337, US 4,495,285 and US 4,609,546. Additional illustrative polymers include polyoxyethylated polyols and polyethylene glycol (PEG) (e.g., a PEG with a molecular weight of between about 1,000 and about
10   40,000, such as between about 2,000 and about 20,000, e.g., about 3,000-12,000 g/mol).

In one embodiment, anti-AβpE3 antibodies comprising one or more radiolabeled amino acids are provided. A radiolabeled anti-AβpE3 antibody may be used for
15   both diagnostic and therapeutic purposes (conjugation to radiolabeled molecules is another possible feature). Non-limiting examples of labels for polypeptides include, but are not limited to 3H, 14C, 15N, 35S, 90Y, 99Tc, and 125I, 131I, and 186Re. Methods for preparing radiolabeled amino acids and related peptide derivatives are known in the art (see for instance Junghans et al., in Cancer
20   Chemotherapy and Biotherapy 655-686 (2nd edition, Chafner and Longo, eds., Lippincott Raven (1996)) and US 4,681,581, US 4,735,210, US 5,101,827, US 5,102,990 (US RE35,500), US 5,648,471 and US 5,697,902. For example, a radioisotope may be conjugated by a chloramine T method.

25   In a further aspect, the invention relates to an expression vector encoding an antibody of the invention. Such expression vectors may be used for recombinant production of antibodies of the invention.

An expression vector in the context of the present invention may be any suitable
30   vector, including chromosomal, non-chromosomal, and synthetic nucleic acid vectors (a nucleic acid sequence comprising a suitable set of expression control elements). Examples of such vectors include derivatives of SV40, bacterial plasmids, phage DNA, baculovirus, yeast plasmids, vectors derived from combinations of plasmids and phage DNA, and viral nucleic acid (RNA or DNA)

vectors. In one embodiment, an anti-AβpE3 antibody-encoding nucleic acid is comprised in a naked DNA or RNA vector, including, for example, a linear expression element (as described in, for instance, Sykes and Johnston, *Nat Biotech* 12, 355-59 (1997)), a compacted nucleic acid vector (as described in for

5   instance US 6,077,835 and/or WO 00/70087), a plasmid vector such as pBR322, pUC 19/18, or pUC 118/119, a "midge" minimally-sized nucleic acid vector (as described in, for instance, Schakowski et al., *Mol Ther* 3, 793-800 (2001)), or as a precipitated nucleic acid vector construct, such as a CaPO$_4$-precipitated construct (as described in, for instance, WO 00/46147, Benvenisty and Reshef,

10   *PNAS USA* 83, 9551-55 (1986), Wigler et al., *Cell* 14, 725 (1978), and Coraro and Pearson, *Somatic Cell Genetics* 2, 603 (1981)). Such nucleic acid vectors and the usage thereof are well known in the art (see for instance US 5,589,466 and US 5,973,972).

15   In one embodiment, the vector is suitable for expression of anti-AβpE3 antibody in a bacterial cell. Examples of such vectors include expression vectors such as BlueScript (Stratagene), pIN vectors (Van Heeke & Schuster, *J Biol Chem* 264, 5503-5509 (1989), pET vectors (Novagen, Madison, WI) and the like).

20   An expression vector may also or alternatively be a vector suitable for expression in a yeast system. Any vector suitable for expression in a yeast system may be employed. Suitable vectors include, for example, vectors comprising constitutive or inducible promoters such as alpha factor, alcohol oxidase and PGH (reviewed in: F. Ausubel et al., ed. Current Protocols in Molecular Biology, Greene

25   Publishing and Wiley InterScience New York (1987), and Grant et al., *Methods in Enzymol* 153, 516-544 (1987)).

In an expression vector of the invention, anti-AβpE3 antibody-encoding nucleic acids may comprise or be associated with any suitable promoter, enhancer, and

30   other expression- facilitating elements. Examples of such elements include strong expression promoters (e. g., human CMV IE promoter/enhancer as well as RSV, SV40, SL3-3, MMTV, and HIV LTR promoters), effective poly (A) termination sequences, an origin of replication for plasmid product in E. coli, an antibiotic resistance gene as selectable marker, and/or a convenient cloning site (e.g., a

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polylinker). Nucleic acids may also comprise an inducible promoter as opposed to a constitutive promoter such as CMV IE (the skilled artisan will recognize that such terms are actually descriptors of a degree of gene expression under certain conditions).

5   In an even further aspect, the invention relates to a recombinant eukaryotic or prokaryotic host cell, such as a transfectoma, which produces an antibody of the invention as defined herein or a bispecific molecule of the invention as defined herein. Examples of host cells include yeast, bacterial, and mammalian cells, such as CHO or HEK cells. For example, in one embodiment, the present

10   invention provides a cell comprising a nucleic acid stably integrated into the cellular genome that comprises a sequence coding for expression of an anti-AβpE3 antibody of the present invention. In another embodiment, the present invention provides a cell comprising a non-integrated nucleic acid, such as a plasmid, cosmid, phagemid, or linear expression element, which comprises a

15   sequence coding for expression of an anti-AβpE3 antibody of the invention.

       In a further aspect, the invention relates to a method for producing an anti-AβpE3 antibody of the invention, said method comprising the steps of a) culturing a hybridoma or a host cell of the invention as described herein above, and b)

20   purifying the antibody of the invention from the culture media.

       In an even further aspect, the invention relates to a pharmaceutical composition comprising:
       - an anti-AβpE3 antibody as defined herein or a bispecific molecule as defined

25   herein, and
       - a pharmaceutically-acceptable carrier.

       The pharmaceutical compositions may be formulated with pharmaceutically acceptable carriers or diluents as well as any other known adjuvants and

30   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.

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The pharmaceutically acceptable carriers or diluents as well as any other known adjuvants and excipients should be suitable for the chosen compound of the present invention and the chosen mode of administration. Suitability for carriers and other components of pharmaceutical compositions is determined based on

5   the lack of significant negative impact on the desired biological properties of the chosen compound 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.

10   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.

15

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

20   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 compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in

25   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

30   mode. Suitable routes of administering a compound 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.

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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,

5   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

10   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

15   physiologically compatible with a compound 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,

20   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.

25

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

30   conventional media or agent is incompatible with the active compound, use thereof in the pharmaceutical compositions of the present invention is contemplated.

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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.

5   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

10   (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

15   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

20   preservatives, wetting agents, emulsifying agents, dispersing agents, preservatives or buffers, which may enhance the shelf life or effectiveness of the pharmaceutical composition. The compounds of the present invention may be prepared with carriers that will protect the compound against rapid release, such as a controlled release formulation, including implants, transdermal patches, and

25   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

30   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.

35

In one embodiment, the compounds 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

5   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 compound, use thereof in the pharmaceutical compositions of the present invention is contemplated. Supplementary active compounds may also be incorporated into the

10   compositions.

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

15   suitable to high drug concentration. The carrier may be a aqueous or nonaqueous 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,

20   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

25   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 active compound 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

30   are prepared by incorporating the active compound 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

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ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Sterile injectable solutions may be prepared by incorporating the active
5   compound 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 active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the
10   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.

15   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
20   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 active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier.
25   The specification for the dosage unit forms of the present invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.
30

The effective dosages and the dosage regimens for the anti-AβpE3 antibodies 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

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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.

5

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 anti-AβpE3 antibody employed in the pharmaceutical composition at levels lower than that

10   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 compound 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

15   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

20   compound of the present invention to be administered alone, it is preferable to administer the compound as a pharmaceutical composition as described above.

Labeled antibodies of the invention can be used for diagnositc purposes to detect, diagnose, or monitor diseases or disorders. The invention provides for the

25   detection or diagnosis of a neurodegenerative or cognitive disease or disorder, including but not limited to Alzheimer's Disease, comprising: (a) assaying the existence of pyroglutamated Aβ fragments in cells or tissue samples of a subject using one or more antibodies that immunospecifically bind to AβpE3; and (b) comparing the level of the antigen with a control level, e.g. levels in normal tissue

30   samples, whereby an increase in the assayed level of antigen compared to the control level of antigen is indicative of the disease or disorder, or indicative of the severity of the disease or disorder.

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Antibodies of the invention can be use to assay pyroglutamated Aβ fragments in a biological sample using immunohistochemical methods well-known in the art. Other antibody-based methods useful for detecting protein include immunoassays sucha s the enzyme linked immunoassay (ELISA) and the

5   radioimmunoassay (RIA). Suitable antibody labels may be used in such kits and methods, and labels known in the art include enzyme labels, such as alkaline phophatase and glucose oxidase; radioisotope labels, such as iodine (^{125}I, ^{131}I), carbon (^{14}C), sulfur (^{35}S), tritium (^{3}H), indium (^{121}In), and technetium (^{99m}Tc); and luminescent labels, such as luminol and luciferase; and flourescent labels, such

10   as flourescein and rhodamine.

Presence of labeled antibodies may be detected *in vivo* for diagnosis purposes. In one embodiment, diagnosis comprises: a) administering to a subject an effective amount of a labeled antibody; b) waiting for a time interval following

15   administration for permitting labeled antibody to concentrate at sites where Aβ may be detected and to allow for unbound labeled antibody to be cleared to background level; c) determining a background level; and d) detecting the labeled antibody in the subject, such that detection of labeled antibody above the background level is indicative that the subject has the disease or disorder, or is

20   indicative of the severity of the disease or disorder. In accordance with such embodiment, the antibody is labeled with an imaging moiety suitable for detection using a particular imaging system known to those skilled in the art. Background levels may be determined by various methods known in the art, including comparing the amount of labeled antibody detected to a standard value

25   previously determined for a particular imaging system. Methods and systems that may be used in the diagnostic methods of the invention include, but are not limited to, computed tomography (CT), whole body scan such as positron emission tomography (PET), magnetic resonance imaging (MRI), and sonography.

30

EXAMPLES
Example 1: Immunization and screening.
Pyroglutamated human Aβ 3-11, with the addition of a C-terminal cysteine resi-
due, pE-FRHDSGYE-C and pyroglutamated murine Aβ 3-11, with the addition of

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a C-terminal cysteine residue, pE- FRHDSGYE-C was used in the immunisa-
tions. As such, mice were immunized with pE-FRHDSGYE-C or with pE-
FRHDSGYE-C each conjugated to KLH. The peptides were made and amplified
using PCR and purified as disclosed in Sambrook (2000) Molecular Cloning, third
5   edition, Cold Spring Harbor Laboratory Press.

The modified peptide was conjugated to a carrier protein, such as KLH. 10 Balb/c
mice were immunized and boosted with a standard immunization protocol as out-
lined below
10

Procedure	Scheduled days	Routes and dosages
1st Immunization	0	10-100ug/0.25ml animal, CFA
2nd Immunization	14	10-50ug/0.25ml animal, IFA
3rd Immunization	35	10-50ug/0.25ml animal, IFA
Final immuniza-tion	56	10-50ug/0.25ml animal, Saline,

Tail bleedings were screened by ELISA or other method if needed with pyroglu-
tamate and non- pyroglutamate -peptide.

15   Fusion and screening.
2 fusions (1-2 spleens per fusion) were performed on the best mice that re-
sponded the best to the pyroglutamate-peptide in Example 1.

The hybridoma cells were screened by ELISA or other method if needed with py-
20   roglutamate and non- pyroglutamate -petide and all positive clones were ex-
panded into 24-well plates. The clones with best ELISA value and specificity to
pyroglutamate -peptide were selected for subcloning.

Subcloning, expansion, cryopreservation and delivery.

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Hybridoma subclones were screened by ELISA with pyroglutamate and non- py-roglutamate -petide. Up to five positive parental clones by limiting dilution, isotype the final positive supernatants, and expand and cryopreserve two subclones for each parental.

5

Antibodies were purified by protein A chromatography of media from cultured hy-bridoma cell.

Example 2: Characterization of Monoclonal Antibodies

10   Affinity measurements using SPR technology : Binding affinity of anti-Aβ antibod-ies was determined using BIAcore 3000 (Biacore, Upsula, Sweden). Human and murine pyroglutaminyl and unmodified Aβ peptides with a C-terminal Cystein were immobilized on a CM5 chip using the 2-(2-pyridinyldithio) ethaneamine hy-drochloride (PDEA) coupling technology (GE healthcare).

15

Approximately 100 RU of peptide was immobilised. Antibody binding was deter-mined at different concentrations and kinetic constant calculated from the com-bined set of data using bivalent binding kinetics. Association and dissociation constants were determined using BIAevaluation software (version 3.1).

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The BIAcore binding is shown in Fig 1.

Example 3: Immunohistochemistry Methods

Paraffin blocks of frontal cortex tissue from Alzheimer patients and from age-

25   matched controls were obtained from Cambridge Bioscience (UK) and sectioned at 4 μm. Likewise, sections were prepared from brains of APP/PS1 double-transgenic mice carrying the APP Swedish mutation. Deparaffinised sections were subjected to antigen retrieval by microwave treatment in 10 mM Citrate buffer, pH 6, followed by 88% formic acid for 3 min. Sections were incubated o/n

30   at 4°C with primary antibodies: Anti-Abeta #6E10 at 1:100 (Covance, NJ, USA); pE3-Abeta antibodies (clone 2E8G6 and 5C9) at dilutions ranging from 3-20 μg/ml. This was followed by biotin-conjugated anti-mouse antibodies (#0464, DAKO, Denmark) at 1:500 and StreptAvidin-Biotin complex (Vector Laboratoreis,

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UK). The immunoreaction was developed in 0.05% diaminobenzidine+0.01% H_2O_2.

5 Binding studies indicated that the anti-AßpE3 antibodies, generated from the AßpE3-11 immunogen, are highly specific to pyroglutamated pE3 peptide. As such, the generated anti-AßpE3 antibodies bind with little or no affinity to the un-modified Aß 3-11 peptide.

10 Further, antibodies raised against the human peptide did not bind the murine py-roglutamated peptide, and vice versa (see Figure 1). Comparison of the human and the murine 3-11 amino acid sequences of Aß reveals two amino acid residue differences at Aß positions 4 and 10. We observe that the amino acid residue Y at position 10 in the human peptide may contribute to the higher degree of speci-ficity seen by anti-human AßpE3 antibodies.

15

It was demonstrated by immunohistochemistry in brain samples from Alzheimer patients as well as APP/PS1 transgenic mice that the two anti-AβpE3 monoclonal antibodies 5C9 and 2E8 (which is another specific monoclonal antibody not claimed in this application) were able to bind to Abeta plaques (fig 2 and 3). Im-

20 munostaining using a recognised commercial anti-Abeta antibody (clone 6E10) was compared to the anti-AβpE3 monoclonal antibodies 2E8 and 5C9. AβpE3 antibodies were shown to label only a subset of plaques at the dilutions tested, whereas intracellular Abeta and diffuse plaques were also stained by the 6E10 antibody. The intracellular Abeta and diffuse plaques were not detected by

25 AβpE3 antibodies. All 3 antibodies clearly stained dense-core plaques. pE3-Abeta antibodies did not label structures in human cortex from non-demented controls.

Those skilled in the art will recognize that various changes and/or modifications

30 may be made to aspects or embodiments of this invention and that such changes and/or modifications may be made without departing from the spirit of this invention. Therefore, it is intended that the appended claims cover all such equivalent variations as will fall within the spirit and scope of this invention.

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Each reference cited in the present application, including literature references, books, patents and patent applications, is incorporated herein by reference in its entirety.

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CLAIMS

1. An antibody, or a fragment thereof, specific for human AβpE3 with a binding
   affinity (K_D) of about 100 nM to about 50 nM, or about 50 nM to about 10 nM,
5   or about 10 nM to about 5 nM for the human pyroglutamated Aβ fragment
   pEFRHDSGYE, which antibody comprises of:

   a heavy chain variable region (V_H) CDR1 comprising GYTFTDYYX$_1$N
   a heavy chain variable region (V_H) CDR2 comprising
10   WX$_2$X$_3$PGSGNX$_4$KYNEKFKG
   a heavy chain variable region (V_H) CDR3 comprising EGX$_5$X$_6$X$_7$Y
   a light chain variable region (V_L) CDR1 comprising
   KSSQSLLX$_8$SNGX$_9$X$_{10}$YLN
   a light chain variable region (V_L) CDR2 comprising X$_{11}$VSKLDS, and
15   a light chain variable region (V_L) CDR3 comprising VQGTHX$_{12}$PFT

   wherein X$_1$ to X$_{12}$ symbolizes a natural amino acid.

2. The antibody according to claim 1, wherein X$_1$ is I or L.
20
3. The antibody according to any one of claims 1 or 2, wherein X$_2$ is I or L, X$_3$ is
   Y or N and/or X$_4$ is V or T.

4. The antibody according to any one of the previous claims, wherein X$_5$ is I or
25   L, X$_6$ is I or P and/or X$_7$ is V or D.

5. The antibody according to any one of the previous claims, wherein X$_8$ is H or
   Y, X$_9$ is E or K and/or X$_{10}$ is T or S.

30 6. The antibody according to any one of the previous claims, wherein X$_{11}$ is A or
   V.

7. The antibody according to any one of the previous claims, wherein X$_{12}$ is Y
   or F.

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8. An antibody according to any one of the previous claims, or a fragment thereof, comprising of:

    a heavy chain variable region (V$_H$) CDR1 comprising SEQ ID NO: 12;

    a heavy chain variable region (V$_H$) CDR2 comprising SEQ ID NO: 13;

    a heavy chain variable region (V$_H$) CDR3 comprising SEQ ID NO: 14;

    a light chain variable region (V$_L$) CDR1 comprising SEQ ID NO: 15;

    a light chain variable region (V$_L$) CDR2 comprising SEQ ID NO: 16; and

    a light chain variable region (V$_L$) CDR3 comprising SEQ ID NO: 17.

9. An antibody according claim 8, wherein said sequences are each having no more than 4 amino acid differences, or no more than 3 amino acid differences, or no more than 2 amino acid differences, or no more than 1 amino acid difference from said CDR1-3 (V$_H$ and/or V$_L$) sequences.

10. An antibody according claim 8, or fragment thereof, comprising of a heavy chain variable region (V$_H$) having the nucleotide sequence of SEQ NO: 4 or having the amino acid sequence encoded by SEQ ID NO:5.

11. An antibody according to claim 8, or fragment thereof, comprising of a light chain variable region (V$_L$) having the nucleotide sequence of SEQ NO: 6 or having the amino acid sequence encoded by SEQ ID NO:7.

12. An antibody, or fragment thereof, comprising of the heavy chain variable region (V$_H$) of claim 10 and the light chain variable region (V$_L$) of claim 11.

13. An antibody according to any one of claims 1-7, or a fragment thereof, comprising of:

    a heavy chain variable region (V$_H$) CDR1 comprising SEQ ID NO: 18;

    a heavy chain variable region (V$_H$) CDR2 comprising SEQ ID NO: 19;

    a heavy chain variable region (V$_H$) CDR3 comprising SEQ ID NO: 20;

    a light chain variable region (V$_L$) CDR1 comprising SEQ ID NO: 21;

    a light chain variable region (V$_L$) CDR2 comprising SEQ ID NO: 22; and

    a light chain variable region (V$_L$) CDR3 comprising SEQ ID NO: 23.

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14. An antibody according claim 13, wherein said sequences are each having no
more than 4 amino acid differences, or no more than 3 amino acid
differences, or no more than 2 amino acid differences, or no more than 1
amino acid difference from said CDR1-3 (V_H and/or V_L) sequences.

15. An antibody according to claim 13, or a fragment thereof, comprising of a
heavy chain variable region (V_H) having the nucleotide sequence of SEQ NO:
8 or having the amino acid sequence encoded by SEQ ID NO:9.

16. An antibody according to claim 13, or fragment thereof, comprising of a light
chain variable region (V_L) having the nucleotide sequence of SEQ NO: 10 or
having the amino acid sequence encoded by SEQ ID NO:11.

17. An antibody, or fragment thereof, comprising of the heavy chain variable
region (V_H) of claim 15 and the light chain variable region (V_L) of claim 16.

18. An antibody, or a fragment thereof, specific for murine AβpE3 with a binding
affinity (K_D) of about 100 nM to about 50 nM, or about 50 nM to about 10 nM,
or about 10 nM to about 5 nM for the murine pyroglutamated Aβ fragment
pEFGHDSGFE, which antibody comprises of

a heavy chain variable region (V_H) CDR1 comprising $GX_1TLX_2DAWMX_3$
a heavy chain variable region (V_H) CDR2 comprising
$EIRX_4KAX_5X_6HATX_7YAESVKG$
a heavy chain variable region (V_H) CDR3 comprising HX_8X_9
a light chain variable region (V_L) CDR1 comprising $X_{10}ASQGIX_{11}X_{12}X_{13}X_{14}G$
a light chain variable region (V_L) CDR2 comprising HGTKLED,and
a light chain variable region (V_L) CDR3 comprising $VQYX_{15}QFPYT$

wherein X_1 to X_{15} symbolizes a natural amino acid.

19. The antibody according to claim 18, wherein X_1 is I or L, X_2 is S or N, and/or
X_3 is N or T.

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20. The antibody according to claim 18-19, wherein X_4 is S or N, X_5 is Y or N, X_6 is K or N, and/or X_7 is Y or N.

5   21. The antibody according to claim 18-20, wherein X_8 is G or S and/or S X_9 is S or Y.

22. The antibody according to claim 18-21, wherein X_{10} is R or H, X_{11} is S or R, X_{12} is S or N, X_{13} is K or N, and/or X_{14} is M or I.

10

23. The antibody according to claim 18-22, wherein X_{15} is A or D.

24. An antibody, or a fragment thereof, according to claims 18-23 comprising of:
        a heavy chain variable region (V_H) CDR1 comprising SEQ ID NO: 32;
15   a heavy chain variable region (V_H) CDR2 comprising SEQ ID NO: 33;
        a heavy chain variable region (V_H) CDR3 comprising SEQ ID NO: 34;
        a light chain variable region (V_L) CDR1 comprising SEQ ID NO: 35;
        a light chain variable region (V_L) CDR2 comprising SEQ ID NO: 36; and
        a light chain variable region (V_L) CDR3 comprising SEQ ID NO: 37.

20

25. An antibody according claim 24, wherein said sequences are each having no more than 4 amino acid differences, or no more than 3 amino acid differences, or no more than 2 amino acid differences, or no more than 1 amino acid difference from said CDR1-3 (V_H and/or V_L) sequences.

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26. An antibody according claim 18-25, or fragment thereof, comprising or consisting of a heavy chain variable region (V_H) having the nucleotide sequence of SEQ NO: 24 or having the amino acid sequence encoded by SEQ ID NO:25.

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27. An antibody according to claim 18-26 , or fragment thereof, comprising or consisting of a light chain variable region (V_L) having the nucleotide sequence of SEQ NO: 26 or having the amino acid sequence encoded by SEQ ID NO:27.

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28. An antibody, or fragment thereof, comprising or consisting of the heavy chain variable region (V_H) of claim 26 and the light chain variable region (V_L) of claim 27.

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29. An antibody, or a fragment thereof, according to claims 18-23 comprising of:

a heavy chain variable region (V_H) CDR1 comprising SEQ ID NO: 38;

a heavy chain variable region (V_H) CDR2 comprising SEQ ID NO: 39;

a heavy chain variable region (V_H) CDR3 comprising SEQ ID NO: 40;

10   a light chain variable region (V_L) CDR1 comprising SEQ ID NO: 41;

a light chain variable region (V_L) CDR2 comprising SEQ ID NO: 42; and

a light chain variable region (V_L) CDR3 comprising SEQ ID NO: 43.

30. An antibody according claim 29, wherein said sequences are each having no

15   more than 4 amino acid differences, or no more than 3 amino acid differences, or no more than 2 amino acid differences, or no more than 1 amino acid difference from said CDR1-3 (V_H and/or V_L) sequences.

31. An antibody according claim 29, or fragment thereof, comprising or consisting

20   of a heavy chain variable region (V_H) having the nucleotide sequence of SEQ NO: 28 or having the amino acid sequence encoded by SEQ ID NO:29.

32. An antibody according to claim 29-31, or fragment thereof, comprising or consisting of a light chain variable region (V_L) having the nucleotide sequence

25   of SEQ NO: 30 or having the amino acid sequence encoded by SEQ ID NO:31.

33. An antibody, or fragment thereof, comprising or consisting of the heavy chain variable region (V_H) of claim 31 and the light chain variable region (V_L) of

30   claim 32.

34. The antibody, or fragment thereof, according to any one of the previous claims which is isolated or recombinant.

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35. The antibody, or fragment thereof, according to any one of the previous
claims, wherein the antibody is a humanized, chimeric, or single-chain
antibody.

5   36. The antibody according to any one of the previous claims for use in the
treatment of neurodegenerative or cognitive disease or disorder.

37. A method of treating a neurodegenerative or cognitive disease or disorder in
a patient, comprising administering to the patient in need of such treatment, a
10   therapeutically effective amount of an antibody of claims 1-35.

38. The use or method according claims 36 or 37, wherein the neurodegenerative
or cognitive disease or disorder is characterized by amyloid or tau deposits in
the central nervous system (CNS).

15

39. The use or method according claims 36-38, wherein the disease or disorder
is selected from a prion diseases (such as kuru, Creutzfeld-Jacob disease
and bovine spongiform encephalitis), Parkinson's disease, Huntington's
disease and/or fronto-temporal dementia.

20

40. The use or method according claims 36-39, wherein the disease or disorder
is Alzheimer disease.

41. A composition comprising the antibody of any one of the previous claims and
25   a pharmaceutically acceptable carrier.

42. The composition according to claim 41 for the use or in a method according
to claims 37-40.

30   43. Use of an antibody according to any one of claims 1-35 in a diagnostic
method or as a diagnostic imagining ligand.

44. Use of an immunogenic fragment consisting of the AβpE3-11
(pEFRHDSGYE) fragment and/or AβpE3-11-C (pE-FRHDSGYEC) fragment

49

for generating antibodies having a binding affinity (K_D) of about 100 nM to
about 50 nM, or about 50 nM to about 10 nM, or about 10 nM to about 5 nM.

45. A therapeutic composition consisting of an AβpE3-11 (pEFRHDSGYE)
    fragment and/or the AβpE3-11-C (pE-FRHDSGYEC) fragment in an amount
    sufficient to elicit the production of antibodies, or an antibody that
    immunospecifically binds to such fragment, and a pharmaceutically
    acceptable adjuvant or carrier.

46. The therapeutic composition of claim 45, wherein the antibody binds to
    AβpE3-11 (pEFRHDSGYE) fragment and/or AβpE3-11-C (pE-FRHDSGYEC)
    fragment with a binding affinity (K_D) to such fragment of about 100 nM to
    about 50 nM, or about 50 nM to about 10 nM, or about 10 nM to about 5 nM.

47. An in vitro method for making an antibody of claim 1 or 18, or a fragment
    thereof, specific for human pyroglutamated Aβ fragment pEFRHDSGYE
    and/or pE-FRHDSGYEC or specific for murine pyroglutamated Aβ fragment
    pEFGHDSGFE and/or pEFGHDSGFEC, which method comprises the step
    varying one or more of the amino acids "X" in claims .

48. An in vitro method for making an antibody of claim 1 or 18, or a fragment
    thereof, specific for human pyroglutamated Aβ fragment pEFRHDSGYE
    and/or pE-FRHDSGYEC or specific for murine pyroglutamated Aβ fragment
    pEFGHDSGFE and/or pEFGHDSGFEC, which method comprises the step
    varying one or more of the amino acids "X" according to claims 1-7 or 19-23.

49. The in vitro method according to claims 47 or 48, wherein the antibody binds
    with an affinity (K_D) to said fragment of about 100 nM to about 50 nM, or
    about 50 nM to about 10 nM, or about 10 nM to about 5 nM.

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A

Clone	Isotype Type (mouse)	Ka_1 (1/M)	Kd_1 (1/s)	KD_1 (M)	Ka_2 (1/RUs)	Kd_2 (1/s)	KD_2 (RU)	KD (M)
LuAb1h	IgG1, κ	3,64e4	4,22e-6	1,16e-10	1,32e-4	9,43e-3	71,4	8,2e-9
LUAb2h	IgG2a, κ	1,35e5	4,64e-4	3,46e-9	5,94e-2	1,1	18,52	64e-9

B

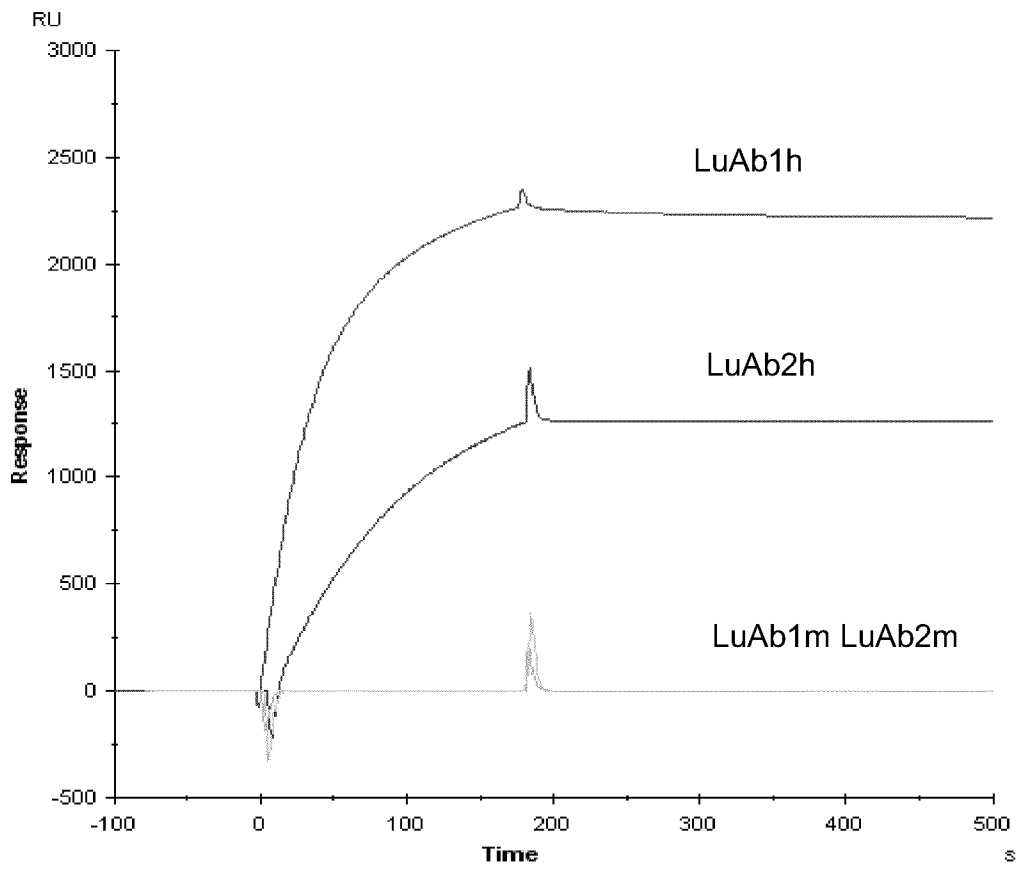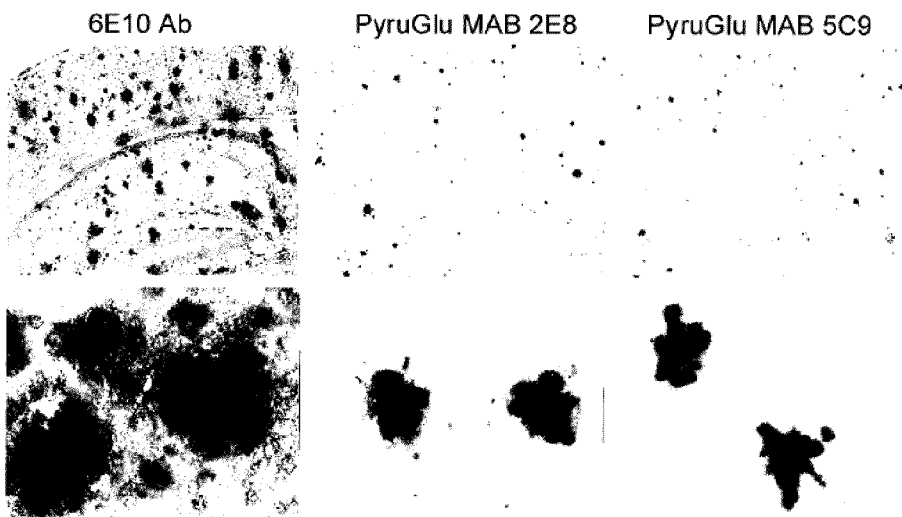| Clone	Isotype Type (mouse)	Ka_1 (1/M)	Kd_1 (1/s)	KD_1 (M)	Ka_2 (1/RUs)	Kd_2 (1/s)	KD_2 (RU)	KD (M)
LuAb1m	IgG1, κ	3,04e5	5,03e-3	1,65e-8	1,57	5,6	3,57	58,9e-9
LuAb2m	IgG1, κ	5,06e5	7,5e-3	1,48e-8	0,0155	0,0676	4,36	64,6e-9

Fig. 1

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A

FIG. 2A
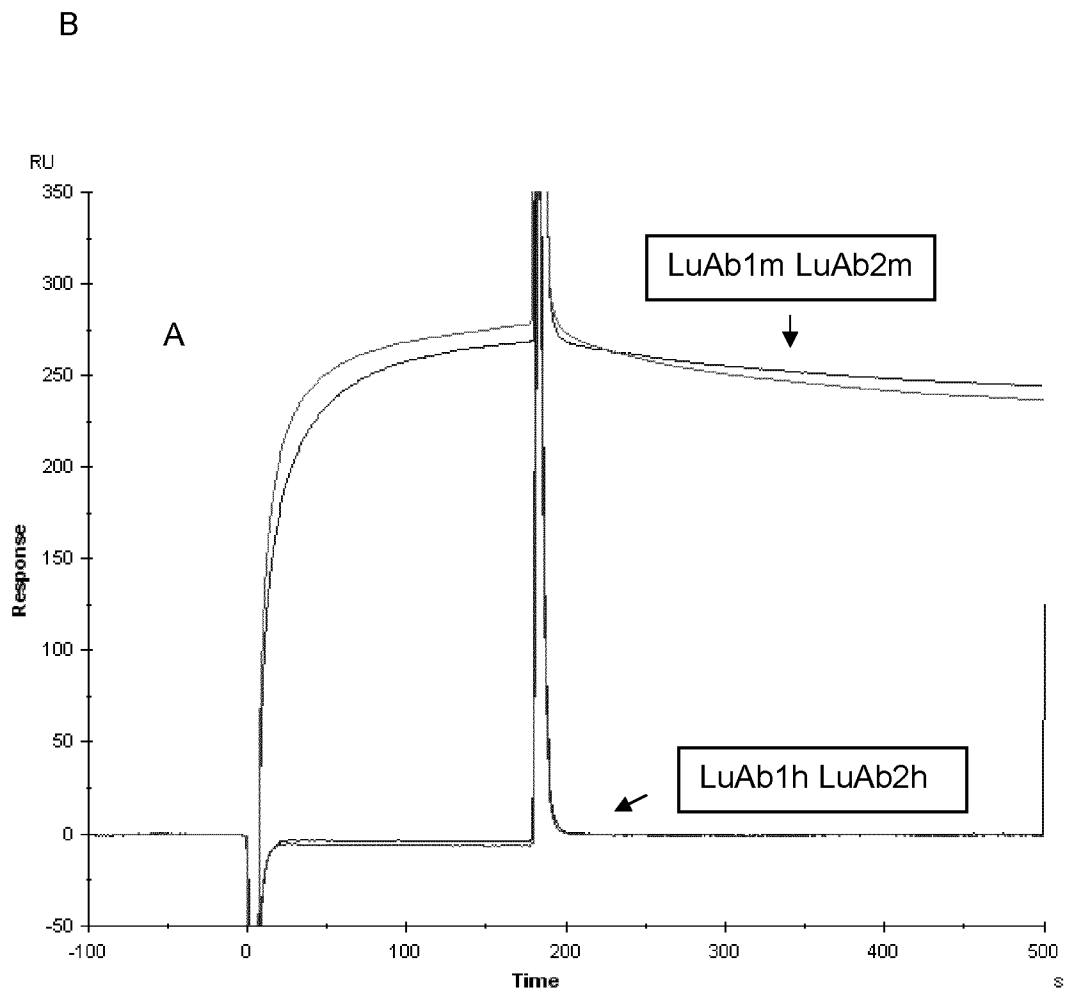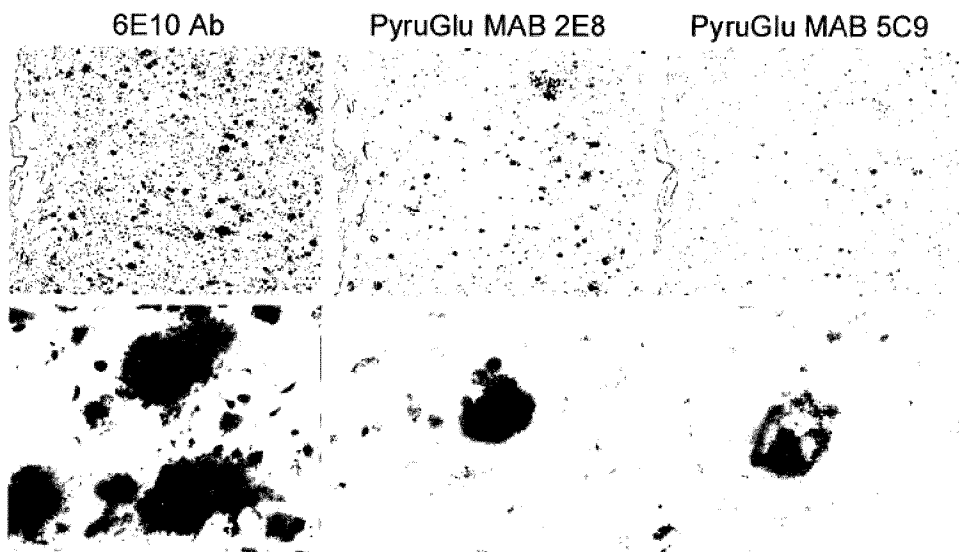
Fig. 2B

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A

B

Fig. 3

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

INV. C07K16/18
ADD. G01N33/577   G01N33/68   A61P25/28

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07K

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

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

EPO-Internal, BIOSIS, EMBASE, WPI Data, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2010/129276 A2 (CASE WESTERN RESERVE UNIVERSITY) 11 November 2010 (2010-11-11) cited in the application example 1 -----	1-17, 36-49
X	WO 2010/009987 A2 (PRO-BIODRUG AG) 28 January 2010 (2010-01-28) cited in the application examples ----- -/--	1-17, 36-49

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

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 June 2012	02/07/2012

| Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016 | Authorized officer Nooij, Frans |

1

Form PCT/ISA/210 (second sheet) (April 2005)

page 1 of 2

C(Continuation).   DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	O. WIRTHS ET AL.: "Pyroglutamate Abeta pathology in APP/PS1KI mice, sporadic and familial Alzheimer's disease cases.", JOURNAL OF NEURAL TRANSMISSION, vol. 117, no. 1, January 2010 (2010-01), pages 85-96, XP019783126, Austria page 89, left-hand column, paragraph 2 - right-hand column, paragraph 1 figure 2	1-17, 36-49
A	O. WIRTHS ET AL.: "Identification of low molecular weight pyroglutamate Abeta oligomers in Alzheimer disease.", THE JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 285, no. 53, 31 December 2010 (2010-12-31), pages 41517-41524, XP002658320, USA cited in the application abstract figure 1	1-49
A	Q. SHI ET AL.: "Intra-cerebral injection of AbetaN3pE antibody reduces Abeta deposits in 3xTg AD mice.", SOCIETY FOR NEUROSCIENCE ABSTRACT VIEWER AND ITINERARY PLANNER 2010, vol. 40, 17 November 2010 (2010-11-17), XP008152870, USA Abstract 650	1-49

1

Box No. I   Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1.   With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:

      a.   (means)

         ☐   on paper

         ☒   in electronic form

      b.   (time)

         ☐   in the international application as filed

         ☒   together with the international application in electronic form

         ☐   subsequently to this Authority for the purpose of search

2.   ☒   In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3.   Additional comments:

INTERNATIONAL SEARCH REPORT

Box No. II   Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

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see additional sheet
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1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☒ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest   ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (2)) (April 2005)

INTERNATIONAL SEARCH REPORT
Information on patent family members

Patent document cited in search report			Publication date	Patent family member(s)			Publication date
WO 2010129276	A2		11-11-2010	US	2012045392	A1	23-02-2012
				WO	2010129276	A2	11-11-2010
WO 2010009987	A2		28-01-2010	AU	2009273387	A1	28-01-2010
				CA	2731044	A1	28-01-2010
				CN	102131519	A	20-07-2011
				EA	201100231	A1	30-08-2011
				EP	2320942	A2	18-05-2011
				JP	2011528561	A	24-11-2011
				KR	20110031989	A	29-03-2011
				US	2010021478	A1	28-01-2010
				US	2012064547	A1	15-03-2012
				WO	2010009987	A2	28-01-2010

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-17, 44-46(completely); 34-43, 47-49(partially)

> An antibody or a fragment thereof specific for human AbetapE3 with a binding affinity (KD) of about 100 nM to about 50 nM, or about 50 nM to about 10 nM, or about 10 nM to about 5 nM for the human pyroglutamated Abeta fragment pEFRHDSGYE, wherein said antibody has been defined by consensus amino acid sequences of its 6 CDRs. Also its therapeutic and diagnostic use. Furthermore the human pyroglutamated Abeta fragment pEFRHDSGYE(C) for use in producing said antibody.
> ---

2. claims: 18-33(completely); 34-43, 47-49(partially)

> An antibody or a fragment thereof specific for murine AbetapE3 with a binding affinity (KD) of about 100 nM to about 50 nM, or about 50 nM to about 10 nM, or about 10 nM to about 5 nM for the human pyroglutamated Abeta fragment pEFGHDSGFE, wherein said antibody has been defined by consensus amino acid sequences of its 6 CDRs. Also its therapeutic and diagnostic use.
> ---