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(54) Title: ANTI-N3pGlu AMYLOID BETA PEPTIDE ANTIBODIES AND USES THEREOF

(57) Abstract: The present invention provides anti-N3pGlu A antibodies useful in the treatment of Alzheimers Disease.



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ANTI-N3pGlu AMYLOID BETA PEPTIDE ANTIBODIES AND USES THEREOF

The present invention relates to antibodies that selectively bind N3pGlu Amyloid Beta peptide and their use in treating diseases related to Amyloid Beta ($A\beta$, also referred to as Abeta) peptide.

The $A\beta$ peptide in circulating form is composed of 38-43 amino acids (mostly 38, 40 or 42 amino acids) resulting from the cleavage of a precursor protein, amyloid precursor protein (APP). Conversion of $A\beta$ from soluble to insoluble forms having high β -sheet content, and the deposition of these insoluble forms as neuritic and cerebrovascular plaques in the brain has been associated with a number of conditions and diseases, including Alzheimer's disease (AD), Down's syndrome, and cerebral amyloid angiopathy (CAA).

The deposits found in plaques are comprised mainly of a heterogeneous mixture of $A\beta$ peptides. N3pGlu $A\beta$, also referred to as N3pE or $A\beta_{p3-42}$, is a truncated form of the $A\beta$ peptide found only in plaques. N3pGlu $A\beta$ lacks the first two amino acid residues at the N-terminus of $A\beta$ and has a pyroglutamate which was derived from the glutamic acid at the third amino acid position. Although N3pGlu $A\beta$ peptide is a minor component of the deposited $A\beta$ in the brain, studies have demonstrated that N3pGlu $A\beta$ peptide has aggressive aggregation properties and accumulates early in the deposition cascade.

While polyclonal and monoclonal antibodies that target the N3pGlu $A\beta$ peptide have been previously described (US 7,122,374 and WO2010/009987), there is still a need for high affinity anti-N3pGlu $A\beta$ monoclonal antibodies to engage the target *in vivo* (i.e. plaque binding) and subsequently lower plaque levels. In addition, given that amino-terminal and carboxyl-terminal anti- $A\beta$ antibodies lead to an increase in cerebral amyloid angiopathy (CAA) related microhemorrhage, there is a need for anti-N3pGlu $A\beta$ antibodies that do not result in an increase in microhemorrhage even though chronic treatment results in a significant reduction of deposited plaque.

The antibodies within the scope of the present invention are therapeutically useful N3pGlu $A\beta$ peptide antagonists possessing a number of desirable properties. The present antibodies bind human N3pGlu $A\beta$ peptide with high affinity and exhibit dose-dependent *in vivo* plaque lowering without an increase in cerebral amyloid angiopathy (CAA) related microhemorrhage.

The present invention provides an anti- N3pGlu $A\beta$ antibody, or antigen-binding fragment thereof comprising a light chain variable region (LCVR) and a heavy chain variable region (HCVR), wherein said LCVR and HCVR are polypeptides selected from the group consisting of:

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- a) LCVR of SEQ ID NO: 11 and HCVR of SEQ ID NO: 12;
- b) LCVR of SEQ ID NO: 11 and HCVR of SEQ ID NO: 13;
- c) LCVR of SEQ ID NO: 11 and HCVR of SEQ ID NO: 42;
- d) LCVR of SEQ ID NO: 36 and HCVR of SEQ ID NO: 37; and
- e) LCVR of SEQ ID NO: 47 and HCVR of SEQ ID NO: 48.

In an embodiment, the present invention provides an anti- N3pGlu A β monoclonal antibody or antigen-binding fragment thereof comprising a LCVR of SEQ ID NO: 11 and an HCVR of SEQ ID NO: 12. In an embodiment, the present invention provides an anti- N3pGlu A β monoclonal antibody or antigen-binding fragment thereof comprising a LCVR of SEQ ID NO: 11 and an HCVR of SEQ ID NO: 13. In an embodiment, the present invention provides an anti- N3pGlu A β monoclonal antibody or antigen-binding fragment thereof comprising a LCVR of SEQ ID NO: 11 and an HCVR of SEQ ID NO: 42. In a preferred embodiment, the present invention provides an anti- N3pGlu A β monoclonal antibody or antigen-binding fragment thereof comprising a LCVR of SEQ ID NO: 36 and an HCVR of SEQ ID NO: 37. In a preferred embodiment, the present invention provides an anti- N3pGlu A β monoclonal antibody or antigen-binding fragment thereof comprising a LCVR of SEQ ID NO: 47 and an HCVR of SEQ ID NO: 48.

The present invention also provides an anti- N3pGlu A β monoclonal antibody comprising a light chain (LC) and a heavy chain (HC), wherein the LC and HC polypeptides are selected from the group consisting of:

- a) LC of SEQ ID NO: 14 and HC of SEQ ID NO: 15;
- b) LC of SEQ ID NO: 14 and HC of SEQ ID NO: 16;
- c) LC of SEQ ID NO: 14 and HC of SEQ ID NO: 44;
- d) LC of SEQ ID NO: 38 and HC of SEQ ID NO: 39; and
- e) LC of SEQ ID NO: 49 and HC of SEQ ID NO: 50.

In an embodiment, the present invention provides an anti- N3pGlu A β monoclonal antibody or antigen-binding fragment thereof comprising a LC of SEQ ID NO: 14 and an HC of SEQ ID NO: 15. In an embodiment, the present invention provides an anti- N3pGlu A β monoclonal antibody or antigen-binding fragment thereof comprising a LC of SEQ ID NO: 14 and an HC of SEQ ID NO: 16. In an embodiment, the present invention provides an anti- N3pGlu A β monoclonal antibody or antigen-binding fragment thereof comprising a LC of SEQ ID NO: 14 and an HC of SEQ ID NO: 44. In a preferred embodiment, the present invention provides an anti- N3pGlu A β monoclonal antibody

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or antigen-binding fragment thereof comprising a LC of SEQ ID NO: 38 and an HC of SEQ ID NO: 39. In a preferred embodiment, the present invention provides an anti- N3pGlu A β monoclonal antibody or antigen-binding fragment thereof comprising a LC of SEQ ID NO: 49 and an HCVR of SEQ ID NO:50.

5 In a preferred embodiment, the anti- N3pGlu A β monoclonal antibody comprises two light chains and two heavy chains wherein each LC comprises the polypeptide of SEQ ID NO: 14 and each HC comprises the polypeptide of SEQ ID NO: 15. In a preferred embodiment, the anti- N3pGlu A β monoclonal antibody comprises two light chains and two heavy chains wherein each LC comprises the polypeptide of SEQ ID NO: 14 and each HC comprises the polypeptide of SEQ ID
10 NO: 16. In a preferred embodiment, the anti- N3pGlu A β monoclonal antibody comprises two light chains and two heavy chains wherein each LC comprises the polypeptide of SEQ ID NO: 14 and each HC comprises the polypeptide of SEQ ID NO: 44. In a preferred embodiment, the anti- N3pGlu A β monoclonal antibody comprises two light chains and two heavy chains wherein each LC comprises the polypeptide of SEQ ID NO: 38 and each HC comprises the polypeptide of SEQ ID
15 NO: 39. In a preferred embodiment, the anti- N3pGlu A β monoclonal antibody comprises two light chains and two heavy chains wherein each LC comprises the polypeptide of SEQ ID NO: 49 and each HC comprises the polypeptide of SEQ ID NO: 50.

The present invention also provides a pharmaceutical composition comprising an anti-N3pGlu A β monoclonal antibody of the present invention or antigen-binding fragment thereof. In a
20 preferred embodiment, the pharmaceutical composition comprises an anti- N3pGlu A β monoclonal antibody of the present invention, or antigen-binding fragment thereof and a pharmaceutically acceptable carrier, diluent, or excipient, and optionally one or more other therapeutic agents.

In a further aspect, the present invention provides a method of treating a condition associated with A β peptide activity, comprising administering to a human patient in need thereof an anti-
25 N3pGlu A β monoclonal antibody or antigen-binding fragment of the present invention.

In a further aspect, the present invention provides a method of treating a condition selected from a group consisting of clinical or pre-clinical Alzheimer's disease, prodromal Alzheimer's disease, Down's syndrome, and clinical or pre-clinical CAA, comprising administering to a human in need thereof an anti- N3pGlu A β monoclonal antibody of the present invention or antigen-binding
30 fragment thereof. In a preferred embodiment, the present invention provides a method of treating Alzheimer's disease.

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In a further aspect, the present invention provides an anti- N3pGlu A β monoclonal antibody or antigen-binding fragment thereof, for use in therapy. In a preferred embodiment, the present invention provides an anti- N3pGlu A β monoclonal antibody or antigen-binding fragment thereof, for use in the treatment of a condition selected from clinical or pre-clinical Alzheimer's disease, prodromal Alzheimer's disease, Down's syndrome, or clinical or pre-clinical CAA. In a more preferred embodiment, the present invention provides an anti- N3pGlu A β monoclonal antibody or antigen-binding fragment thereof, for use in the treatment of Alzheimer's disease.

In a further aspect, the present invention provides a use of an anti- N3pGlu A β monoclonal antibody or antigen-binding fragment thereof, in the manufacture of a medicament for the treatment of a condition selected from a group consisting of clinical or pre-clinical Alzheimer's disease, prodromal Alzheimer's disease, Down's syndrome, and clinical or pre-clinical CAA. In a preferred embodiment, the present invention provides a use of an anti- N3pGlu A β monoclonal antibody or antigen-binding fragment thereof, in the manufacture of a medicament for the treatment of Alzheimer's disease.

In a further aspect, the present invention provides a use of an anti- N3pGlu A β monoclonal antibody or antigen-binding fragment thereof, in the manufacture of a medicament for the prevention of a condition selected from a group consisting of clinical or pre-clinical Alzheimer's disease, prodromal Alzheimer's disease, Down's syndrome, and clinical or pre-clinical CAA. In a preferred embodiment, the present invention provides a use of an anti- N3pGlu A β monoclonal antibody or antigen-binding fragment thereof, in the manufacture of a medicament for the prevention of Alzheimer's disease.

A full-length antibody is an immunoglobulin molecule comprising 2 heavy (H) chains and 2 light (L) chains interconnected by disulfide bonds. The amino terminal portion of each chain includes a variable region of about 100-110 amino acids primarily responsible for antigen recognition via the complementarity determining regions (CDRs) contained therein. The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function. The CDRs are interspersed with regions that are conserved, termed framework regions (FR). Each light chain variable region (LCVR) and heavy chain variable region (HCVR) is composed of 3 CDRs and 4 FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The 3 CDRs of the light chain are referred to as "LCDR1, LCDR2, and LCDR3" and the 3 CDRs of the heavy chain are referred to as "HCDR1, HCDR2, and HCDR3." The CDRs contain most of the residues which form specific interactions with the antigen. The numbering and

positioning of CDR amino acid residues within the LCVR and HCVR regions is in accordance with the well-known Kabat numbering convention.

Light chains are classified as kappa or lambda, and are characterized by a particular constant region as known in the art. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, and define the isotype of an antibody as IgG, IgM, IgA, IgD, or IgE, respectively. IgG antibodies can be further divided into subclasses, e.g., IgG1, IgG2, IgG3, or IgG4. Each heavy chain type is characterized by a particular constant region with a sequence well known in the art.

As used herein, the term “monoclonal antibody” (Mab) refers to an antibody that is derived or isolated from a single copy or clone including, for example, any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced. Mabs of the present invention preferably exist in a homogeneous or substantially homogeneous population. Complete Mabs contain 2 heavy chains and 2 light chains. The phrase “antigen-binding fragments” includes, for example, Fab fragments, Fab’ fragments, F(ab’)₂ fragments, and single chain Fv fragments.

Monoclonal antibodies of the present invention and antigen-binding fragments thereof can be produced, for example, by recombinant technologies, phage display technologies, synthetic technologies, e.g., CDR-grafting, or combinations of such technologies, or other technologies known in the art. For example, mice can be immunized with human anti-N3pGlu Aβ or fragments thereof, the resulting antibodies can be recovered and purified, and determination of whether they possess binding and functional properties similar to or the same as the antibody compounds disclosed herein can be assessed by the methods disclosed essentially as described in Examples below. Antigen-binding fragments can also be prepared by conventional methods. Methods for producing and purifying antibodies and antigen-binding fragments are well known in the art and can be found, for example, in Harlow and Lane (1988) *Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, chapters 5-8 and 15, ISBN 0-87969-314-2.

The term “treating” (or “treat” or “treatment”) refers to processes involving a slowing, interrupting, arresting, controlling, stopping, reducing, or reversing the progression or severity of an existing symptom, disorder, condition, or disease, but does not necessarily involve a total elimination of all disease-related symptoms, conditions, or disorders associated with the anti- N3pGlu Aβ antibody.

The antibodies of the present invention can be used as medicaments in human medicine, administered by a variety of routes. Most preferably, such compositions are for parenteral administration. Such pharmaceutical compositions can be prepared by methods well known in the art

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(See, e.g., *Remington: The Science and Practice of Pharmacy*, 19th ed. (1995), A. Gennaro et al., Mack Publishing Co.) and comprise an antibody as disclosed herein or an antigen-binding fragment thereof, and a pharmaceutically acceptable carrier, diluent, or excipient.

The results of the following assays demonstrate that certain monoclonal antibodies and antigen-binding fragments thereof of the present invention are useful for treating a condition associated with A β peptide activity such as Alzheimer's disease, Down's syndrome, and CAA.

Example 1: Production of Antibodies

Antibodies can be made and purified essentially as follows. An appropriate host cell, such as HEK 293 EBNA or CHO, is either transiently or stably transfected with an expression system for secreting antibodies using an optimal predetermined HC:LC vector ratio or a single vector system encoding both HC, such as SEQ ID NO: 56 and SEQ ID NO: 43, and LC, such as SEQ ID NO: 55. Clarified media, into which the antibody has been secreted, is purified using any of many commonly-used techniques. For example, the medium may be conveniently applied to a Protein A or G Sepharose FF column that has been equilibrated with a compatible buffer, such as phosphate buffered saline (pH 7.4). The column is washed to remove nonspecific binding components. The bound antibody is eluted, for example, by pH gradient (such as 0.1 M sodium phosphate buffer pH 6.8 to 0.1 M sodium citrate buffer pH 2.5). Antibody fractions are detected, such as by SDS-PAGE, and then are pooled. Further purification is optional, depending on the intended use. The antibody may be concentrated and/or sterile filtered using common techniques. Soluble aggregate and multimers may be effectively removed by common techniques, including size exclusion, hydrophobic interaction, ion exchange, or hydroxyapatite chromatography. The purity of the antibody after these chromatography steps is greater than 99%. The product may be immediately frozen at -70°C or may be lyophilized. The amino acid sequences for these antibodies of the present invention are provided below.

Table 1-Antibody SEQ ID NOs

Antibody	Light Chain	Heavy Chain	LCVR	HCVR
I (B12L)	14	15	11	12
II (R17L)	14	16	11	13

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III (hE8L)	14	44	11	42
IV (R17)	38	39	36	37
V (CI-C7)	49	50	47	48
VI (mE8)	22	23	20	21
VII (mE8c)	22	24		

Example 2: Binding Affinity to soluble N3pGlu

Surface plasmon resonance measured with the BIACORE® 2000 instrument is used to
 5 measure binding of N3pGlu A β to anti-N3pGlu antibodies. Except as noted, all reagents and
 materials are from BIACORE® AB (Uppsala, Sweden). All measurements are performed at 25°C.
 Samples are dissolved in HBS-EP buffer (150 mM sodium chloride, 3 mM EDTA, 0.005% (w/v)
 surfactant P-20, and 10 mM HEPES, pH 7.4).

A series of Abeta peptides with positional changes (glycine mutants) are synthesized to assess
 10 the impact of a given residue on antibody binding and thereby identify the characteristics and the
 sequence required for antibody recognition:

	<u>Peptide name</u>	<u>Abeta 3-16 Sequence</u>	
	pE3-16	Pyr-EFRHDSGYEVHHQK-biotin	SEQ ID NO: 25
15	E3-16	EFRHDSGYEVHHQK-biotin	SEQ ID NO: 26
	pEG4	Pyr-EGRHDSGYEVHHQK-biotin	SEQ ID NO: 27
	mpE3-16	Pyr-EFGHDSGFVHHQK-biotin (rodent)	SEQ ID NO: 28
	pEG6	Pyr-EFRGDSGYEVHHQK-biotin	SEQ ID NO: 29
	pEG7	Pyr-EFRHGSGYEVHHQK-biotin	SEQ ID NO: 30
20	pEG8	Pyr-EFRHDGGYEVHHQK-biotin	SEQ ID NO: 37
	pEF10	Pyr-EFRHDSGFVHHQK-biotin	SEQ ID NO: 39

The importance of a truncated (des 1,2) and modified form of glutamic acid (3 pyr-E or 3 pyr-Glu) is
 assessed by comparing A β 1-42 binding versus A β 3-16 versus pE3-16 (SEQ ID NO:1 versus SEQ

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ID NO:26 versus SEQ ID NO:25, respectively). Peptides are dissolved in PBS at 5mg/ml prior to dilution for binding experiments.

Binding is evaluated using multiple analytical cycles of antibody capture, peptide injection/association, prolonged buffer flow for dissociation, and surface regeneration. For the antibody capture step, depending on the type of antibody to be captured, a CM5 chip is immobilized with either protein A or goat anti-mouse Fc. Except for mouse antibodies, each cycle consists of: injection of ~5-7 μ L of 10 μ g/mL anti-N3pGlu antibody at 5 μ L/min (capture app. 3,000 RU), injection of 100 μ L of peptide at 50 μ L/min (1000 nM – 62.5 nM in two-fold serial dilutions for each cycle), followed by 10 minutes for dissociation. For a mouse antibody, the flow rate is 50 μ L/min, and 20 μ L of mouse antibody at 50 μ g/ml is injected. In both cases, the chip surface is regenerated using 20 μ L of 10 mM glycine hydrochloride, pH 1.5. The binding affinity (K_D) is then obtained from association and dissociation rates for each cycle using a 1:1 binding model in the BIAevaluation analysis software. The anti-N3pGlu antibodies, B12L and R17L and the parental mouse antibody (mE8C) recognize N3pGlu A β specifically, with a K_D less than 1 nM. Anti-N3pGlu antibodies, B12L and R17L and parental mouse antibody (mE8C) also bind to pE3-16 with similar affinity, indicating the epitope is located within this region of the peptides. Binding analysis of antibodies to glycine mutant peptides shows that the residues critical for binding were from 3 to 7: pyroE at position 3, F at position 4, R at position 5, H at position 6, D at position 7. Detectable binding to A β ₁₋₄₀ is not detected for the antibodies of the present invention.

Example 3: Binding Affinity to aggregated N3pGlu

BIACORE® experiments are also conducted to monitor the binding of anti-N3pGlu antibodies to aggregated N3pGlu A β . In this experiment, N3pGlu A β peptide is immobilized at different densities to flow cells 2 (low density, LD), 3 (medium density, MD), and 4 (high density, HD) on a CM-5 chip through amine coupling chemistry. Different levels of N3pGlu A β peptide are immobilized to examine the impact of surface density on binding of anti-N3pGlu antibodies. Upon immobilization, the majority of N3pGlu A β aggregates on the surface as demonstrated by the lack of the binding of a control Mab which only recognizes the monomeric peptide. This aggregated form of peptide mimics the property of aggregated abeta peptide in fibril or amyloid form, where the N-terminal region of the peptides is exposed and can be targeted with antibodies.

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Binding is evaluated using multiple analytical cycles at 25°C. Each cycle is performed at a flow rate of 50 µL/min and consists of the following steps: injection of 250 µL of N3pGlu antibody solution (starting at 500 nM and using two-fold serial dilutions for each cycle) followed by 20 minutes for dissociation, and regeneration using ~30 µL of 10 mM glycine hydrochloride, pH 1.5.

5 Association and dissociation rates for each cycle are evaluated using a heterogeneous ligand model in the BIAevaluation software. Since 1:1 binding model does not fit the data, the heterogeneous fit yields two binding affinities (a low and a high affinity). The R17L and B12L antibodies and parental murine antibody mE8c bind to aggregated N3pGlu Aβ with high affinity $K_{D,1} < 100$ pM and a lower affinity $K_{D,2} < 10$ nM. The maximum binding signal (Rmax) was calculated as the sum of Rmax from
10 low and high affinity binding. The Rmax is shown to increase as the density of the peptide on the surface increased, as expected when more binding sites are available at higher density surface. These binding studies demonstrate that antibodies of the present invention bind to aggregated N3pGlu Aβ.

Example 4: Ex Vivo Target Engagement Studies

15 Immunohistochemical analysis is performed with exogenously added Aβ antibodies in order to determine ex vivo target engagement on brain sections from a fixed PDAPP brain (24-month old). The PDAPP transgenic mouse has been shown to develop much of the pathology associated with Alzheimer's disease. For murine antibodies, a biotin tag was used as the label since this experiment
20 was conducted on murine tissue, and thus a direct comparison between the non-biotinylated, non-murine anti-N3pGlu antibodies is not appropriate. The biotinylated 3D6 N terminal (1-5) antibody robustly labels significant quantities of deposited Aβ in the PDAPP hippocampus, whereas the biotinylated mE8 labels only a subset of deposits. Unlike the human AD brain, the vast majority of deposited Aβ in PDAPP brain is full length. A similar plaque labeling for the non-biotinylated anti-
25 N3pGlu antibodies, such as B12L and R17L (compared to the mE8), is observed. No specific plaque labeling is observed for either the mouse or human control IgG's. Because the composition and likely structure of the deposited Aβ is dramatically different in AD brain, the non-biotinylated anti-N3pGlu (3 ug/ml) antibodies are investigated to determine whether they bind deposited Aβ on brain sections from a freshly-frozen AD brain. The positive control antibody (biotinylated 3D6) intensely labels
30 many Aβ plaques in the AD brain, whereas the negative control antibodies (murine and human IgG) lacks any appreciable binding. Several of the non-biotinylated anti-N3pGlu antibodies such as B12L

and R17L bind similarly to the deposited A β . These histological studies demonstrate that anti-N3pGlu antibodies of the present invention can engage the deposited A β target ex vivo.

Example 5: In Vivo Target Engagement Studies

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The ability of the anti-N3pGlu antibodies to engage the deposited target in vivo is measured. A sub-chronic 4-week study is performed with biotinylated murine antibodies 3D6 and mE8c at 40 mg/kg administered intraperitoneally (IP) weekly. Brains are harvested at the conclusion of the experiment and the level of target engagement is determined by histological examination of the brain.

10 The animals injected with the biotinylated 3D6 have plaque labeling only along the hippocampal fissure, whereas mice injected with biotinylated mE8c display robust plaque labeling in the hippocampus and cortical regions. Very similar target engagement patterns are observed in a more acute 3-day assay (3D6 hippocampal fissure staining and mE8 labeling both hippocampal and cortical regions). These results strongly suggest that the 3D6 antibody, which binds both soluble and

15 insoluble A β , is becoming saturated with soluble A β and thus is not able to engage the desired deposited target. In stark contrast, the murine anti-N3pGlu antibody mE8c consistently engages the intended target in both of the critical brain regions. High and low doses of the R17L and B12L anti-N3pGlu antibodies are evaluated in a similar 3-day in vivo study. The antibodies are injected IP at either 10mg/kg (low dose) or 40mg/kg (high dose). At the conclusion of the study, plasma and brains

20 are harvested and plasma PK determined. The brains are sectioned and immunohistochemistry is performed on sister sections with an anti-human antibody (to detect the bound anti-N3pGlu antibody) and 3D6 (to detect the total amount of deposited target in the section). In order to better quantify the level of in vivo target engagement, the percent area bound by the anti-N3pGlu antibody is normalized against the total % area of possible target (total deposited A β visualized by exogenous 3D6

25 immunohistochemistry). Additionally, the overall percent target engagement is normalized against the plasma pharmacokinetics (PK) values for each individual mouse since significant exposures are detected at the conclusion of the study. Both the R17L and B12L anti-N3pGlu antibodies are found to engage the deposited plaque with a similar distribution as that observed with the murine anti-N3pGlu antibody (mE8). These results demonstrate that the R17L and B12L anti-N3pGlu antibodies

30 when administered peripherally can cross the blood-brain barrier and engage the intended target of deposited A β , whereas an antibody that binds both soluble and insoluble A β becomes saturated with the soluble and cannot engage the intended deposited target.

Example 6: Therapeutic Plaque Lowering Studies

A therapeutic plaque lowering study in 23-month old PDAPP mice is performed with the following antibodies: negative control antibody (IgG2a), 3D6, mE8 (IgG1), and mE8c (IgG2a). The aged PDAPP mice are injected subcutaneously with 12.5 mg/kg of each antibody weekly for three months. A group of mice is necropsied at the beginning of the study (time zero) in order to determine the initial plaque load at 23-months of age. At the conclusion of the study, plasma is obtained and the brains are processed for biochemical and histological outcomes (one hemi-brain each). The hippocampus and cortical regions are homogenized in 5M guanidine and the A β content measured by acid urea gels followed by Western blotting. An analysis of the hippocampal guanidine lysates from the 23-month old time zero and negative antibody control (26-month old) cohorts show a non-significant increase in deposited A β_{1-42} ; thereby confirming that the brains of the PDAPP mice are at the plaque plateau. Similar to previous studies in aged PDAPP mice, treatment with the comparator antibody 3D6 has no effect on plaque lowering. The treatment with either N3pGlu antibody, mE8 or mE8c, results in significant plaque lowering as compared to the IgG2a negative control antibody (p<0.01 and p<0.001, respectively)(Table 2). The mE8 and mE8c lowers the hippocampal A β_{1-42} by ~38% and ~53%, respectively. The N3pGlu antibody mE8c with maximal effector function trends to being more efficacious than the minimal effector function antibody mE8 (compared to control), however this difference does not reach statistical significance. Also, the mE8c antibody has a significant ~30% lowering of A β_{1-42} in the hippocampus as compared to the time zero mice (t-test; p<0.0066), thus indicating clearance of previously deposited plaque. The analyses of the cortical guanidine lysates yield very similar outcomes with the exception that only the mE8c with maximal effector function significantly decreases A β_{1-42} deposition. These results demonstrate that chronic treatment with N3pGlu antibodies of this Example significantly decreases plaque deposition in aged PDAPP mice in an effector function dependent manner. Additionally, these results support the hypothesis that poor target engagement for A β antibodies that bind both soluble and insoluble A β rather than senescence was the causative factor for their lack of efficacy when used in therapeutic paradigms.

Table 2-Hippocampal and Cortex plaque lowering (ng A β_{1-42} /mg wet weight)

Hippocampal plaque of 23 to 26-month old PDAPP mice					
	Time Zero Control	Negative Control - IgG2a	m3D6	mE8 - IgG1	mE8c - IgG2a

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Number of values	15	27	30	27	23
Mean	48.13	71.96	66.73	44.25	33.62
Std. Deviation	17.12	39.4	29.48	19.64	13.8
Std. Error	4.42	7.583	5.383	3.78	2.877

Cortex plaque of 23 to 26-month old PDAPP mice					
	Time Zero Control	Negative Control - IgG2a	m3D6	mE8 - IgG1	mE8c - IgG2a
Number of values	15	27	30	27	24
Mean	34.43	41.93	40.46	33.66	27.52
Std. Deviation	16.14	19.98	18.14	14.91	16.95
Std. Error	4.168	3.845	3.313	2.869	3.459

Example 7: Analysis of Microhemorrhage in Aged PDAPP Mice

5 A histological study is performed to investigate whether the mechanism of action of the N3pGlu antibodies that leads to decreased plaque lowering in aged PDAPP mice would result in an exacerbation of CAA-related microhemorrhage. Previous studies have demonstrated that treatment of aged APP transgenic mice with certain anti- A β amino-terminal and carboxyl-terminal antibodies will lead to an increase in CAA-related microhemorrhage (Pfeifer et al. 2002; Wilcock et al. 2004; Racke et al. 2005). Although the mechanism underlying this potential adverse event is unclear, two non-mutually exclusive hypotheses have been proposed: the redistribution of A β into the cerebral blood vessels (Wilcock et al. 2004) or the direct binding of antibodies to existing CAA (Racke et al. 2005). Biochemical and histological analyses demonstrate that A β_{p3-x} is a constituent of CAA in both AD patients and aged PDAPP mice. A detailed histological analysis for microhemorrhage in aged PDAPP mice (23 to 26 months of age) that have been therapeutically treated with N3pGlu and control antibodies is performed for three months with weekly subcutaneously injections of 12.5 mg/kg. The positive control for the microhemorrhage analyses is the 3D6-chronically treated animals which have previously demonstrated that this anti- A β amino-terminal antibody significantly exacerbates microhemorrhage (Racke et al. 2005). At the conclusion of the study, one hemi-brain from each animal is drop-fixed in 4% formaldehyde and imbedded in paraffin. Coronal sections encompassing 2mm of tissue are sectioned on to 50 slides (four 10 μ m sections per slide). Eleven slides from even intervals across the 2mm of tissue are stained with Perls Blue in order to visualize hemosiderin (cellular iron accumulation due to microhemorrhage). Two sections per slide are manually counted in

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a blinded fashion. Chronic treatment of aged PDAPP mice with 3D6 (positive control) dramatically increases microhemorrhage ($p < 0.001$). Importantly, it is demonstrated that treatment with either mE8 (IgG1) or mE8c (IgG2a) does not exacerbate microhemorrhage, even though these N3pGlu antibodies significantly lower deposited A β in these animals. These results demonstrate that the N3pGlu
5 antibodies of this Example do not exacerbate CAA-related microhemorrhage in aged PDAPP mice.

We Claim,

1. An anti- N3pGlu A β monoclonal antibody or antigen-binding fragment thereof comprising a light chain variable region (LCVR) and a heavy chain variable region (HCVR), wherein said LCVR and HCVR are polypeptides selected from the group consisting of:
 - a. LCVR of SEQ ID NO: 11 and HCVR of SEQ ID NO: 12;
 - b. LCVR of SEQ ID NO: 11 and HCVR of SEQ ID NO: 13;
 - c. LCVR of SEQ ID NO: 11 and HCVR of SEQ ID NO: 42;
 - d. LCVR of SEQ ID NO: 36 and HCVR of SEQ ID NO: 37; and
 - e. LCVR of SEQ ID NO: 47 and HCVR of SEQ ID NO: 48.
2. An anti- N3pGlu A β monoclonal antibody or antigen-binding fragment thereof of claim 1, wherein said LCVR is the polypeptide of SEQ ID NO: 11 and said HCVR is the polypeptide of SEQ ID NO: 12.
3. An anti- N3pGlu A β monoclonal antibody or antigen-binding fragment thereof of claim 1, wherein said LCVR is the polypeptide of SEQ ID NO: 11 and said HCVR is the polypeptide of SEQ ID NO: 13.
4. An anti- N3pGlu A β monoclonal antibody or antigen-binding fragment thereof of claim 1, wherein said LCVR is the polypeptide of SEQ ID NO: 11 and said HCVR is the polypeptide of SEQ ID NO: 42.
5. An anti- N3pGlu A β monoclonal antibody or antigen-binding fragment thereof of claim 1, wherein said LCVR is the polypeptide of SEQ ID NO: 36 and said HCVR is the polypeptide of SEQ ID NO: 37.
6. An anti- N3pGlu A β monoclonal antibody or antigen-binding fragment thereof of claim 1, wherein said LCVR is the polypeptide of SEQ ID NO: 47 and said HCVR is the polypeptide of SEQ ID NO: 48.
7. An anti- N3pGlu A β monoclonal antibody or antigen-binding fragment thereof of claim 2, wherein said antibody comprises a light chain and a heavy chain, wherein the light chain is the polypeptide of SEQ ID NO: 14 and the heavy chain is the polypeptide of SEQ ID NO: 15.
8. An anti- N3pGlu A β monoclonal antibody or antigen-binding fragment thereof of claim 3, wherein said antibody comprises a light chain and a heavy chain, wherein the light chain is the polypeptide of SEQ ID NO: 14 and the heavy chain is the polypeptide of SEQ ID NO: 16.

9. An anti- N3pGlu A β monoclonal antibody or antigen-binding fragment thereof of claim 4, wherein said antibody comprises a light chain and a heavy chain, wherein the light chain is the polypeptide of SEQ ID NO: 14 and the heavy chain is the polypeptide of SEQ ID NO: 44.
- 5 10. An anti- N3pGlu A β monoclonal antibody or antigen-binding fragment thereof of claim 5, wherein said antibody comprises a light chain and a heavy chain, wherein the light chain is the polypeptide of SEQ ID NO: 38 and the heavy chain is the polypeptide of SEQ ID NO: 39.
11. An anti- N3pGlu A β monoclonal antibody or antigen-binding fragment thereof of claim 6, wherein said antibody comprises a light chain and a heavy chain, wherein the light chain is the polypeptide of SEQ ID NO: 49 and the heavy chain is the polypeptide of SEQ ID NO: 50.
- 10 12. An anti- N3pGlu A β monoclonal antibody or antigen-binding fragment thereof of claim 7, wherein said antibody comprises two light chains and two heavy chains, wherein each light chain is the polypeptide of SEQ ID NO: 14 and each heavy chain is the polypeptide of SEQ ID NO: 15.
- 15 13. An anti- N3pGlu A β monoclonal antibody or antigen-binding fragment thereof of claim 8, wherein said antibody comprises two light chains and two heavy chains, wherein each light chain is the polypeptide of SEQ ID NO: 14 and each heavy chain is the polypeptide of SEQ ID NO: 16.
- 20 14. An anti- N3pGlu A β monoclonal antibody or antigen-binding fragment thereof of claim 9, wherein said antibody comprises two light chains and two heavy chains, wherein each light chain is the polypeptide of SEQ ID NO: 14 and each heavy chain is the polypeptide of SEQ ID NO: 44.
- 25 15. An anti- N3pGlu A β monoclonal antibody or antigen-binding fragment thereof of claim 10, wherein said antibody comprises two light chains and two heavy chains, wherein each light chain is the polypeptide of SEQ ID NO: 38 and each heavy chain is the polypeptide of SEQ ID NO: 39.
- 30 16. An anti- N3pGlu A β monoclonal antibody or antigen-binding fragment thereof of claim 11, wherein said antibody comprises two light chains and two heavy chains, wherein each light chain is the polypeptide of SEQ ID NO: 49 and each heavy chain is the polypeptide of SEQ ID NO: 50.
17. A pharmaceutical composition, comprising an anti- N3pGlu A β monoclonal antibody or antigen-binding fragment thereof of any one of claims 1-16 and a pharmaceutically acceptable carrier, diluent, or excipient.

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18. A method of treating a condition selected from a group consisting of clinical or pre-clinical Alzheimer's disease, prodromal Alzheimer's disease, Down's syndrome, and clinical or pre-clinical amyloid angiopathy (CAA), comprising administering to a human patient in need thereof the anti- N3pGlu A β monoclonal antibody or antigen-binding fragment thereof of any one of claims 1-16.
19. A method of treating Alzheimer's disease, comprising administering to a human patient in need thereof the anti- N3pGlu A β monoclonal antibody or antigen-binding fragment thereof of any one of claims 1-16.
20. A human engineered antibody or antigen-binding fragment thereof, of any one of Claims 1-16 for use in therapy.
21. A human engineered antibody or antigen-binding fragment thereof, of any one of Claims 1-16 for use in the treatment of a condition associated with A β peptide activity.
22. A human engineered antibody or antigen-binding fragment thereof, of any one of Claims 1-16 for use in the treatment of a condition selected from clinical or pre-clinical Alzheimer's disease, prodromal Alzheimer's disease, Down's syndrome, or clinical or pre-clinical CAA.
23. A human engineered antibody or antigen-binding fragment thereof, for use according to claim 22 in the treatment of Alzheimer's disease.