



(51) International Patent Classification:

C07K 14/47 (2006.01) C07K 16/00 (2006.01)
A61K 47/68 (2017.01) C07K 16/46 (2006.01)
A61P 25/28 (2006.01) C07K 19/00 (2006.01)
C07K 14/00 (2006.01) C12N 15/62 (2006.01)
C07K 14/46 (2006.01)

(21) International Application Number:

PCT/IB2018/050576

(22) International Filing Date:

30 January 2018 (30.01.2018)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/452,015 30 January 2017 (30.01.2017) US
62/530,980 11 July 2017 (11.07.2017) US

(71) Applicant: **NATIONAL RESEARCH COUNCIL OF CANADA** [CA/CA]; 1200 Montreal Road, Ottawa, Ontario K1A 0R6 (CA).

(72) Inventors: **CHAKRAVARTHY, Balu**; 6127, Lariviere Crescent, Ottawa, Ontario K1W 1C6 (CA).
STANIMIROVIC, Danica; 6892 Sparkling Lake Way, Ot-

tawa, Ontario K4P 1R7 (CA). **DUROCHER, Yves**; 5335 place d'Argenteuil, Montreal, Québec H4J 1M1 (CA).

(74) Agent: **THOMAS, Roula** et al.; 1200 Montreal Road, Ottawa, Ontario K1A 0R6 (CA).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, 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, KM, ML, MR, NE, SN, TD, TG).

(54) Title: BLOOD-BRAIN BARRIER TRANSMIGRATING COMPOUNDS AND USES THEREOF

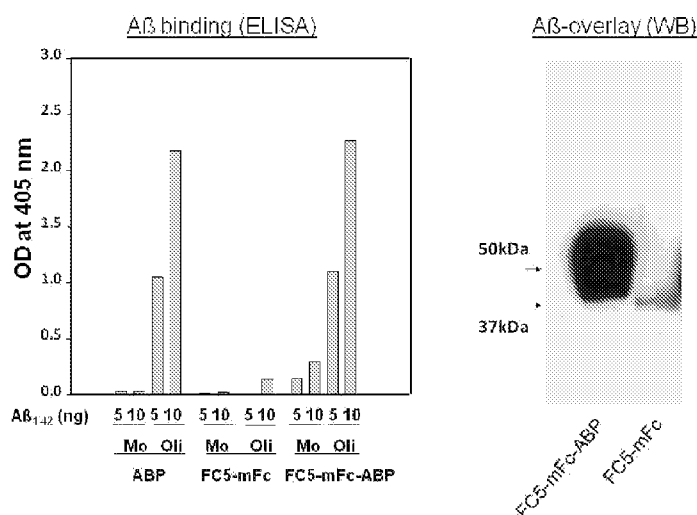


FIG. 2B

(57) Abstract: A brain-penetrating composition of amyloid-β binding peptide is disclosed. This may be useful in the treatment of Alzheimer's disease, for example as a bifunctional molecule, comprising a blood-brain barrier crossing antibody and an amyloid-β targeting peptide linked via an Fc fragment that is able to transmute across the blood-brain barrier into the brain, and compositions comprising same. Methods of using this composition for treating Alzheimer's disease are disclosed.



Declarations under Rule 4.17:

- *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

Published:

- *with international search report (Art. 21(3))*
- *in black and white; the international application as filed contained color or greyscale and is available for download from PATENTSCOPE*

BLOOD-BRAIN BARRIER TRANSMIGRATING COMPOUNDS AND USES THEREOFFIELD OF THE INVENTION

The present invention relates to compounds that transmigrate the blood-brain barrier, and uses thereof. More specifically, the present invention relates to compounds that may comprise an antibody or fragment thereof that crosses the blood-brain barrier, an immunoglobulin Fc domain or fragment thereof, and a polypeptide binding to beta-amyloid, fusion proteins and compositions thereof and their use in the treatment of Alzheimer's disease.

BACKGROUND OF THE INVENTION

Neurodegenerative diseases, such as Alzheimer's and Parkinson's disease, are an increasing burden on our ageing society because there are currently no effective treatments for these disabling conditions. Alzheimer's disease (AD) is an irreversible neurodegenerative disorder affecting approximately 15% of the population over 65 years of age and is the predominant cause of progressive intellectual and cognitive failure in the ageing population (Hardy et al, 2014).

In AD, there is a severe loss of cholinergic neurons with a consequent decline in the levels of acetylcholine (ACh), a key neurotransmitter involved in memory processing and storage. In addition, excitotoxicity induced by neurotransmitter glutamate is also implicated in the pathogenesis of AD. Therefore, cholinergic augmentation and/or inhibition of glutamate toxicity might improve cognition in AD. Indeed, the only FDA approved drugs for the treatment of AD are acetylcholine esterase (AChE) inhibitors (e.g., donepezil, rivastigmine, galantamine) to prevent the loss of ACh and inhibitors of specific glutamate receptors (e.g., memantine) (Mangialasche et al, 2010; Ji and Ha, 2010; Savonenko et al, 2012). However, the beneficial effects of these symptomatic drugs are limited and transient, providing temporary improvement in cognitive functions and do not stop the progression of the disease. While other treatments including antioxidants, anti-inflammatory drugs (NSAIDS), cholesterol-lowering drugs and estrogen therapy are considered, none of these treatments appear to have any long-term beneficial effects, particularly in improving memory and cognitive function in AD patients (Magialasche et al, 2010; Ji and Ha, 2010).

A major hallmark of Alzheimer's disease is the accumulation of a 39-43 amino acid peptide β -amyloid ($A\beta$) in the brain in the form of aggregates and plaques. A considerable body of

evidence based on genetic, pathological and biochemical studies indicate that A β , particularly its oligomeric aggregates, plays a central role in the development of AD pathology (Hardy et al, 2014; DeLaGarza, 2003; Selkoe and Hardy, 2016). According to amyloid hypothesis, a chronic imbalance in the production and clearance of A β in the brain results in its accumulation and aggregation with ageing. These A β aggregates are believed to initiate a cascade of events leading to synaptic loss and neuronal functions, leading to a progressive loss of memory and other cognitive functions (Hardy et al, 2014; DeLaGarza, 2003; Selkoe and Hardy, 2016; Sengupta et al, 2016).

The generation of A β from its precursor protein APP is achieved by the sequential proteolysis of APP by proteases β and γ secretases (Barageb and Sonawane, 2015). Inhibitors of these enzymes have been shown to reduce A β production and are being developed as potential drugs for treating AD (Hardy et al, 2014; Mangialasche et al, 2010; Selkoe and Hardy, 2016; Ji and Ha, 2010). Similarly, agents that sequester and/or promote A β clearance are also being developed. Notable among these are the development of immunotherapies with AD vaccine. Both active (A β peptides) and passive immunization (A β -antibodies) have been shown to be effective in preventing amyloid deposition as well as clearing of preformed amyloid plaques in transgenic animal models of AD and in clinical trials involving AD patients (Mangialasche et al, 2010; Ji and Ha, 2010; Morrone et al, 2015; Lannfelt et al, 2014; Selkoe and Hardy, 2016; Goure et al, 2014).

Inhibitors of β and γ secretases that prevent proteolytic cleavage of amyloid precursor protein (APP) and thereby reduce or suppress brain A β production are being developed (e.g., tarenflurbil, semagacestat, verubecestat). However, their therapeutic efficacy in reducing A β burden is not yet known and many of these drugs have failed in pre-clinical or clinical trials (Savonenko et al, 2012; Musiek and Holtzman, 2015). Moreover, since these enzymes are also involved in the processing of other enzymes and signaling molecules such as Notch that are linked to neuronal development (Savonenko et al, 2012; Musiek and Holtzman, 2015), these inhibitors may have serious non-specific side effects.

Immunotherapeutic approaches such as active (A β vaccine, AN1792) and passive immunization (e.g., Bapineuzumab, Solanezumab, Crenezumab, aducanumab etc) have been shown to be quite effective in reducing A β deposition and partial elimination of memory deficits in transgenic animals (Monsonogo and Weiner, 2003; Bard et al, 2000, Sevigny J et al., 2016). Several clinical trials using both active and passive immunization have shown reduction in brain A β deposition with moderate improvement in cognition. However, clinical trials had to be abandoned due to severe inflammatory reactions (meningo-encephalitic presentation), vasogenic edema, and micro-haemorrhages in AD patients. Despite these limitations, the

immunotherapy approach indicates that agents that effectively sequester A β , and prevent its deposition and toxicity, could potentially serve as effective drugs in arresting the progression of AD, and even prevent its development (Rafii and Aisen, 2015, Selkoe and Hardy, 2016).

5 Treatment as well as early diagnosis of AD and other diseases that originate in the brain remain challenging because the majority of suitable therapeutic molecules and diagnostics cannot penetrate the tight and highly restrictive blood-brain barrier (BBB) (Abbott, 2013). The BBB constitutes a physical barricade that is formed by brain endothelial cells (BECs) that line the blood vessels and connect with each other through tight junctions (Abbott, 2013). The tight junctions formed between the BECs are essential for the integrity of the BBB and prevent the
10 paracellular transport of molecules larger than 500 daltons (Da). Because brain endothelial cells exhibit very low pinocytosis rates (Abbott, 2013), transcellular transport of larger molecules is limited to the highly specific receptor mediated transcytosis (RMT) pathway, and the passive, charge-based adsorption mediated transcytosis (Abbott, 2013; Pardridge, 2002). Additionally, the high density of efflux pumps, such as P-glycoprotein or the multi-drug
15 resistance protein -1 (MDR-1), contribute to the removal of unwanted substances from the brain (Abbott, 2013).

While all these characteristics protect the brain from pathogens and toxins, they equally prevent the entry of most therapeutics. In fact, less than 5% of small molecule therapeutics and virtually none of the larger therapeutics can cross the BBB in pharmacologically relevant
20 concentrations (i.e., sufficient to engage a central nervous system (CNS) target and elicit pharmacologic/therapeutic response) unless they are specifically 'ferried', that is, coupled to a transporter molecule. Due to the lack of effective 'carriers' to transport molecules across the BBB, numerous drugs against neurodegenerative diseases have been 'shelved' or eliminated from further development as they cannot be delivered to the brain in sufficient amount.

25 Despite considerable progress in understanding the molecular mechanism of AD pathology, there are no effective drugs or treatments currently available that can prevent the progression of or cure the disease. Furthermore, the lack of high-capacity and high-selectivity BBB carriers delays the development of new therapeutics and diagnostics for diseases originating in the brain, including brain tumors and neurodegenerative diseases.

30 SUMMARY OF THE INVENTION

The present invention relates to compounds or compositions that transmigrate the blood-brain barrier, and uses thereof.

The present invention provides polypeptides which binds beta-amyloid (β -amyloid). The polypeptides (or proteins) that bind β -amyloid may selectively bind pathologically relevant β -amyloid₁₋₄₂ ($A\beta_{1-42}$) aggregates, and may be abbreviated and referred to herein as ABP or ABP variants.

- 5 The present invention provides fusion proteins (also referred to herein as compounds, compositions or constructs) comprising a beta-amyloid (β -amyloid) binding polypeptide (ABP or ABP variant) linked to an antibody or fragment thereof that crosses the blood-brain barrier (BBB), wherein BBB herein refers to an abbreviation for the carrier antibody or fragment that transmigrates the blood brain barrier. In a preferred embodiment, the fusion protein
- 10 comprising an ABP or ABP variant and a BBB carrier, or fragment thereof, wherein the ABP and BBB components of the fusion protein may be linked via an Fc region or portion thereof. In a preferred embodiment, the fusion protein comprising an ABP or ABP variant and a BBB, further comprises an immunoglobulin protein effector domain, known as Fc, or fragment thereof, wherein the ABP and BBB components of the fusion protein may be linked via the Fc
- 15 region or portion thereof. For example, a construct of the present invention may further comprise a linker (L), wherein L is a small linking peptide or peptide-like chain. For example, the BBB-Fc-L-ABP construct provided may be a single chain polypeptide or a dimeric polypeptide, wherein the single chain polypeptide comprising BBB-Fc-L-ABP may form a dimer by Fc dimerization. Fc dimerization may be mediated by interaction of a large tightly packed
- 20 hydrophobic interface between two Fc CH3 domains. For example, a construct of the present invention may comprise BBB-Fc-ABP. The BBB-Fc-ABP construct provided may be a single chain polypeptide (monovalent) or a dimeric polypeptide (bivalent), wherein the single chain polypeptide comprising BBB-Fc-ABP may form a dimer through Fc which allows the dimerization of the fusion protein. A compound of the present invention may be referred to as a
- 25 fusion protein, construct, fusion molecule, formulation or composition.

The compounds of the present invention may be used in the treatment of Alzheimer's disease (AD).

The polypeptide that binds β -amyloid may comprise a sequence selected from the group consisting of:

- 30 SGKTEYMAFPKPFESSSSIGAEKPRNKKLPEEEESSRTPWLYEQEGEVEKPFIKTGFS
VSVEKSTSSNRKNQLDTNGRRRQFDEESLESFSSMPDPVDPTTVTKTKTRKASAQAS
LASKDKTPKSKSKRNSTQLKSRVKNITHARRILQQSNRNACNEAPETGSDFSMFEA
(SEQ ID NO:27);

FSSMPDPVDPTTVTKFKTRKASQAASLASKDKTPKSKSK (SEQ ID NO:28);

KDKTPKSKSKKRNSTQLKSRVKNIHARRILQQSNRNACN (SEQ ID NO:29);

KTFKTRKASQAASLASKDKTPKSKSKKRNSTQLKSRVKNI (SEQ ID NO:30);

The polypeptide that binds β -amyloid may be a variant that comprises a sequence:

5 X_1 TFX₂TX₃X₄ASAQAASLASKDKTPKSKSKKX₅X₆STQLX₇SX₈VX₉NI

where X_1 = G or A, X_2 = G or V, X_3 = G or A, X_4 = G or A, X_5 = G or V, X_6 = G or V, X_7 = G or V, X_8 = G or A, X_9 = G or A (SEQ ID NO: 31)

In specific non-limiting embodiments, the ABP, or its variants, may comprise a sequence selected from any one of:

10 KTFKTRKASQAASLASKDKTPKSKSKKRGSTQLKSRVKNI (SEQ ID NO:32);

KTFKTRKASQAASLASKDKTPKSKSKKGGSTQLKSRVKNI (SEQ ID NO:33);

KTFKTRGASQAASLASKDKTPKSKSKKRGSTQLKSRVKNI (SEQ ID NO:34);

KTFKTGGASQAASLASKDKTPKSKSKKRGSTQLKSRVKNI (SEQ ID NO:35);

GTFGTGGASQAASLASKDKTPKSKSKKGGSTQLKSRVKNI (SEQ ID NO:36);

15 KTFKTRKASQAASLASKDKTPKSKSKKGGSTVKNI (SEQ ID NO:37);

KTFKTRKASQAASLASKDKTPKSKSKKRG (SEQ ID NO:38); and

a sequence substantially identical to any of the above sequences.

The polypeptide that binds β -amyloid (ABP) variant may comprise a polypeptide sequence selected from the group consisting of SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ
 20 ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, and SEQ ID NO: 38. An ABP comprising a polypeptide sequence selected from the group consisting of SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, and SEQ ID NO: 38 may be referred to as an ABP or ABP variant. The present invention additionally comprises an ABP variant sequence having consensus sequence SEQ ID NO: 31, and may be for example SEQ
 25 ID NO: 35, or SEQ ID NO: 36, or any equivalently stable polypeptide sequence. An equivalently stable polypeptide sequence exhibits peptide stability during expression and production of fusion protein in mammalian expression system. For example, an ABP variant of

the present invention may exhibit improved peptide stability over ABP peptides of the prior art (Ref. WO2006/133566).

The present invention provides an isolated polypeptide that binds β -amyloid, the isolated polypeptide that binds β -amyloid may comprise a sequence selected from the group consisting of SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, and SEQ ID NO: 38, or a sequence comprising an equivalently stable polypeptide sequence.

The present invention provides a fusion protein comprising an ABP or ABP variant selected from the group consisting of SEQ ID NO: 31, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, and SEQ ID NO: 38. In a non-limiting example, the ABP variant may comprise the sequence KTFKTGGASAQASLASKDKTPKSKSKKRGSTQLKSRVKNI (SEQ ID NO:35); GTFGTGGASAQASLASKDKTPKSKSKKGGSTQLKSRVKNI (SEQ ID NO:36).

A fusion protein of the present invention may comprise an antibody, or fragment thereof, that transmigrates across the blood brain barrier (BBB) (as noted above, BBB is an abbreviation of the antibody carrier that transmigrates the blood brain barrier). The antibody, or fragment thereof, (BBB) may bind surface receptor epitopes on brain endothelial cell that allow for transmigration across the blood brain barrier. For example, such surface receptor epitopes may be TMEM30A or an Insulin-Like Growth Factor 1 Receptor (IGF1R) epitope, or isoforms, variants, portions, or fragments thereof.

The antibody, or fragment thereof, may comprise a sequence selected from the group consisting of:

an antibody or fragment thereof comprising a complementarity determining region (CDR) 1 sequence of GFKITHYTMG (SEQ ID NO:1), a CDR2 sequence of RITWGGDNTFYNSVKG (SEQ ID NO:2), a CDR3 sequence of GSTSTATPLRVDY (SEQ ID NO:3);

an antibody or fragment thereof comprising CDR1 sequence of EYPSNFYA (SEQ ID NO:4), a CDR2 sequence of VSRDGLTT (SEQ ID NO:5), a CDR3 sequence of AIVITGVWNKVDVNSRSYHY (SEQ ID NO:6);

an antibody or fragment thereof comprising CDR1 sequence of GGTVSPTA (SEQ ID NO:7), a CDR2 sequence of ITWSRGTT (SEQ ID NO:8), a CDR3 sequence of AASTFLRILPEESAYTY (SEQ ID NO:9); and

an antibody or fragment thereof comprising CDR1 sequence of GRTIDNYA (SEQ ID NO:10), a CDR2 sequence of IDWGDGGX; where X is A or T (SEQ ID NO:11), a CDR3 sequence of AMARQSRVNLDVARYDY (SEQ ID NO:12).

The antibody or fragment thereof may comprise a sequence selected from the group
5 consisting of:

X₁VQLVX₂SGGGLVQPGGSLRLSCAASGFKITHYTMGWX₃RQAPGKX₄X₅EX₆VSRTW
GGDNTFYSNSVKGRFTISRDNKNTX₇YLQMNSLRAEDTAVYYCAAGSTSTATPLRVD
YWGQGTLLTVSS (SEQ ID NO:13), where X₁=D or E, X₂=A or E, X₃=F or V, X₄=E or
G, X₅=R or L, X₆=F or W, X₇=L or V;

10 X₁VX₂LX₃ESGGGLVQX₄GGSLRLSCX₅ASEYPSNFYAMSWX₆RQAPGKX₇X₈EX₉VX₁₀G
VSRDGLTTLTLYADSVKGRFTX₁₁SRDNX₁₂KNTX₁₃X₁₄LQMNSX₁₅X₁₆AEDTAVYYCAIVITG
VWNKVDVNSRSYHYWGQGT₁₇TVSS, (SEQ ID NO:18), where X₁ is E or Q; X₂ is K
or Q; X₃ is V or E; X₄ is A or P; X₅ is V or A; X₆ is F or V; X₇ is E or G; X₈ is R or L; X₉ is
F or W; X₁₀ is A or S; X₁₁ is M or I; X₁₂ is A or S; X₁₃ is V or L; X₁₄ is D or Y; X₁₅ is V or
15 L; X₁₆ is K or R; and X₁₇ is Q or L;

X₁VX₂LX₃ESGGGLVQX₄GGSLRLSCX₅X₆SGGTVSPTAMGWX₇RQAPGKX₈X₉EX₁₀VX₁₁
HITWSRGTTTRX₁₂ASSVKX₁₃RFTISRDX₁₄X₁₅KNTX₁₆YLQMNSLX₁₇X₁₈EDTAVYYCAAS
TFLRILPEESAYTYWGQGT X₁₉TVSS, (SEQ ID NO:21), where X₁ is E or Q; X₂ is K or
Q; X₃ is V or E; X₄ is A or P; X₅ is A or E; X₆ is V or A; X₇ is V or F; X₈ is G or E; X₉ is L
20 or R; X₁₀ is F or W; X₁₁ is G or S; X₁₂ is V or Y; X₁₃ is D or G; X₁₄ is N or S; X₁₅ is A or S;
X₁₆ is L or V; X₁₇ is K or R; X₁₈ is A or S; and X₁₉ is L or Q; and

X₁VX₂LX₃ESGGGLVQX₄GGSLRLSCAASGRTIDNYAMAWX₅RQAPGKX₆X₇EX₈VX₉T
IDWGDGGX₁₀RYANSVKGRFTISRDNX₁₁KX₁₂TX₁₃YLQMNX₁₄LX₁₅X₁₆EDTAVYX₁₇CA
MARQSRVNLDVARYDYWGQGT₁₈TVSS (SEQ ID NO: 24), where X₁ is E or Q;
25 X₂ is K or Q; X₃ is V or E; X₄ is A or P; X₅ is V or S; X₆ is D or G; X₇ is L or R; X₈ is F
or W; X₉ is A or S; X₁₀ is A or T; X₁₁ is A or S; X₁₂ is G or N; X₁₃ is M or L; X₁₄ is N or
R; X₁₅ is E or R; X₁₆ is P or A; X₁₇ is S or Y; and X₁₈ is Q or L.

In specific non-limiting embodiments, the antibody, or fragment thereof, may comprise a
sequence selected from any one of:

30 DVQLQASGGGLVQAGGSLRLSCAASGFKITHYTMGWFRQAPGKEREFVSRTWGGD
NTFYSNSVKGRFTISRDNKNTVYLQMNSLKPEDTADYYCAAGSTSTATPLRVDYWG
KGTQVTVSS (SEQ ID NO:14);

EVQLVESGGGLVQPGGSLRLSCAASGFKITHYTMGWWRQAPGKGLEWSRITWGGD
NTFYNSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAAGSTSTATPLRVDYWG
QGTLVTVSS (SEQ ID NO: 15)

5 EVQLVESGGGLVQPGGSLRLSCAASGFKITHYTMGWWRQAPGKGLEWSRITWGGD
NTFYNSVKGRFTISRDN SKNTVYLQMNSLRAEDTAVYYCAAGSTSTATPLRVDYWG
QGTLVTVSS (SEQ ID NO:16);

EVQLVESGGGLVQPGGSLRLSCAASGFKITHYTMGWFRQAPGKLEFVSRITWGGD
NTFYNSVKGRFTISRDN SKNTVYLQMNSLRAEDTAVYYCAAGSTSTATPLRVDYWG
QGTLVTVSS (SEQ ID NO:17);

10 QVKLEESGGGLVQAGGSLRLSCVASEYPSNFYAMSWFRQAPGKEREVAGVSRDGL
TTLYADSVKGRFTMSRDNAKNTVDLQMNSVKAEDTAVYYCAIVITGVWNKVDVNSRS
YHYWGQGTQVTVSS (SEQ ID NO: 19);

EVQLVESGGGLVQPGGSLRLSCAASEYPSNFYAMSWFRQAPGKEREVSGVSRDGL
TTLYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAIVITGVWNKVDVNSRSY
15 HYWGQGTQVTVSS (SEQ ID NO: 20);

QVKLEESGGGLVQAGGSLRLSCEVSGGTVSPTAMGWFRQAPGKEREVGHITWSR
GTTRVASSVKDRFTISRDSA KNTVYLQMNSLKSEDTAVYYCAASTFLRILPEESAYTY
WGQGTQVTVSS (SEQ ID NO: 22);

20 QVQLVESGGGLVQPGGSLRLSCAVSGGTVSPTAMGWFRQAPGKLEFVGHITWSR
GTTRYASSVKGRFTISRDN SKNTVYLQMNSLRAEDTAVYYCAASTFLRILPEESAYTY
WGQGTQVTVSS (SEQ ID NO: 23);

QVKLEESGGGLVQAGGSLRLSCAASGRTIDNYAMAWSRQAPGKDREFVATIDWGDG
GARYANSVKGRFTISRDN AKGTMYLQMNNLEPEDTAVYSCAMARQSRVNLDVARYD
YWGQGTQVTVSS (SEQ ID NO: 25);

25 QVQLVESGGGLVQPGGSLRLSCAASGRTIDNYAMAWWRQAPGKGLEWWATIDWGD
GGTRYANSVKGRFTISRDN SKNTMYLQMNSLRAEDTAVYYCAMARQSRVNLDVARY
DYWGQGTQVTVSS (SEQ ID NO: 26); and

a sequence substantially identical to any of the above sequences.

The BBB may be an antibody, or fragment thereof, in a preferred embodiment, the BBB may
30 be a single-domain antibody (sdAb). The sdAb may be humanized.

In a preferred embodiment, the antibody or fragment thereof (BBB) may be linked to an Fc, or fragment thereof, wherein the BBB-Fc construct may form a dimer. The invention further provides a fusion peptide comprising BBB-Fc-L-ABP, wherein the BBB-Fc portion of the fusion peptide is linked to the ABP via a short peptidic linker (for example a linker having less than 12 amino acids) and the Fc or Fc fragment allows for the dimerization of said fusion peptides to provide dimers, which accordingly protects the construct from degradation and increases its serum half-life. The Fc fragment may be any suitable Fc fragment, selected in order to impart desirable pharmacokinetics, in which the Fc or Fc fragment contributes to the long half-life of the fusion molecule. Other preferred Fc or Fc fragment embodiments may modulate, modify or suppress an immunological effector function (Shields et al., 2001). Other highly preferred Fc fragment embodiments may mediate clearance of the fusion peptide from the brain (Caram-Salas N, 2011). In a non-limiting example Fc or Fc fragments may be Fc mouse Fc2a, or a human Fc1, selected from any one of SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, and a sequence substantially identical thereto with attenuated effector function, which, when included in said fusion peptide, may have enhanced clearance of amyloid from the brain.

In a compound of the present invention, the BBB may be linked to the ABP via an Fc fragment, and/or any additional suitable linker L.

A compound of the present invention comprises a fusion protein; wherein the fusion protein comprises an antibody or fragment thereof, an Fc fragment, and the polypeptide that binds β -amyloid. The fusion protein may comprise an antibody or fragment thereof linked to the N-terminus of Fc fragment, and the polypeptide that binds β -amyloid is linked to the C-terminus of the Fc fragment via L, a linking peptide or a chemical linker. The antibody or fragment thereof may be linked to the C-terminus of Fc fragment and the polypeptide that binds β -amyloid linked to the N-terminus of the Fc fragment.

Accordingly, the present invention provides fusion proteins comprising an antibody or fragment thereof selected from the group consisting of SEQ ID NO:13 to SEQ ID NO: 26 ; a polypeptide that binds b-amyloid selected from the group consisting of SEQ ID NO: 27 to SEQ ID NO:38 ; and an Fc fragment selected from the group consisting of SEQ ID No: 39 to SEQ ID NO:41. The provided Fc may comprise an Fc with attenuated effector functions.

For example, the fusion protein may comprise SEQ ID NOs: 42 to SEQ ID NO:52, or a sequence substantially identical thereto. The fusion protein may be a single chain polypeptide, and the single chain polypeptide of the fusion protein may form a dimeric polypeptide. It is noted that the (GGGSGGGGS or GGGGSGGGGS) linker provided in the fusion protein comprised in SEQ ID NO: 42 to SEQ ID NO: 52 may be any suitable linker sequence. For

example, the linker sequence highlighted (ex. GGGSGGGGS) may be any equivalent peptide linking sequence that allows for the linking of the components of the fusion protein provided, as exemplified in SEQ ID NO: 53, wherein the linker may be any peptide or chemical linker.

5 In an embodiment, the BBB may be linked to an Fc fragment, thus forming a dimer. The Fc fragment may be any suitable Fc fragment, for example mouse Fc2a or human Fc1, with attenuated effector function (Shields et al., 2001).

10 An ABP variant of the present invention may comprise SEQ ID NO: 31, for example, may be a sequence selected from any one of SEQ ID NO: 32 to 38. An ABP variant as provided herein exhibits unexpected and significant advantages over ABP polypeptides of the prior art. More specifically, the present ABP variants exhibit increased stability and bio-manufacturability. Moreover, a compound or composition of the present invention comprising an ABP or variant, as provided herein, when coupled to BBB, via a linker and/or Fc fragment as provided herein, exhibits a synergistic and unexpected efficacy in transmigrating the blood brain barrier. Such an ABP variant, when coupled to BBB, via a selected Fc fragment as provided herein, provides
15 an unexpected, rapid and improved clearance of A β from the brain. Moreover, a fusion protein of the present invention comprising an ABP or ABP variant as provided herein exhibits a synergistic and unexpected efficacy in transmigrating the BBB and improved clearance of A β from the brain.

20 Accordingly, there is provided a therapeutic composition comprising a blood brain barrier transmigrating antibody or fragment thereof (BBB), an Fc (Fc), linked to a polypeptide that binds β -amyloid (ABP or ABP variant), wherein said polypeptide confers a synergistic increase in stability and efficacy of the therapeutic composition provided. As shown, unexpectedly and most significantly, a single bolus of BBB-Fc-ABP reduced brain A β burden by 50% within 24 hrs of treatment (Fig. 9B) compared to three months of multiple treatments with free ABP to
25 achieve similar results in animals, (mouse model of AD) (Fig 9A). Thus, the compound as provided herein was shown to be far more potent than free ABP in reducing brain A β burden. Additionally, this fusion protein comprising Fc provided a substantially longer serum half-life compared to free ABP or BBB-ABP (WO 2006/133566), thereby providing an improved therapeutic compound. (Fig 10).

30 The ABP variants of the present invention, and fusion proteins (constructs) comprising ABP overcome the disadvantages of the prior art. In the prior art, the linking of ABP with a BBB carrier (WO 2006/133566) alone does not assure the generation of an effective molecule. Fusions comprising an Fc aimed at enhancing serum half-life do not ensure efficient transport of ABP across the blood brain barrier. Specific engineering and formulation of a fusion

molecule comprising a BBB carrier-, Fc fragment- and ABP provide an efficient BBB-permeable therapeutic compound. The efficient blood brain barrier-permeable therapeutic compound provided comprises a specifically engineered formulation of a BBB-Fc-ABP, wherein the formulation exhibits a synergistic improvement in compound stability and efficacy.

- 5 An unexpected increase in the stability of the fusion compositions, and a synergistic increase in efficacy of transmigration the blood brain barrier and faster clearance (within 24 hrs) of A β from the brain, as shown in Fig 9, is provided in a composition comprising said fusion protein constructs.

The compound provided herein may be referred to as a compound, a fusion protein, formulation, composition or construct. The provided construct may comprise an antibody or
10 fragment thereof (which may be abbreviated BBB), an Fc fragment (abbreviated Fc), and a polypeptide that binds β -amyloid (abbreviated ABP). The construct or composition provided (which may be abbreviated herein as BBB-Fc-ABP or BBB-Fc-L-ABP), comprises components that synergistically overcome the deficiencies encountered in the prior art with respect to blood
15 brain barrier transmigration, efficacy and compound stability. Accordingly, compounds provided herein comprise a novel formulation having superior and unexpected efficacy in transmigration the blood brain barrier, therapeutic efficacy and compound stability.

There are instances in the prior art where Fc fusions may increase serum half-life, although serum half-life is not necessarily increased in Fc fusions. Moreover, Fc fusions do not
20 necessarily improve transmigration across the blood brain barrier.

The present invention provides a polypeptide that binds β -amyloid, the polypeptide sequence may be selected from the group consisting of SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, and SEQ ID NO: 38 and a sequence substantially identical in
25 peptide stability.

The present invention provides a polypeptide that binds β -amyloid, wherein when the polypeptide sequence is referred to as an ABP or an ABP variant, ABP variant sequence may comprise SEQ ID NO: 31, and may be selected from the group consisting of SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID
30 NO: 37, SEQ ID NO: 38, and sequence substantially identical in peptide stability, for example, any sequence variant of SEQ ID NO:31. An ABP variant having SEQ ID NO: 31 of the present invention overcomes the disadvantages of the prior art with respect to polypeptide stability. Moreover, a compound comprising an ABP of the present invention exhibits improved compound stability and bio-manufacturability. Furthermore, a compound comprising an ABP

or ABP variant as provided herein exhibits a synergistic and unexpected therapeutic improvement.

An ABP variant of the present invention may comprise a sequence selected from any one of SEQ ID NO: 31 to SEQ ID NO: 38, and an equivalently stable polypeptide sequence. An ABP
5 variant of the present invention is superior over ABP sequences of the prior art, wherein the present ABP variant polypeptides exhibit improved stability (as shown in comparison between Fig. 2A and Fig 16).

The present invention also provides fusion proteins (also referred to as compounds, constructs, or fusion molecules) comprising an ABP, or ABP variant, of the present invention.
10 The fusion protein may comprise an Fc fragment. The fusion protein may comprise an antibody or fragment thereof that transmigration the blood brain barrier, an Fc fragment, and a β -amyloid binding polypeptide sequence that may be selected from the group consisting of SEQ ID NO: 31, SEQ ID NO: 32-38. The provided BBB-Fc-ABP formulations exhibit synergistic improvement in compound stability and therapeutic efficacy in removing toxic
15 amyloid from the brain.

The fusion protein or construct, which may be referred to herein as BBB-Fc-ABP, may vary in orientations with respect to the components comprised therein. In a compound of the present invention the fusion protein forms a dimer (as shown in Fig.1) wherein the fusion peptide dimerizes via the Fc region. For example, in an embodiment, the provided compound may
20 comprise a fusion protein comprising a BBB linked to the N-terminus of the Fc fragment (Fc) and an ABP or ABP variant (ABP) linked to the C-terminus of the Fc fragment via a short peptidic linker attached to the C-terminus (Fig 1A, wherein the compound is shown as a dimer of the fusion protein). In a further embodiment, the provided compound may comprise a BBB linked to the C-terminus of the Fc fragment (Fc) wherein the ABP or ABP variant (ABP) may
25 be linked to the N-terminus of the Fc fragment via a suitable linker (L) (Fig 1B). It is noted that in configuration 1A, the ABP or ABP variant may be N-terminally (fusion) or C-terminally (chemical linking) fused/linked to Fc. In other possible configurations, the BBB may be linked to the N-terminus of the ABP, and the ABP linked to the N-terminus of the Fc fragment (Fig 1C). In yet another possible configuration the BBB may be linked to the C-terminus of the
30 ABP, and the ABP is linked to the C-terminus of the Fc fragment (Fig 1D).

The compounds provided herein comprise fusion proteins comprising a sequence selected from any one of SEQ ID NO:42 SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO: 45, SEQ ID NO:46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO: 53 and a sequence substantially identical to any of these sequences.

The fusion protein of the present is a fusion protein (BBB-Fc-ABP or BBB-Fc-L-ABP, wherein L may be any suitable linker) dimer (as shown in Fig 1, and preferably, Fig 1A or 1B)". In a non-limiting example, the compound or fusion protein provided may comprise a polypeptide comprising sequence SEQ ID NO:35 (ABP-GG-G) or SEQ ID NO:36 (ABP-6G); an antibody or
5 fragment thereof comprising sequence SEQ ID NO:17 (FC5-H3); and an Fc fragment comprising sequence SEQ ID NO:40 (hFc1X7).

In a specific non-limiting embodiment of the present invention, the compound may comprise sequence SEQ ID NO:46 [FC5-H3-hFc1X7-L-ABP(GG-G)] or SEQ ID NO:47 [FC5-H3-hFc1X7-L-ABP(6G)], wherein the L may be any suitable linker.

10 The compounds of the present invention transmute the blood-brain barrier.

The present invention encompasses a nucleic acid molecule encoding any compound of the present invention as described herein. Vectors comprising the nucleic acid molecule of a fusion protein or compound of the present invention are also included in the scope of the present invention.

15 The present invention encompasses a composition comprising a compound or fusion protein of the present invention and a pharmaceutically-acceptable carrier, diluent, or excipient.

Kits comprising a pharmaceutical composition of the present invention are also included in the scope of the present invention.

20 The composition of the present invention may be used for treating Alzheimer's disease in a patient.

The composition of the present invention may be used for reducing toxic β -amyloid (A β) levels in the brain or CSF of a subject having increased levels of brain A β .

25 The present invention provides a method of treating Alzheimer's disease, wherein a pharmaceutical composition of the present invention may be administered to a subject in need thereof.

The present invention provides a method of reducing toxic β -amyloid (A β) levels in the brain of a subject having increased levels of brain A β . The method of the present invention comprises the administration of a compound of the present invention to a patient with AD. More specifically, the present method comprises the steps of repeated parenteral administration of a
30 sufficient amount of a pharmaceutical composition of the present invention to a subject.

In the method of the present invention, parenteral administration is subcutaneous or intravenous administration.

The method of the present invention reduces toxic β -amyloid levels, after repeated parenteral administration of a composition provided herein, in the brains of subjects having increased
5 brain levels of A β . More specifically, toxic β -amyloid levels are reduced within four weeks of repeated parenteral administration of the composition of the present invention.

The method of the present invention reduces toxic β -amyloid levels in the cerebrospinal fluid (CSF) of subjects after parenteral administration of the composition of the present invention. More specifically, toxic β -amyloid levels in the cerebrospinal fluid (CSF) of subjects is
10 significantly reduced within 24 hours of a single parenteral administration of the composition of the present invention; wherein significant reduction is up to 50% within 24 hours.

The present invention provides a blood brain barrier-permeable single domain antibody (sdAb), wherein the sdAb is either camelid FC5 or a humanized version thereof. FC5 displayed in bi-valent format on Fc has been shown to have improved blood brain barrier-crossing properties
15 compared to FC5 in V_HH format (Farrington et al., 2014).

The present invention provides a compound comprising a BBB that facilitates blood brain barrier transmigration *in vitro* and an Fc fragment and increases serum half-life of the fusion molecule, wherein serum half-life of the Fc fusion becomes similar to that of a full IgG. Prolonged serum half-life of FC5-Fc-ABP also increases overall brain exposure, which is
20 particularly important for treating chronic diseases such as Alzheimer's Disease.

The FC5-Fc-ABP fusion molecule as well as a humanized version FC5(H3)-hFc-ABP, and IGF1R5-H2-ABP fusion molecules were produced in CHO cells. The ABP variants of the present invention, as provided in SEQ ID NO: 31, are methodically re-engineered with specific point-mutations or deletions that enhance bio-manufacturability and stability of the fusion
25 molecule (SEQ ID NO: 45, SEQ ID NO: 46; SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53). The fusion molecules of the present invention comprising the ABP variants not only retain A β binding ability, the provided fusion molecules comprising the ABP variants not only penetrate the blood brain barrier, but advantageously allow for the clearance of A β from the brain, for example, the rapid
30 clearance of A β from the brain within 24 hours of administration.

The ABP variants of the present invention, for example ABP(GG-G) (SEQ ID NO: 35) or ABP(6G) (SEQ ID NO: 36), were specifically engineered to enhance stability and manufacturability of the construct (as shown in FIG. 16). The enhanced stability and bio-

manufacturability of the construct of the present invention is significant in the present art. The present invention accordingly encompasses ABP and ABP variants that enhance stability and bio-manufacturability of the fusion molecules provided, but also retain therapeutic activity (as provided in FIG. 15A).

5 Therefore, the fusion molecules comprising different ABP variants, as provided in SEQ ID NO:31, such as in SEQ ID NOs: 32-38 retain the A β oligomer binding ability of the parent ABP *in vitro* (Figs. 2B,2C, 12 and 15 A,B and C) as well as the BBB-penetrating property of the parent FC5 both *in vitro* and *in vivo* (Figs. 4, 5A, 6, 7, 17A, 17B, 18 and 19). The ABP is transported across the BBB by the carrier, for example FC5, *in vitro* and *in vivo*, as established
10 by its presence in the rat and dog CSF (Figs. 5 and 6). The ABP fusion was also transported and delivered to the target regions in the brain, such as hippocampus and cortex in both wild type and AD transgenic mice (Fig. 8 and 19). The brain-delivered ABP also promoted A β clearance in the CSF (Figs. 9C and 11A and 11B) and in cortical and hippocampal regions of AD transgenic mice and rat (Figs. 9B and 11C). In addition, and most importantly, A β
15 clearance in rat AD model correlated with enhanced hippocampal volume and neuronal connectivity (Fig. 12A and 12B) indicating desired pharmacological/physiological response to treatment.

Unexpectedly and most significantly, a single bolus of BBB-Fc-ABP reduced brain A β burden by 50% within 24 hrs of treatment (Fig 9B) compared to three months of multiple treatments
20 with free ABP to achieve similar results in animals (Fig 9A). Thus, the BBB-enabled ABP was shown to be far more potent than free ABP in reducing brain A β burden. Additionally, this compound has a substantially longer serum half-life compared to free- or FC5- ABP, an essential characteristic of a better therapeutic, due to its fusion with Fc (Fig 10).

In the prior art, the linking of ABP with a BBB carrier (WO 2006/133566) alone does not ensure
25 the generation of an effective molecule. Fusion with Fc to enhance serum half-life also does not ensure efficient transport of ABP across blood brain barrier. Suitable engineering and formulation of BBB carrier-, Fc fragment- and ABP fusion molecule, as provided in the fusion protein constructs of present invention, provide an efficient BBB-permeable therapeutic compound.

30 This novel bi-functional fusion molecule has distinct advantage over conventional therapeutic antibodies currently under development. First, the BBB-fused therapeutic ABP can penetrate the brain at much higher levels and at a faster rate, which substantially improves the therapeutic efficacy. In addition, ABP and BBB (ex. FC5) have relatively lower affinity towards their respective receptors and thus are likely cleared from the brain faster, and accordingly

facilitate faster clearance of ABP-bound A β . Unlike therapeutic antibodies that mainly employ reactive microglia/astrocytes for A β clearance (e.g., aducanumab), which is a slower process, the fusion molecule of the present invention likely employs faster perivascular drainage pathway for A β clearance. This is supported by a nearly 50% reduction in CNS A β within 24 hr of treatment compared to free ABP (Fig 9B) and antibody-based therapeutics, which require months of treatment with repeated multiple doses to achieve similar results.

Moreover, the present compound is less likely to elicit neuro-inflammatory response compared to antibody-based therapeutics.

BRIEF DESCRIPTION OF THE DRAWINGS

These and other features of the invention will now be described by way of example, with reference to the appended drawings, wherein:

Fig 1: shows schematic drawings of a blood brain barrier-crossing, amyloid-binding fusion protein. The fusion protein comprises a BBB-crossing single-domain antibody (BBB or BBB carrier), an Fc fragment (Fc) and an amyloid-binding peptide (ABP). Fig.1 shows the corresponding dimers of the fusion protein comprising BBB-Fc-ABP. The 3 components (i.e. BBB, Fc, and ABP) are depicted in various configurations, as illustrated in FIG 1A, 1B, 1C, and 1D.

Fig 2: shows the production of FC5-mFc-ABP and humanized FC5(H3)-hFc-ABP (ABP SEQ ID NO: 32) fusion molecule in CHO cells. As shown in FIG. 2A, a Coomassie blue stained gel after separation of FC5 fusion molecules by SDS-PAGE (NR- non-reducing and R- reducing conditions) showing successful production of recombinant fusion molecule. FIG. 2B and FIG. 2C: A β -oligomer binding of free ABP and BBB-Fc-ABP fusion protein by ELISA and Western blot (WB) Overlay assay. Free or fused ABP was immobilized on ELISA plate and exposed to A β . Bound A β was detected with A β -specific antibody 6E10 or 4G8. The fusion molecules were also separated by SDS-PAGE, transferred to PVDF paper and exposed to A β . Bound A β was detected with specific antibody as above. Results show that ABP retained its A β oligomer binding ability after fusion with the BBB carrier. Mo: A β monomers; Oli: A β Oligomers

Fig 3: shows an immunohistofluorescence assay of the binding of FC5-Fc-ABP to amyloid deposits in AD- Tg mice (B6.Cg-Tg, Jackson Lab). ABP retains the ability to bind naturally produced A β aggregates in AD-Tg mouse brain as shown by immunohistofluorescence assay. Brain sections from wild type and AD-transgenic mice were incubated with IR 800-labelled FC5-mFc-ABP and the bound fusion molecule was visualized under fluorescence microscope.

Selective binding (bright spots) were seen in brain sections from AD-Tg mice that produce A β deposits and not in brain sections from wild type mice that does not produce amyloid deposits.

Fig 4: shows BBB permeability of FC5 is retained after fusion with ABP *in vitro*. Blood brain barrier crossing of FC5-ABP fusion molecules was assessed in *in vitro* BBB models from rat and human. Fusion molecules crossing BBB were detected by nanoLC-MRM method (FIG. 4A, FIG. 4B and FIG. 4C) and by Western blot analysis using Fc-specific antibody (FIG. 4D, done in triplicate). FC5-mFc-ABP crossed the BBB as effectively as FC5-mFc, whereas Fc-ABP without the BBB carrier moiety FC5 did not traverse across the brain endothelial cell monolayer. As expected, control single domain antibodies EG2 and A20.1, or control full IgG (anti-HEL) did not cross the blood brain barrier. Similar results were obtained with humanized FC5-H3-hFc-ABP fusion protein (FIG. 4C and FIG. 4D).

Fig 5: shows serum and CSF pharmacokinetics of FC5-Fc-ABP *in vivo*. FC5-mFc-ABP was administered intravenously into rats via tail vein injection at the indicated doses (2.5, 6.25, 12.5, and 25 mg/kg). Serum and CSF were serially collected. FC5-Fc-ABP levels were quantified using nanoLC- MRM method. As shown in Fig 5, FC5-mFc-ABP appeared in the CSF in a time- and dose-dependent manner with C_{max} between 12 and 24 h, indicating transport of ABP by FC5 into brain and CSF compartments *in vivo*. Serum PK parameters (FIG. 5 and Table 1) show that alpha- and beta- half-life of FC5-mFc-ABP is similar to that of a full IgG (a benchmark antibody containing rat Fc).

Fig 6: shows serum and CSF PK profile of FC5-mFc-ABP in beagle dog. FC5-mFc-ABP was administered by intravenous injection to 10-12-year old beagle dogs and serum and CSF were serially collected and analyzed by nanoLC-MRM (FIG. 6A) and by Western blot using Fc-specific antibody (FIG. 6B). Asterisks indicates blood-contaminated sample (not shown in MRM analyses). As can be seen, FC5-mFc-ABP appeared in the CSF in a time-dependent manner indicating transport of ABP by FC5 across dog blood brain barrier *in vivo*. The PK parameters and CSF exposure were analyzed by WinNonlin software and are shown in Table 2 below.

Fig 7: shows BBB permeability and CSF appearance of FC5 fused with human Fc (hFc) and chemically linked to ABP (FC5-hFc-ABP) *in vivo* (rat model). FC5-hFc was linked with ABP-cystamide using a heterobifunctional cross-linker sulfo-SMCC (sulfosuccinimidy] 4-[N-maleimidomethyl] cyclohexane-1-carboxylate) according manufacturer's instructions (ThermoFisher Scientific). Chemically conjugated molecule was administered intravenously into rats via tail vein at 6.25 mg/kg and serum and CSF samples were collected at 4 and 24 hrs and analyzed by nanoLC-MRM. FC5-hFc-ABP appears in the CSF in a time-dependent

manner in contrast to Fc-ABP without the BBB carrier. It should be noted that in this chemically linked construct, C-terminus of ABP is linked to Fc fragment (random region) and N-terminus is free, unlike in the fusion construct, wherein it is N-terminus of ABP that is fused to C-terminus of Fc and C-terminus of ABP is free. This reversal in the orientation of ABP did not affect its

5 A β -binding ability and transport across the blood brain barrier.

Fig. 8: shows levels of FC5-mFc-ABP measured in various brain regions (cortex and hippocampus) after intravenous injection in mice. 15mg/kg dose of FC5-mFc-ABP was injected intravenously into the tail vein of either wild type (WT) or AD-transgenic (AD-Tg, B6.Cg-Tg, Jackson Lab) mice and brains were collected at 4 and 24 hrs after intra-cardiac saline perfusion. Hippocampal and cortical tissues were dissected and analyzed by nanoLC-MRM (FIG. 8A) and by Western blot using Fc-specific antibodies (FIG. 8B). Specific peptides belonging to all the three components of the fusion molecule (BBB, in this example FC5, Fc and ABP) were detected by MRM in both cortex and hippocampus, indicating that FC5 carrier

10 successfully delivered ABP to the target areas of the brain. Measured levels ranged between 750-1400 ng/g brain tissue at different time points, compared to ~50 ng/g tissue typically measured for control single-domain antibody A20.1 fused to Fc, or Fc fragment alone. This was further confirmed by Western blot analysis probing for Fc and ABP in tissue extracts (FIG. 8B). No protein signal of the fusion molecule was detected in animals receiving just saline by

15 Western blot. There was a dose-dependent increase in FC5-mFc-ABP levels detected by Western blot in the target regions of the brain.

Fig 9: shows the effect of ABP on A β levels in transgenic (Tg) mice: Comparison between treatment with ABP alone (FIG. 9A) or ABP fused with BBB carrier FC5 (FIG. 9B and FIG. 9C). Two different AD Tg mouse models, triple transgenic (3X Tg-AD, sv129/ C57BL6 mice harboring PS1M146V, APP_{Swe} and tauP301L transgenes, Dr. F.M. LaFerla, University of California) and double transgenic (B6.Cg-Tg, harboring PSEN1dE9 and APP_{Swe} transgenes, Jackson Lab) were used; mice were dosed subcutaneously (sc) with 300 nmol/kg of free ABP every second day over a 3-month or a 2-month period, respectively. At the end of the treatment period, A β levels in the brain were measured by ELISA. The treatment with ABP

25 alone resulted in 25-50% reduction in brain A β after 2-3 months of multiple treatments (every second day) (FIG. 9A). The FC5-mFc-ABP construct was injected intravenously into double-transgenic AD mice (B6.Cg-Tg, 15 mg/kg; equivalent of 220 nmol/kg) and brain A β levels were measured by both ELISA and nanoLC-MRM 24 h after injection. Unexpectedly, about 50% amyloid reduction was observed within 24 hr of treatment with FC5-mFc-ABP (FIG. 9B),

30 indicating that efficient brain delivery of ABP by FC5, suitably linked to Fc, dramatically increased the efficacy of ABP in reducing brain A β levels. CSF analysis also indicated a

significant decrease in A β ₁₋₄₂ levels within 24 hrs following FC5-mFc-ABP treatment (FIG. 9C). The signature peptide or epitope of A β detected by MRM or ELISA analyses is remote/different from the A β epitope recognized by ABP (therefore, not interfering with its quantification by either ELISA or MRM).

5 **Fig 10:** shows an example of enhanced serum half- life of FC5-Fc-ABP construct compared to FC5-ABP (i.e. without Fc component): FC5-ABP and FC5-Fc-ABP were injected into rats via tail vein and serial serum samples were collected at various time points and analyzed by direct ELISA with FC5-specific antibody. As can be seen in Fig 10 A, FC5-ABP construct was rapidly cleared in the serum (less than 1 hr) compared to FC5-Fc-ABP (Fig 10 B), indicating
10 substantial increase in the serum stability of the molecule comprising the Fc fragment.

Fig 11: shows the effect of FC5-mFc-ABP on brain amyloid burden in Tg rats: AD-Tg rats were dosed with either saline or FC5-mFc-ABP via tail vein every week over a period of four weeks (loading dose of 30mg/kg and subsequent four weekly doses of 15 mg/kg). CSF levels of FC5-mFc-ABP and A β were analyzed by nanoLC MRM (FIG. 11A and FIG 11B). Before
15 and after four weeks of treatment, brain A β levels were determined by PET scan using a specific A β -binding agent [18F] NAV4694. Following tracer injection, 60 min Dynamic images were acquired, transmission scans were obtained, images were reconstructed and Binding Potential (BP_{ND}) parametric maps were generated. FC5-mFc-ABP reduced CSF A β level in
20 FC5-mFc-ABP and A β was observed, as in Tg rats, suggesting target engagement and rapid clearance of A β by ABP delivered to the brain and CSF by FC5 (FIG. 11B). This was further corroborated by PET scan which clearly indicated a significant reduction (30-50%) of rat brain A β levels following four weeks of treatment with FC5-mFc-ABP (FIG. 11C).

Fig 12: shows volumetric Magnetic Resonance Imaging (MRI, using Fast Imaging with Steady-state Precession) and functional MRI (fMRI) of Tg rats before and after treatment with saline or
25 FC5-mFc-ABP. As shown in Fig 12 A, increased hippocampal volume was observed in ABP-treated Tg rats (Tg-ABP) compared to saline-treated Tg rats (Tg-Sal) after four weeks of treatment. As shown in Fig 12B, group comparison after four weeks of treatment showed that the ABP-treated Tg rats (Tg-ABP) had greater Anterior Cingulate Cortex (ACC) connectivity
30 compared to saline-treated Tg rats (Tg-Sal).

Fig 13: shows time- and dose -dependent appearance of FC5-mFc-ABP in the CSF of beagle dog and a decrease in CSF A β levels as seen Tg mice (Fig 9C) and Tg rats (Fig 11A and B). FC5-mFc-ABP was administered by intravenous injection to 10-12-year old beagle dogs at 15 mg/kg and 30 mg/kg and serum and CSF were serially collected and analyzed by nanoLC-

MRM for FC5-mFc-ABP and A β levels. As can be seen, FC5-mFc-ABP appeared in the CSF in a time-and dose-dependent manner. Importantly, as seen in Tg mice and Tg rats, there was a significant decrease in CSF A β levels within 24 hrs after the FC5-mFc2a-ABP injection, suggesting translational nature of FC5 carrier in larger animals and also cross-species efficacy of ABP in reducing CNS A β burden.

Fig 14: shows the generation of ABP fusion molecule with a different BBB carrier. To assess the versatility of ABP fusion molecule, ABP was successfully fused with another humanized BBB carrier IGF1R5 (H2). As shown, the bi-functionality of the molecule was retained, ABP's ability to bind A β oligomer (ELISA and overlay assays) and also IGF1R5's ability deliver ABP across BBB model *in vitro* (data not shown). This clearly indicates that ABP can be fused to different BBB-crossing single-domain antibodies to be delivered to the brain.

Fig 15: shows A β oligomer binding by different single-domain antibody-Fc-ABP constructs (FIG. 15A, 15B and 15C). In some of these constructs, ABP has been modified by site-specific mutations or removal of C-terminus portion of the molecules as indicated in the SEQ ID NOs. All constructs retained similar potency in binding A β oligomers by ELISA method.

Fig 16: shows the production of FC5-hFc1X7-ABP with specific mutations to improve stability and bio-manufacturability. FC5-hFc1X7-ABP carrying specific mutation (such as the ABP of SEQ ID NO: 35 or SEQ ID NO: 36) were produced in CHO cells and separated on SDS-PAGE under reducing (R) and non-reducing (NR) conditions and stained with Coomassie blue as described in Fig 2. Separated protein transferred to nitrocellulose membrane and immunoblotted with either FC5-specific or hFc-specific or ABP-specific antibodies. In another set, A β -binding of ABP in the fusion molecule was also tested by overlay assay. Bound A β was detected with A β -specific antibody 6E10. As can be seen, the methodic modification of ABP with specific mutation substantially enhanced the stability of the molecule generated, as indicated by single protein band under reducing and non-reducing conditions.

Fig 17: shows blood brain barrier permeability of various FC5-Fc-ABP constructs and IGF1R5-Fc-ABP construct *in vitro*. BBB- crossing was assessed in *in vitro* rat BBB model as described in Fig 4 and molecules crossing blood brain barrier were detected by nanoLC-MRM method. All ABP variants fused to humanized FC5 and IGF1R carriers crossed the blood brain barrier effectively. As expected, A20.1, a non-BBB permeable sdAb did not cross blood brain barrier, likewise ABP fused to A20.1 did not cross BBB (FIG. 17A). In Fig 17B, it is shown that "fingerprint" peptides for all the three components of the fusion molecule, FC5, Fc and ABP were detected by nanoLC-MRM, thereby indicating the transmigration of the intact FC5, Fc and ABP across the BBB.

Fig. 18: shows humanized FC5(H3)-hFc1X7-ABP construct (ABP, SEQ ID NO: 35 and SEQ ID NO: 36) are transported across *in vitro* blood-brain barrier intact by FC5. Blood brain barrier - crossing was assessed in *in vitro* rat BBB model as described for Fig. 4 and molecule crossing BBB was detected by Western blot and ELISA assays. Fig 18 (1) A and B show immunoblots probed with hFc- and ABP-specific antibodies, respectively. The molecular size is exactly identical to that of the fusion molecule that was applied to *in vitro* blood brain barrier model. Fig 18(1) C shows sandwich ELISA in which the molecule after crossing the BBB was captured by FC5-specific antibody on ELISA plate and detected with ABP-specific antibody. This sandwich ELISA confirmed that FC5(H3)-hFc1X7-ABP remained intact after crossing rat blood brain barrier *in vitro*. Similar results were obtained with FC5(H3)-hFc1X7-ABP (ABP, SEQ ID NO: 36); Fig 18(2) A, B, and C.

Fig. 19: shows humanized FC5(H3)-hFc1X7-ABP constructs (ABP, SEQ ID NO: 35 and SEQ ID NO: 36) are transported across *in vivo* blood-brain barrier and delivered to the brain intact by FC5. The FC5-ABP fusion molecule was administered intravenously into wild type and AD-Tg mice via tail vein and brains were collected following intra-cardiac perfusion as described for Fig. 8. Brain cortex was homogenized and extracted in RIPA buffer and the extract was subjected to Western blot and sandwich ELISA analysis as described for Fig. 18. Fig. 19A shows immunoblot probed with ABP-specific antibody. The molecular size is exactly identical to that of the fusion molecule that was injected into the animals. Fig. 19 B shows sandwich ELISA of the same extract, molecule in the extract captured with FC5-specific antibody on the ELISA plate and detected with ABP-specific antibody. This confirms immunoblot results that FC5(H3)-hFc1X7-ABP is transported across the BBB *in vivo* and delivered to the brain intact.

Fig. 20: shows immunohistochemical analysis of ex-vivo binding (A) and *in vivo* binding (B) of FC5(H3)-hFc1X7-ABP (ABP, SEQ ID NO: 36) to endogenous A β deposits in AD-Tg mouse brain (B6.Cg-Tg, Jackson Lab). Brain sections from AD-transgenic mice were incubated with FC5(H3)-hFc1X7-ABP (ABP, SEQ ID NO: 36) and the bound fusion molecule was visualized with HRP-conjugated FC5-specific antibody. Selective binding (black spots) was seen with FC5(H3)-hFc1X7-ABP construct but not with FC5(H3)-hFc1X7 without ABP (A), indicating ABP-dependent binding of A β deposits (target engagement) in the brain. No binding was seen in brain sections from wild type mice that does not produce amyloid deposits (data not shown). Similar binding of A β deposits were detected (using ABP-specific antibody) following intra-hippocampal injection (4hrs post-injection) of FC5(H3)-hFc-ABP construct into AD transgenic mice (B).

Fig. 21: shows target engagement by FC5(H3)-hFc1X7-ABP (ABP, SEQ ID NO: 32) *in vivo*. Fig 21 A shows binding of Alexa 647-labeled FC5-hFc-ABP construct to natural amyloid- β (A β)

deposits in AD transgenic mice *in vivo* after intra-hippocampal injection. Identity of A β was confirmed by probing the brain sections with A β -specific antibody 6E10 labelled with Alexa 488 and demonstrating co-localization of two signals (Merge). Fig 21 B shows demonstration of target engagement by ELISA. Following intra-hippocampal injection (4hrs post-injection) of FC5(H3)-hFc-ABP construct into wild type and AD transgenic mice, hippocampal formation was dissected and homogenized. FC5-ABP fusion construct was detected by sandwich ELISA using FC5 antibody as capturing antibody and ABP antibody as the detection antibody. *In vivo* binding of ABP to endogenous A β was detected by the same sandwich ELISA but with A β -specific antibody as the detection antibody. It is clear from the FIG 21 A and 21B that the FC5-ABP construct remains intact 4hrs post-injection in both wild type and AD transgenic mice. Most importantly, in Tg mice which expresses human A β , injected ABP binds A β and is pulled down as a complex (FC5(H3)-hFc-ABP*A β) indicating A β -target engagement by ABP *in vivo*.

Fig. 22: shows PK, PD comparison between non-humanized and humanized FC5-Fc-ABP constructs. FC5-mFc2a-ABP or FC5(H3)-hFc1x7-ABP was administered intravenously into rats via tail vein injection at 15 mg/kg as described for Fig 5. Serum and CSF were serially collected. FC5-Fc-ABP levels were quantified using nanoLC- MRM method. As shown in Fig 22 A, serum and CSF PK profile were very similar for non-humanized and humanized constructs. FC5-mFc2a-ABP or FC5(H3)-hFc1x7-ABP was administered intravenously into Tg mice via tail vein injection at 15 mg/kg as described for Fig 9B. FC5-Fc-ABP and A β levels in the CSF were measured by nanoLC-MRM as described in Fig 9B. As shown in Fig 22 B, the levels of non-humanized and humanized FC5-Fc-ABP in the CSF were similar, and most importantly, changes (decrease) in CSF A β levels were also very similar, indicating that humanization of FC5-Fc-ABP construct did not affect the PK and PD profile of the fusion construct.

25 DETAILED DESCRIPTION OF THE INVENTION

The present invention provides polypeptides, fusion proteins comprising said polypeptides, and fusion proteins comprising said polypeptides and antibodies or fragments thereof that transmute the blood-brain barrier.

The present invention provides polypeptides which binds beta-amyloid (β -amyloid). The polypeptides (or proteins) that bind β -amyloid may selectively bind pathologically relevant β -amyloid₁₋₄₂ (A β ₁₋₄₂) aggregates, and may be abbreviated and referred to herein as ABP or ABP variants (or collectively as ABP).

The present invention provides fusion proteins comprising ABP or an ABP variant linked to an antibody or fragment thereof that crosses the blood-brain barrier. In a preferred embodiment, the fusion protein comprising ABP and a BBB additionally comprises an Fc or fragment thereof, wherein the ABP and BBB components of the fusion protein may be linked via an Fc region or portion thereof. For example, a construct of the present invention may comprise BBB-Fc-ABP or BBB-Fc-L-ABP, wherein L may be any suitable linker. The BBB-Fc-ABP or BBB-Fc-L-ABP construct provided may be a single chain polypeptide or a dimeric polypeptide, wherein the single-chain polypeptide comprising BBB-Fc-ABP may form a multimer (preferably a dimer) via the component Fc region.

- 10 The present invention relates to compounds that transmigrate the blood-brain barrier, and uses thereof. More specifically, the present invention relates to compounds comprising a BBB and an ABP and their use in the treatment of Alzheimer's disease (AD).

There is a need for therapeutic formulations that can efficiently transmigrate ABP across the blood brain barrier, and provide the clearing of A β through the binding of ABP. In the prior art, a 40-amino acid A β -binding peptide (ABP) was identified that selectively binds A β ₁₋₄₂ oligomers implicated in AD development (WO 2006/133566). This A β -binding peptide inhibits A β binding to cellular proteins and inhibits A β ₁₋₄₂-induced cell toxicity *in vitro* (Chakravarthy et al, 2013). This A β -binding peptide binds amyloid deposits in AD transgenic mice brain, as well as binds amyloid deposits in the brains from AD patients *in vitro*. More importantly when directly injected into live AD transgenic mice brain (Chakravarthy et al, 2014) ABP targets natural amyloid deposits *in vivo*. Thus, ABP can potentially target CNS A β , can assist in clearing A β from the brain, and reduce its toxic effect. However, systemically-administered ABP has limited ability to cross BBB and access the brain parenchyma by itself.

Accordingly, although ABP has been shown to bind A β deposits when directly applied, in order to bind and clear A β from the brain, parenterally administered ABP needs to permeate the blood brain barrier. The present invention advantageously provides an ABP fused to a BBB-permeable single-domain antibody, such as FC5 or IGF1R, via an Fc fragment to provide a bi-specific blood brain barrier-permeable therapeutic (Farrington et al, 2014). The BBB-Fc-ABP construct of the present invention may dimerize, i.e. the BBB-Fc-ABP single-chain fusion protein may form dimers of two single chain fusion proteins to yield a dimeric compound wherein each single chain of the dimer comprises a BBB, a Fc fragment and an ABP, to provide a BBB-Fc-ABP dimer. The BBB-Fc-ABP or BBB-Fc-L-ABP construct and dimers thereof, allows for the efficient transmigration of ABP across the blood brain barrier. Accordingly, the advantageous therapeutic clearing of A β through the binding of ABP in CSF and brain parenchyma is provided in the constructs and methods of the present invention.

In order to enable brain delivery of ABP and improve its efficacy, the 40-amino acid ABP polypeptide is presently fused to the C-terminus of an Fc fragment, whereas a BBB-permeable single-domain antibody, such as FC5 (WO 2002/057445), is fused to the N-terminus of the same Fc fragment, to create a bi-specific BBB-permeable therapeutic (Farrington et al, 2014).

- 5 In a non-limiting embodiment of the present invention, the Fc fragment may be mouse (SEQ ID NO. 39) or human (SEQ ID NO: 40; SEQ ID NO: 41). In a preferred embodiment, the Fc fragment of the present invention was engineered to reduce effector functions (Shields et al., 2001). For example the Fc fragment may be hFc1x7 (SEQ ID NO:40) wherein the Fc fragment in the BBB-Fc-ABP fusion protein advantageously allows for the dimerization of the fusion
- 10 protein to yield a therapeutically effective fusion molecule (BBB-Fc-ABP dimer) capable of transmigrating the blood brain barrier. In an embodiment, the BBB-Fc-ABP fusion protein may be FC5-H3-hFc1x7-ABP(6G) (SEQ ID NO: 47) and dimers thereof. The fusion protein may comprise a linker sequence L that is as embodied in SEQ ID NO: 47 or SEQ ID NO:53, or any suitable linker sequence.
- 15 The present invention provides an isolated polypeptide which binds beta-amyloid (β -amyloid). A polypeptide of the present invention may comprise a sequence.

X_1 TFX₂TX₃X₄ASAQASLASKDKTPKSKSKX₅X₆STQLX₇SX₈VX₉NI (SEQ ID NO: 31)

where X_1 = G or A, X_2 = G or V, X_3 = G or A, X_4 = G or A, X_5 = G or V, X_6 = G or V, X_7 = G or V, X_8 = G or A, X_9 = G or A

- 20 A polypeptide (ABP and ABP variant) of the present invention may be selected from the group consisting of: SEQ ID NO:27 to SEQ ID NO:38 or a sequence substantially identical thereto. In an embodiment, the polypeptide provided is an ABP variant comprising a sequence substantially equivalent to SEQ ID NO: 31. A sequence that is substantially equivalent thereto may confer equivalent stability to the fusion molecules.
- 25 The present invention provides a compound, namely a fusion protein, comprising an antibody or fragment thereof that transmigrates across the blood brain barrier (BBB) and a polypeptide that binds β -amyloid. The present invention provides fusion proteins comprising a BBB, a polypeptide that binds β -amyloid, and an Fc.

- The term "antibody", also referred to in the art as "immunoglobulin" (Ig), as used herein refers
- 30 to a protein constructed from paired heavy and light polypeptide chains; various Ig isotypes exist, including IgA, IgD, IgE, IgG, and IgM. When an antibody is correctly folded, each chain folds into a number of distinct globular domains joined by more linear polypeptide sequences. For example, the immunoglobulin light chain folds into a variable (V_L) and a constant (C_L)

domain, while the heavy chain folds into a variable (V_H) and three constant (C_H , C_{H2} , C_{H3}) domains. Interaction of the heavy and light chain variable domains (V_H and V_L) results in the formation of an antigen binding region (Fv). Each domain has a well-established structure familiar to those of skill in the art.

- 5 The light and heavy chain variable regions are responsible for binding the target antigen and can therefore show significant sequence diversity between antibodies. The constant regions show less sequence diversity, and are responsible for binding a number of natural proteins to elicit important biochemical events. The variable region of an antibody contains the antigen-binding determinants of the molecule, and thus determines the specificity of an antibody for its
- 10 target antigen. The majority of sequence variability occurs in six hypervariable regions, three each per variable heavy (V_H) and light (V_L) chain; the hypervariable regions combine to form the antigen-binding site, and contribute to binding and recognition of an antigenic determinant. The specificity and affinity of an antibody for its antigen is determined by the structure of the hypervariable regions, as well as their size, shape, and chemistry of the surface they present
- 15 to the antigen. Various schemes exist for identification of the regions of hypervariability, the two most common being those of Kabat and of Chothia and Lesk. Kabat et al (1991) define the "complementarity-determining regions" (CDR) based on sequence variability at the antigen-binding regions of the V_H and V_L domains. Chothia and Lesk (1987) define the "hypervariable loops" (H or L) based on the location of the structural loop regions in the V_H and V_L domains.
- 20 These individual schemes define CDR and hypervariable loop regions that are adjacent or overlapping, those of skill in the antibody art often utilize the terms "CDR" and "hypervariable loop" interchangeably, and they may be so used herein. The CDR/loops are identified herein according to the Kabat scheme.

- An "antibody fragment" as referred to herein may include any suitable antigen-binding antibody
- 25 fragment known in the art. The antibody fragment may be a naturally-occurring antibody fragment, or may be obtained by manipulation of a naturally-occurring antibody or by using recombinant methods. For example, an antibody fragment may include, but is not limited to a Fv, single-chain Fv (scFv; a molecule consisting of V_L and V_H connected with a peptide linker), Fab, $F(ab')_2$, single-domain antibody (sdAb; a fragment composed of a single V_L or V_H), and
- 30 multivalent presentations of any of these. Antibody fragments such as those just described may require linker sequences, disulfide bonds, or other type of covalent bond to link different portions of the fragments; those of skill in the art will be familiar with the requirements of the different types of fragments and various approaches and various approaches for their construction.

In a non-limiting example, the antibody fragment may be an sdAb derived from naturally-occurring sources. Heavy chain antibodies of camelid origin (Hamers-Casterman et al, 1993) lack light chains and thus their antigen binding sites consist of one domain, termed V_HH . sdAb have also been observed in shark and are termed V_{NAR} (Nuttall et al, 2003). Other sdAb may
5 be engineered based on human Ig heavy and light chain sequences (Jespersen et al, 2004; To et al, 2005). As used herein, the term "sdAb" includes those sdAb directly isolated from V_H , V_HH , V_L , or V_{NAR} reservoir of any origin through phage display or other technologies, sdAb derived from the aforementioned sdAb, recombinantly produced sdAb, as well as those sdAb generated through further modification of such sdAb by humanization, affinity maturation,
10 stabilization, solubilization, camelization, or other methods of antibody engineering. Also encompassed by the present invention are homologues, derivatives, or fragments that retain the antigen-binding function and specificity of the sdAb.

SdAb possess desirable properties for antibody molecules, such as high thermostability, high detergent resistance, relatively high resistance to proteases (Dumoulin et al, 2002) and high
15 production yield (Arbabi-Ghahroudi et al, 1997); they can also be engineered to have very high affinity by isolation from an immune library (Li et al, 2009) or by *in vitro* affinity maturation (Davies & Riechmann, 1996). Further modifications to increase stability, such as the introduction of non-canonical disulfide bonds (Hussack et al, 2011a,b; Kim et al, 2012), may also be brought to the sdAb.

A person of skill in the art would be well-acquainted with the structure of a single-domain antibody (see, for example, 3DWT, 2P42 in Protein Data Bank). An sdAb comprises a single immunoglobulin domain that retains the immunoglobulin fold; most notably, only three CDR/hypervariable loops form the antigen-binding site. However, and as would be understood
20 by those of skill in the art, not all CDR may be required for binding the antigen. For example, and without wishing to be limiting, one, two, or three of the CDR may contribute to binding and recognition of the antigen by the sdAb of the present invention. The CDR of the sdAb or variable domain are referred to herein as CDR1, CDR2, and CDR3.
25

The antibody or fragment thereof as described herein may transmute the blood-brain barrier. The brain is separated from the rest of the body by a specialized endothelial tissue known as
30 the blood-brain barrier (BBB). The endothelial cells of the BBB are connected by tight junctions and efficiently prevent many therapeutic compounds from entering the brain. In addition to low rates of vesicular transport, one specific feature of the BBB is the existence of enzymatic barrier(s) and high level(s) of expression of ATP-dependent transporters on the abluminal (brain) side of the BBB, including P-glycoprotein (Gottesman and Pastani, 1993; Watanabe,
35 1995), which actively transport various molecules from the brain into the blood stream

(Samuels, 1993). Only small (<500 Daltons) and hydrophobic (Pardridge, 1995) molecules can more readily cross the BBB. Thus, the ability of the antibody or fragment thereof as described above to specifically bind the surface receptor, internalize into brain endothelial cells, and undergo transcytosis across the blood brain barrier by evading lysosomal degradation is useful
5 in the neurological field. The antibody or fragment thereof that crosses the blood-brain barrier may be used to carry other molecules, such as therapeutics, for delivery to the brain tissue. The antibody or fragment thereof may be any suitable antibody or fragment thereof known in the art to transmigrate the blood brain barrier.

The present invention provides a compound, or fusion protein, comprising an antibody or
10 fragment thereof that transmigrates the blood brain barrier (BBB). An antibody or fragment of the present invention may bind to, for example, transmembrane protein 30A (TMEM30A), as described in WO 2007/036021, or to an Insulin-Like Growth Factor 1 Receptor (IGF1R) epitope, or isoforms, variants, portions, or fragments thereof.

The antibody or fragment thereof in the compound of the present invention may comprise a
15 complementarity determining region (CDR) 1 sequence of HYTMG (SEQ ID NO:1); a CDR2 sequence of RITWGGDNTFYNSVKG (SEQ ID NO:2); and a CDR3 sequence of GSTSTATPLRVDY (SEQ ID NO:3); or

a CDR1 sequence of EYPSNFYA (SEQ ID NO:4), a CDR2 sequence of VSRDGLTT (SEQ ID NO:5), a CDR3 sequence of AIVITGVWNKVDVNSRSYHY (SEQ ID NO:6); or

20 a CDR1 sequence of GGTVSPTA (SEQ ID NO:7), a CDR2 sequence of ITWSRGTT (SEQ ID NO:8), a CDR3 sequence of AASTFLRILPEESAYTY (SEQ ID NO:9); or

a CDR1 sequence of GRTIDNYA (SEQ ID NO:10), a CDR2 sequence of IDWGDGGX; where X is A or T (SEQ ID NO:11), a CDR3 sequence of AMARQSRVNLDVARYDY (SEQ ID NO:12).

25 As previously stated, the antibody or fragment thereof may be an sdAb of camelid origin or derived from a camelid V_HH , and thus may be based on camelid framework regions; alternatively, the CDR described above may be grafted onto V_{NAR} , V_HH , V_H or V_L framework regions. In yet another alternative, the hypervariable loops described above may be grafted onto the framework regions of other types of antibody fragments (Fv, scFv, Fab) of any source
30 (for example, mouse or human) or proteins of similar size and nature onto which CDR can be grafted (for example, see Nicaise et al, 2004).

The present invention further encompasses an antibody or fragment thereof that is chimeric (or chimerized), veneered, or humanized. Chimeric antibodies or fragments thereof are constructs in which the native variable domain (of mouse or camelid origin) is linked to human constant domain(s) (see Gonzales et al 2005). Veneering or re-surfacing of antibodies involves replacing exposed residues in the framework region of the native antibody or fragment thereof with the amino acid residues in their human counterpart (Padlan, 1991; Gonzales et al 2005). Humanization of an antibody or antibody fragment comprises replacing an amino acid in the sequence with its human counterpart, as found in the human consensus sequence, without loss of antigen-binding ability or specificity; this approach reduces immunogenicity of the antibody or fragment thereof when introduced into human subjects. In this process, one or more than one of the CDR defined herein may be fused or grafted to a human variable region (V_H , or V_L), to other human antibody (IgA, IgD, IgE, IgG, and IgM), to human antibody fragment framework regions (Fv, scFv, Fab), or to human proteins of similar size and nature onto which CDR can be grafted (Nicaise et al, 2004). In such a case, the conformation of said one or more than one hypervariable loop is likely preserved, and the affinity and specificity of the sdAb for its target (i.e., an epitope on brain endothelial cells, such as TMEM30A, or an IGF1R epitope) brain endothelial cells) is likely minimally affected. As is known by those of skill in the art, it may be necessary to incorporate certain native amino acid residues into the human framework in order to retain binding and specificity. Humanization by CDR grafting is known in the art (for example, see Tsurushita et al, 2005; Jones et al, 1986; Tempest et al, 1991; Riechmann et al, 1988; Queen et al, 1989; reviewed in Gonzales et al, 2005 – see also references cited therein), and thus persons of skill would be amply familiar with methods of preparing such humanized antibody or fragments thereof.

The provided antibody or fragment thereof may be a humanized version of the FC5 antibody (described in WO 2002/057445) or an IGF1R antibody. FC5 (comprising a sequence of any one of SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17), and IGF1R (comprising a sequence of any one of SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, or SEQ ID NO: 26) bind to the surface of receptor epitopes on brain endothelial cells and subsequently transmigrates the blood-brain barrier (BBB). FC5 has also been shown to act as a carrier to usher molecules of various sizes across the BBB (see for example, WO 2011/127580). The antigen mediating FC5 transmigration was tentatively identified as transmembrane domain protein 30A (TMEM30A; WO 2007/036021), which is enriched on the surface of brain endothelial cells.

For example, and without wishing to be limiting, the antibody or fragment thereof may comprise the sequence:

5 X₁VQLVX₂SGGGLVQPGGSLRLSCAASGFKITHYTMGWX₃RQAPGKX₄X₅EX₆VSRT
WGGDNFTFYSNSVKGRFTISRDN SKNTX₇YLQMNSLRAEDTAVYYCAAGSTSTATPL
RVDYWGQGT LTVSS (SEQ ID NO:13), where X₁=D or E, X₂=A or E, X₃=F or V,
X₄=E or G, X₅=R or L, X₆=F or W, X₇=L or V, or a sequence substantially identical
thereto;

10 X₁VX₂LX₃ESGGGLVQX₄GGSLRLSCX₅ASEYPSNFYAMSWX₆RQAPGKX₇X₈EX₉VX₁₀
GVSRDGLTTLYADSVKGRFTX₁₁SRDNX₁₂KNTX₁₃X₁₄LQMNSX₁₅X₁₆AEDTAVYYCAI
VITGVWNKVDVNSRSYHYWGQGT X₁₇VTVSS, (SEQ ID NO:18), where X₁ is E or Q;
X₂ is K or Q; X₃ is V or E; X₄ is A or P; X₅ is V or A; X₆ is F or V; X₇ is E or G; X₈ is R
or L; X₉ is F or W; X₁₀ is A or S; X₁₁ is M or I; X₁₂ is A or S; X₁₃ is V or L; X₁₄ is D or
Y; X₁₅ is V or L; X₁₆ is K or R; and X₁₇ is Q or L; or a sequence substantially identical
thereto;

15 X₁VX₂LX₃ESGGGLVQX₄GGSLRLSCX₅X₆SGGTVSPTAMGWX₇RQAPGKX₈X₉EX₁₀V
X₁₁HITWSRGTTTRX₁₂ASSVKX₁₃RFTISRDX₁₄X₁₅KNTX₁₆YLQMNSLX₁₇X₁₈EDTAVYYC
AASTFLRILPEESAYTYWGQGT X₁₉VTVSS, (SEQ ID NO:21), where X₁ is E or Q; X₂
is K or Q; X₃ is V or E; X₄ is A or P; X₅ is A or E; X₆ is V or A; X₇ is V or F; X₈ is G or
E; X₉ is L or R; X₁₀ is F or W; X₁₁ is G or S; X₁₂ is V or Y; X₁₃ is D or G; X₁₄ is N or S;
20 X₁₅ is A or S; X₁₆ is L or V; X₁₇ is K or R; X₁₈ is A or S; and X₁₉ is L or Q; or a
sequence substantially identical thereto;

25 X₁VX₂LX₃ESGGGLVQX₄GGSLRLSCAASGRTIDNYAMAWX₅RQAPGKX₆X₇EX₈VX₉TID
WGDGGX₁₀RYANSVKGRFTISRDNX₁₁KX₁₂TX₁₃YLQMNX₁₄LX₁₅X₁₆EDTAVYX₁₇CAMAR
QSRVNLDVARYDYWGQGT X₁₈VTVSS (SEQ ID NO: 24), where X₁ is E or Q; X₂ is K or
Q; X₃ is V or E; X₄ is A or P; X₅ is V or S; X₆ is D or G; X₇ is L or R; X₈ is F or W; X₉ is A
or S; X₁₀ is A or T; X₁₁ is A or S; X₁₂ is G or N; X₁₃ is M or L; X₁₄ is N or R; X₁₅ is E or R;
X₁₆ is P or A; X₁₇ is S or Y; and X₁₈ is Q or L; or a sequence substantially identical
thereto.

30 More specifically, and without wishing to be limiting in any manner, the antibody or fragment
thereof may comprise a sequence selected from any one of:

DVQLQASGGGLVQAGGSLRLSCAASGFKITHYTMGWFRQAPGKEREFVSRTWGGDN
TFYSNSVKGRFTISRDN AKNTVYLQMNSLKPEDTADYYCAAGSTSTATPLRVDYWG
KGTQVT VSS (SEQ ID NO:14);

EVQLVESGGGLVQPGGSLRLSCAASGFKITHYTMGWWRQAPGKGLEWWSRITWGGDN
TFYSNSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAAGSTSTATPLRVDYWGQ
TLVTVSS (SEQ ID NO: 15);

5 EVQLVESGGGLVQPGGSLRLSCAASGFKITHYTMGWWRQAPGKGLEWWSRITWGGDN
TFYSNSVKGRFTISRDN SKNTVYLQMNSLRAEDTAVYYCAAGSTSTATPLRVDYWG
QGTLVTVSS (SEQ ID NO:16);

EVQLVESGGGLVQPGGSLRLSCAASGFKITHYTMGWFRQAPGKGLEFVSRITWGGDN
TFYSNSVKGRFTISRDN SKNTVYLQMNSLRAEDTAVYYCAAGSTSTATPLRVDYWG
QGTLVTVSS (SEQ ID NO:17);

10 QVKLEESGGGLVQAGGSLRLSCVASEYPSNFYAMSWFRQAPGKEREVAGVSRDGLT
TLYADSVKGRFTMSRDNAKNTVDLQMNSVKAEDTAVYYCAIVITGVWNKVDVNSRSYH
YWGQGTQVTVSS (SEQ ID NO: 19);

15 EVQLVESGGGLVQPGGSLRLSCAASEYPSNFYAMSWFRQAPGKEREVSGVSRDGLT
TLYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAIVITGVWNKVDVNSRSYH
WGQGTTLVTVSS (SEQ ID NO: 20);

QVKLEESGGGLVQAGGSLRLSCEVSGGTVSPTAMGWFRQAPGKEREVGHITWSRGT
TRVASSVKDRFTISRDSA KNTVYLQMNSLKSEDTAVYYCAASTFLRILPEESAYTYWGQ
GTQVTVSS (SEQ ID NO: 22);

20 QVQLVESGGGLVQPGGSLRLSCAVSGGTVSPTAMGWFRQAPGKGLEFVGHITWSRGT
TRYASSVKGRFTISRDN SKNTVYLQMNSLRAEDTAVYYCAASTFLRILPEESAYTYWGQ
GTLVTVSS (SEQ ID NO: 23)

QVKLEESGGGLVQAGGSLRLSCAASGRTIDNYAMAWSRQAPGKDREFVATIDWGDGG
ARYANSVKGRFTISRDN AKGTMYLQMNNLEPEDTAVYSCAMARQSRVNLDVARYDYW
GQGTQVTVSS (SEQ ID NO: 25);

25 QVQLVESGGGLVQPGGSLRLSCAASGRTIDNYAMAWWRQAPGKGLEWWATIDWGDG
GTRYANSVKGRFTISRDN SKNTMYLQMNSLRAEDTAVYYCAMARQSRVNLDVARYDY
WGQGTTLVTVSS (SEQ ID NO: 26); and

a sequence substantially identical thereto. The antibody or fragment thereof may be a single-domain antibody.

A substantially identical sequence may comprise one or more conservative amino acid mutations. It is known in the art that one or more conservative amino acid mutations to a reference sequence may yield a mutant peptide with no substantial change in physiological, chemical, physico-chemical or functional properties compared to the reference sequence; in such a case, the reference and mutant sequences would be considered “substantially identical” polypeptides. A conservative amino acid substitution is defined herein as the substitution of an amino acid residue for another amino acid residue with similar chemical properties (e.g. size, charge, or polarity). These conservative amino acid mutations may be made to the framework regions of the sdAb while maintaining the CDR sequences listed above and the overall structure of the CDR of the antibody or fragment; thus the specificity and binding of the antibody are maintained.

A substantially equivalent sequence may comprise one or more conservative amino acid mutations; wherein the mutant peptide is substantially equivalent with respect to peptide stability and bio-manufacturability. Substantially equivalent may refer to equivalent with respect to fusion molecule stability; for example, the lack of a degradation product, or a low molecular weight band, as seen in SDS PAGE (reducing and non-reducing conditions). It is known in the art that one or more conservative amino acid mutations to a reference sequence may yield a mutant peptide with no substantial change in physiological, chemical, physico-chemical or functional properties compared to the reference sequence; in such a case, the reference and mutant sequences would be considered “substantially equivalent” polypeptides.

In a non-limiting example, a conservative mutation may be an amino acid substitution. Such a conservative amino acid substitution may substitute a basic, neutral, hydrophobic, or acidic amino acid for another of the same group. By the term “basic amino acid” it is meant hydrophilic amino acids having a side chain pK value of greater than 7, which are typically positively charged at physiological pH. Basic amino acids include histidine (His or H), arginine (Arg or R), and lysine (Lys or K). By the term “neutral amino acid” (also “polar amino acid”), it is meant hydrophilic amino acids having a side chain that is uncharged at physiological pH, but which has at least one bond in which the pair of electrons shared in common by two atoms is held more closely by one of the atoms. Polar amino acids include serine (Ser or S), threonine (Thr or T), cysteine (Cys or C), tyrosine (Tyr or Y), asparagine (Asn or N), and glutamine (Gln or Q). The term “hydrophobic amino acid” (also “non-polar amino acid”) is meant to include amino acids exhibiting a hydrophobicity of greater than zero according to the normalized consensus hydrophobicity scale of Eisenberg (1984). Hydrophobic amino acids include proline (Pro or P), isoleucine (Ile or I), phenylalanine (Phe or F), valine (Val or V), leucine (Leu or L), tryptophan (Trp or W), methionine (Met or M), alanine (Ala or A), and glycine (Gly or G).

"Acidic amino acid" refers to hydrophilic amino acids having a side chain pK value of less than 7, which are typically negatively charged at physiological pH. Acidic amino acids include glutamate (Glu or E), and aspartate (Asp or D).

Sequence identity is used to evaluate the similarity of two sequences; it is determined by
5 calculating the percent of residues that are the same when the two sequences are aligned for maximum correspondence between residue positions. Any known method may be used to calculate sequence identity; for example, computer software is available to calculate sequence identity. Without wishing to be limiting, sequence identity can be calculated by software such as NCBI BLAST2 service maintained by the Swiss Institute of Bioinformatics (and as found at
10 ca.expasy.org/tools/blast/), BLAST-P, Blast-N, or FASTA-N, or any other appropriate software that is known in the art.

The substantially identical sequences of the present invention may be at least 90% identical; in another example, the substantially identical sequences may be at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% identical, or any percentage therebetween, at the amino acid level to
15 sequences described herein. Importantly, the substantially identical sequences retain the activity and specificity of the reference sequence. In a non-limiting embodiment, the difference in sequence identity may be due to conservative amino acid mutation(s). In a non-limiting example, the present invention may be directed to an antibody or fragment thereof comprising a sequence at least 95%, 98%, or 99% identical to that of the antibodies described herein.

20 The antibody or fragment thereof in the compound of the present invention may be linked to an Fc domain, for example, but not limited to human Fc domains. The Fc domains may be selected from various classes including, but not limited to, IgG, IgM, or various subclasses including, but not limited to IgG1, IgG2, etc. In this approach, the Fc gene is inserted into a vector along with the sdAb gene to generate a sdAb-Fc fusion protein (Bell et al, 2010; Iqbal et al, 2010); the fusion protein is recombinantly expressed then purified. For example, and
25 without wishing to be limiting in any manner, multivalent display formats may encompass chimeric formats of FC5-H3 and its mutational variants linked to an Fc domain. Such antibodies are easy to engineer and to produce, can greatly extend the serum half-life of sdAb (Bell et al., 2010).

30 The Fc domain in the compound as just described may be any suitable Fc fragment known in the art. The Fc fragment may be from any suitable source; for example, the Fc may be of mouse or human origin. Other preferred Fc or Fc fragment embodiments may modulate, modify or suppress an immunological effector function (Shields 2001). Other highly preferred Fc fragment embodiments may mediate clearance of the fusion peptide from the brain (Caram-

Salas N 2011). In a specific, non-limiting example, the Fc may be the mouse Fc2a fragment or human Fc1 fragment (Bell et al, 2010; Iqbal et al, 2010). In a specific, non-limiting example, the multimerized construct may comprise the isolated or purified antibody or fragment as described herein and an Fc of sequence of SEQ ID NO:39; SEQ ID NO:40; SEQ ID NO:41.

- 5 Accordingly, the BBB-Fc-ABP fusion proteins provided herein may form dimers via the Fc to provide a bivalent, bifunctional BBB-Fc-ABP.

The compound of the present invention comprises an antibody or fragment thereof linked to a polypeptide that binds β -amyloid. The linker may be any polypeptide, comprising neutral or hydrophilic amino acids, of suitable length. In a non-limiting example, the length is preferably
10 less than 12 amino acids, and the polypeptide is GGGSGGGS. Other chemical linkers may be employed using non-peptidic forms such as amide or ester linkages in a correct orientation. For example, SEQ ID NO: 53 provides a fusion protein comprising any suitable linker, wherein any fusion protein of the present invention, i.e. SEQ ID NO: 42 – 52 may comprise any suitable linker as exemplified in SEQ ID NO: 53. The polypeptide that binds β -amyloid ($A\beta$) may bind
15 pathologically relevant β -amyloids, such as $A\beta_{1-42}$ aggregates, which are implicated in AD pathology; the polypeptide may bind $A\beta$ with high affinity (in the nM range), inhibit $A\beta$ binding to cellular proteins and $A\beta_{1-42}$ -induced cell toxicity *in vitro*, and binds amyloid deposits in AD transgenic mice brain as well as in the brains from AD patients *in vitro*. The polypeptide in the compound of the present invention does not bind the reverse peptide $A\beta_{42-1}$.

- 20 In a compound provided herein, the polypeptide that binds β -amyloid may comprise a sequence selected from the group consisting of: (SEQ ID NO:27); (SEQ ID NO:28); (SEQ ID NO:29); (SEQ ID NO:30); (SEQ ID NO:31); (SEQ ID NO:32); (SEQ ID NO:33); (SEQ ID NO:34); (SEQ ID NO:35); (SEQ ID NO:36); (SEQ ID NO:37); (SEQ ID NO:38); and a sequence substantially identical thereto. A “substantially identical” sequence is as described
25 above.

Accordingly, the present invention provides a polypeptide that binds β -amyloid and variants thereof (i.e. ABP and ABP variants). The ABP variant may comprise a sequence having SEQ ID NO: 31, for example, the ABP variant provided may comprise a sequence selected from the group consisting of: SEQ ID NO: 31 to SEQ ID NO:38 or a sequence substantially equivalent
30 thereto.

Accordingly, there is provided ABP polypeptide sequences comprising specific systematic and methodical modifications based on detailed biophysical characterization of the ABP. The specific and methodically directed modifications to the ABP polypeptide comprise the novel and unobvious ABP variants of the present invention, as provided in SEQ ID NO:31.

The peptide provided herein may comprise an ABP comprising a sequence that may be selected from the group consisting of SEQ ID NO: 27 – SEQ ID NO: 38, and a sequence substantially equivalent thereto.

5 The peptide provided herein may comprise an ABP variant, for example, an ABP comprising a sequence that may be selected from any one of SEQ ID NO: 32 to SEQ ID NO:38, or any equivalent sequence to SEQ ID NO:31. A construct comprising an ABP or an ABP variant, as provided herein, exhibits advantageous improvement in compound stability and bio-manufacturability (as can be seen in Fig.14).

10 The fusion proteins and compounds provided herein, exhibit improved therapeutic efficacy. More specifically, the compounds provided comprise specifically modified ABP that allows the generation of stable BBB-Fc-ABP fusion molecule for enhanced bio-manufacturability (production in human mammalian expression system). The specific modifications to ABP provided herein were systematic and methodical modifications based on detailed biophysical characterization of the ABP. The modified ABP provided herein, may comprise, for example, a
15 sequence selected from SEQ ID NO: 32 - SEQ ID NO: 38, and a sequence substantially equivalent thereto, such as SEQ ID NO: 31. The compounds provided herein advantageously exhibit improved stability and bio-manufacturability, and a most significant increase in the efficacy of reducing A β levels in brain; wherein a 50% amyloid reduction was observed within 24 hr of treatment with a construct of the present invention.

20 By the term “linked”, also referred to herein as “conjugated”, it is meant that two moieties are joined directly or indirectly (e.g., via a linker), covalently or non-covalently (e.g., adsorption, ionic interaction). A covalent linkage may be achieved through a chemical cross-linking reaction, or through fusion using recombinant DNA methodology combined with any peptide expression system, such as bacteria, yeast or mammalian cell-based systems. When
25 conjugating the antibody or fragment thereof to the polypeptide binding A β or Fc, a suitable linker may be used. For example, a suitable linker may be any polypeptide, comprising neutral or hydrophilic amino acids, of suitable length that allows for the conjugation of the components of the BBB-Fc-ABP protein fusion. For example, the linker that allows the components of the fusion protein (for example, in SEQ ID NO: 42-52) to be according linked, and is not limited to
30 the GGGGSGGGGS or (GGGS)_n linker highlighted therein and may be any suitable linker (i.e. as in the non-limited fusion protein of SEQ ID NO: 53). In a non-limiting example, the length is preferably less than 12 amino acids, and the polypeptide may be GGGSGGGGS. Other chemical linkers may be employed using non-peptidic forms such as amide or ester linkages in a correct orientation. One of skill in the present art would be well aware of linkers or method of

linking an antibody or fragment thereof to a polypeptide. Methods for linking an antibody or fragment thereof to a polypeptide or Fc are well-known to a person of skill in the art.

The compound provided herein comprises an antibody or fragment thereof, a polypeptide that binds β -amyloid, and an Fc fragment, linked to provide a construct (also referred to herein as a compound or fusion molecule), wherein the construct comprises a fusion protein and dimers thereof. The antibody or fragment thereof may be linked to a polypeptide that binds β -amyloid via an Fc fragment, or a suitable linker.

The antibody or fragment thereof comprises a sequence selected from any one of: SEQ ID NO:14, SEQ ID NO:14, SEQ ID NO: 15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO 23, SEQ ID NO:25, SEQ ID NO:26, and a sequence substantially identical thereto. The antibody or fragment thereof transmigrates the blood brain barrier. The antibody or fragment may be a sdAb; wherein the sdAb may be humanized.

The polypeptide that binds β -amyloid comprises a sequence selected from the group consisting of: SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, and a sequence substantially identical thereto, and preferably a sequence comprising SEQ ID NO: 31.

The Fc fragment comprises a sequence selected from any one of SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, and a sequence substantially identical thereto. The Fc fragment of the present invention may be any suitable Fc fragment with attenuated effector function. The Fc fragment provided in the fusion protein allows for the formation of dimeric structures; wherein for example a single-chain fusion protein comprising a sequence selected from the group consisting of SEQ ID NO: 42 to SEQ ID NO: 53 may form dimeric structures conjugated via the Fc fragment therein.

Accordingly, a compound or construct of the present invention may comprise a sequence selected from the group consisting of SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44 SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53 and a sequence substantially identical thereto, or dimeric structures thereof.

The present invention provides fusion proteins comprising a BBB-crossing single-domain antibody (BBB), an Fc fragment (Fc) and an amyloid-binding peptide (ABP), wherein each moiety may be linked to provide fusion molecules, as illustrated in FIG. 1. For example, in a non-limiting embodiment of the represent invention, the BBB may linked to the N-terminus of

the Fc and the ABP linked to the C-terminus of the Fc fragment (FIG. 1A). Fig.1 shows the corresponding dimers of the single-chain fusion protein comprising BBB-Fc-ABP. The 3 components (i.e. BBB, Fc, and ABP) are depicted in various configurations, as illustrated in FIG 1A, 1B, 1C, and 1D. In a specific, non-limiting example of the compound of the present invention, the polypeptide that binds β -amyloid may comprise the sequence SEQ ID NO: 31. In an embodiment the ABP variant comprises the sequence:

GTFGTGGASQAASLASKDKTPKSKSKGGSTQLKSRVKNI (SEQ ID NO:36) referred to herein as ABP(6G) and a sequence substantially equivalent thereto.

The antibody or fragment thereof may comprise the sequence

10 EVQLVESGGGLVQPGGSLRLSCAASGFKITHYTMGWFRQAPGKGLEFVSRITWGGD
NTFYSNSVKGRFTISRDN SKNTVYLQMNSLRAEDTAVYYCAAGSTSTATPLRVDYWG
QGTLVTVSS (SEQ ID NO:17) referred to herein as FC5-H3.

The antibody or fragment thereof may further comprise the sequence of a human Fc, such as

15 AEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEGPE
VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL
PAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ
PENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL
SPG (SEQ ID NO:40), also referred to herein as hFc1X7.

Without wishing to be limiting in any manner, the compound of the present invention may
20 comprise the sequence:

25 EVQLVESGGGLVQPGGSLRLSCAASGFKITHYTMGWFRQAPGKGLEFVSRITWGGD
NTFYSNSVKGRFTISRDN SKNTVYLQMNSLRAEDTAVYYCAAGSTSTATPLRVDYWG
QGTLVTVSSAEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCV
VDVSHEGPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY
KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIA
VEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN
HYTQKSLSLSPGGGGSGGGSGTFTGGASQAASLASKDKTPKSKSKGGSTQLKS
RVKNI (SEQ ID NO:47), also referred to herein as FC5-H3-hFc1X7-ABP(6G).

30 Table Summary of some representative BBB-Fc-ABP fusion protein constructs.

BBB	Fc	ABP
FC5 (SEQ ID NO: 14)	mFc2a (SEQ ID NO: 39)	ABP (SEQ ID NO: 30)
FC5(H3) (SEQ ID NO: 17)	mFc2a (SEQ ID NO: 39)	ABP (SEQ ID NO: 30)
FC5(H3) (SEQ ID NO: 17)	hFc1x7 (SEQ ID NO: 40)	ABP (SEQ ID NO: 30)
FC5(H3) (SEQ ID NO: 17)	hFc1x7 (SEQ ID NO: 40)	ABP(G) SEQ ID NO: 32)
FC5(H3) (SEQ ID NO: 17)	hFc1x7 (SEQ ID NO: 40)	ABP(GG-G) SEQ ID NO: 35
FC5(H3) (SEQ ID NO: 17)	hFc1x7 (SEQ ID NO: 40)	ABP(6G) SEQ ID NO: 36)
FC5(H3) (SEQ ID NO: 17)	hFc1X0 (SEQ ID No: 41)	ABP(GG-G) SEQ ID NO: 35
FC5(H3) (SEQ ID NO: 17)	hFc1x7 (SEQ ID NO: 40)	ABP(trc) SEQ ID NO: 38)
IGF1R-5(H2) (SEQ ID NO: 26)	mFc2a (SEQ ID NO: 39)	ABP (SEQ ID NO: 30)

It is noted that in the above-table, the BBB-Fc-ABP fusion protein constructs may also comprise a linker (L) wherein the BBB-Fc-ABP construct is a BBB-Fc-L-ABP fusion protein of the present invention, wherein L may be GGGSGGGGS or any suitable linker, as exemplified
5 in the consensus linker sequence in SEQ ID NO:53.

The compound of the present invention, as provided in the fusion protein of SEQ ID NO:47 also referred to herein as FC5-H3-hFc1X7-L-ABP(6G), may comprise variations in each of the components comprised therein, for example, ABP may be ABP (GG-G), as provided in SEQ ID NO: 46. The linker sequence provided therein (as highlighted for example, in SEQ ID NO:42
10 to SEQ ID NO: 53) may be any linker that allows for the linking of the BBB-Fc-ABP fusion protein. The compound of the present invention may also comprise additional sequences to aid in expression, detection or purification of a recombinant antibody or fragment thereof. Any such sequences or tags known to those of skill in the art may be used. For example, and without wishing to be limiting, the antibody or fragment thereof may comprise a targeting or
15 signal sequence (for example, but not limited to ompA), a detection/purification tag (for example, but not limited to c-Myc, His₅, or His₆), or a combination thereof. In another example, the additional sequence may be a biotin recognition site such as that described by Cronan et al (WO 95/04069) or Voges et al (WO/2004/076670). As is also known to those of skill in the art,

linker sequences may be used in conjunction with the additional sequences or tags, or may serve as a detection/purification tag.

The present invention also encompasses nucleic acid sequences encoding the compounds as described herein. Given the degeneracy of the genetic code, a number of nucleotide sequences would have the effect of encoding the polypeptide, as would be readily understood by a skilled artisan. The nucleic acid sequence may be codon-optimized for expression in various micro-organisms. The present invention also encompasses vectors comprising the nucleic acids as just described. Furthermore, the invention encompasses cells comprising the nucleic acid and/or vector as described.

- 10 The present invention further encompasses a composition comprising one or more than one compound as described herein and a pharmaceutically acceptable diluent, excipient, or carrier. The composition may also comprise a pharmaceutically acceptable diluent, excipient, or carrier. The diluent, excipient, or carrier may be any suitable diluent, excipient, or carrier known in the art, and must be compatible with other ingredients in the composition, with the method of delivery of the composition, and is not deleterious to the recipient of the composition. The composition may be in any suitable form; for example, the composition may be provided in suspension form or powder form (for example, but limited to lyophilised or encapsulated). For example, and without wishing to be limiting, when the composition is provided in suspension form, the carrier may comprise water, saline, a suitable buffer, or additives to improve solubility and/or stability; reconstitution to produce the suspension is effected in a buffer at a suitable pH to ensure the viability of the antibody or fragment thereof. Dry powders may also include additives to improve stability and/or carriers to increase bulk/volume; for example, and without wishing to be limiting, the dry powder composition may comprise sucrose or trehalose. It would be within the competency of a person of skill in the art to prepare suitable compositions comprising the present compounds.

- A method of treating Alzheimer's disease is also provided, in which a compound or composition of the present invention is administered to a subject in need thereof. Any appropriate route of administration may be utilized, including but not limited to intravenous, intraperitoneal, parenteral, intracranial, intramuscular, subcutaneous, oral, or nasal. The optimal dose for administration and route of administrations are generally determined experimentally.

A method of reducing toxic β -amyloid levels in the cerebrospinal fluid (CSF) and brain parenchyma of subjects having increased β -amyloid levels is provided. More specifically, toxic β -amyloid levels in the cerebrospinal fluid (CSF) and brain parenchyma of subjects is reduced

as early as 24 hours of a single parenteral administration of the composition of the present invention.

The present invention will be further illustrated in the following examples. However, it is to be understood that these examples are for illustrative purposes only and should not be used to
5 limit the scope of the present invention in any manner.

Example 1: Construction of the BBB-Fc-L-ABP fusion molecules

Fusion molecules comprising:

- a) the FC5 sdAb (SEQ ID NO:14), a murine Fc (SEQ ID NO:39) and ABP (SEQ ID NO: 30),
- 10 b) a humanized version of FC5 (FC5-H3; SEQ ID NO: 17), a human Fc (SEQ ID NO: 40) and ABP (SEQ ID NO: 30; SEQ ID NO: 31; SEQ ID NO: 32; SEQ ID NO: 33; SEQ ID NO: 34; SEQ ID NO: 35; SEQ ID NO: 36; SEQ ID NO: 37; SEQ ID NO: 38),
- c) a humanized version of IGF1R-5 sdAb (IGF1R-5-H2; SEQ ID NO: 26), a murine Fc (SEQ ID NO:39) and ABP (SEQ ID NO: 30)

15 were prepared. A schematic of the fusion protein construct is shown in Figure 1. The fusion protein comprises a BBB-crossing single-domain antibody, an Fc fragment and an amyloid-binding peptide.

Example 2: Production of BBB-Fc-L-ABP fusion molecules

The constructs FC5-mFc-ABP and humanized FC5(H3)-hFc-ABP described in Example 1
20 were expressed in CHO cells, and the expressed compounds were purified on MabSelect Sure affinity columns

Constructs comprising FC5 variants FC5 and FC5-H3 V_HH fused to N-terminal of mouse or human Fc antibody fragment fused to ABP variants at their C-terminus as described in example 1 were prepared, expressed, and purified.

25 The FC5-Fc-ABP variant DNAs (DNA synthesis suppliers) were cloned into mammalian expression vector pTT5 (Durocher 2002). Polyplexes for a final concentration of 1 mg of DNA per liter of cells were pre-formed by mixing a combination of plasmid vector (80%), pTT-AKTdd (15%, activated mutant of Protein Kinase B), and pTTo-GFP (5%, to monitor transfection efficiency) with PEI MAX solution (Polysciences cat no 24765). The PEI:DNA ratio was 4:1
30 (W:W), both prepared in supplemented F17 medium (4 mM Glutamine, 0.1% Kolliphor). The mixture was incubated for 5 minutes at room temperature prior to addition to the cell culture. The volume of the DNA/PEI polyplexes represent 10% of final culture volume (e.i. 100 ml per 1L

culture). Twenty four hours post-transfection, the cultures were fed with tryptone N1 at a final concentration of 1% (with 40% w/v solution, Organotechnie) and 0.5 mM valproic acid (200 mM solution). The transfection/production were monitored for cell density and viability as well as for productivity titer (mg of Fc per L) and were harvested (supernatant) by centrifugation when the cell viability reach a minimum of 65%. Clarified cell culture medium was filtered through a 0.45 µm membrane prior to its application on a column packed with 5 ml of protein-A MabSelect SuRe resin (GE Healthcare). After loading, the column was washed with 5 volumes of phosphate-buffered saline pH 7.1 (PBS) and the antibody was eluted with 100 mM sodium citrate buffer pH 3.0. Fractions containing the eluted antibody were pooled and a buffer exchange was performed by loading on a desalting Econo-Pac column (BioRad) equilibrated in PBS. Desalted antibody was then sterile-filtered by passing through a Millex GP (Millipore) filter unit (0.22 µm) and aliquoted.

SDS-PAGE and Aβ overlay: Protein samples, prepared in Laemmli sample buffer (heated at 70°C for non-reducing and at 95°C for reducing gels, βME or DTT), were separated by SDS-PAGE on 12% Tris-Tricine gels or TGX4-15% gels (BioRad). Gels were either stained with Coomassie blue or the proteins were transferred to PVDF or nitrocellulose membranes for Western blot/Aβ overlay assay. The immunoblots were blocked with non-fat dry milk and then exposed to Aβ preparations for 45 min at room temperature (50-100nM) and the bound Aβ was detected using 6E10 antibody as described previously (Chakravarthy et al. 2013).

ELISA: Aβ-binding assays were carried out as described by Chakravarthy et al (2013). Maxisorp 96-well ELISA plates (Nunc) were coated with (100-500 ng/well) either free ABP (synthetic) or various FC5-ABP constructs overnight at 4°C in PBS. The wells were blocked with 1% BSA in TBS-T for 30 min and then incubated with Aβ₁₋₄₂ preparations consisting predominantly of either monomer and dimers (Mo) or higher oligomers (Oli) in TBS-T at RT for 45 min with gentle agitation. Following three TBS-T washes, bound Aβ was detected by incubating HRP-conjugated Aβ-specific antibody (6E10 or 4G8) for 90 min at RT in TBS-T. The bound antibody was detected with SureBlue™ TMB reagent kit (KPL) by colorimetric measurement at 450nm according to manufacturer's instructions.

A Coomassie blue stained gel after separation of FC5 fusion molecules by SDS-PAGE (NR- non-reducing and R- reducing conditions) indicating successful production of recombinant fusion molecule is shown in Fig. 2A. Aβ-oligomer binding of free ABP and FC5-Fc-ABP fusion protein by ELISA and Western blot (WB) Overlay assay is shown in Fig. 2B and C. Free or fused ABP was immobilized on ELISA plate by coating samples in phosphate buffered saline (PBS) overnight at 4°C and exposed to Aβ preparations as described by Chakravarthy et al.,

2013. Bound A β was detected with A β -specific antibody 6E10 or 4G8 (Chakravarthy et al., 2013). The fusion molecules were also separated by SDS-PAGE, transferred to PVDF paper and exposed to A β oligomers. Bound A β was detected with specific antibody as above. Results show that ABP retained its A β oligomer binding ability after fusion with the BBB carrier.

5 Mo: A β monomers; Oli: A β Oligomers

Example 3: Binding of BBB-Fc-L-ABP fusion molecules to A β deposits in AD-Tg mice (B6.Cg-Tg, Jackson Lab) in vitro

The constructs produced in Example 2 were submitted to immunohistofluorescence assay to evaluate whether the FC5-Fc-ABP fusion molecules retained the ability to bind naturally-produced amyloid deposits in mouse brain as described (Chakravarthy et al., 2014). Frozen hemi-brains from wild type (Wt) and AD transgenic (AD-Tg) mice were embedded in OCT and 10- μ m sections were prepared using a Jung CM 3000 cryostat and stored at -80°C. Tissue sections were thawed and OCT peeled from sections with a razor blade and then incubated with Dako protein blocking reagent for 30 min at room temperature. Blocking agent was removed and sections were gently washed in TBS. IR 800-labelled FC5-mFc-ABP (1:250 dilution of 5.0 μ g/ μ l solution) in antibody diluent was added and incubated for 1 h at room temperature. Sections were then washed twice with TBS, rinsed in Milli Q water, excess rinse solution removed and sections were cover-slipped with Dako Fluorescent Mounting Media. Sections visualized under fluorescence microscope (Fig. 3). Selective binding (bright spots) was seen in brain sections from AD-Tg mice that produce A β deposits and not in brain sections from Wt mice that does not produce amyloid deposits, indicating that ABP in the FC5-Fc-ABP construct retains the ability to bind naturally produced A β aggregates in AD-Tg mouse brain. In the BBB-Fc-ABP constructs provided, the BBB may be FC5 or an anti-IGF1R antibody.

25

Example 4: BBB transmigration of FC5-Fc-L-ABP fusion molecules in vitro

BBB- crossing of FC5-Fc-ABP fusion molecules was assessed in *in vitro* BBB models from rat and human (Fig. 4). Fusion molecules crossing BBB were screened in *in vitro* BBB permeability assay, using a single-time point for Papp determination. The quantification of variants was done by MRM-ILIS (FIG. 4A, 4B and 4C).

30

SV40-immortalized Adult Rat Brain (SV-ARBEC) and Human Brain Endothelial Cells (HBEC) were used to generate an *in vitro* blood-brain barrier (BBB) model as described (Garberg et al., 2005; Haqqani et al., 2013). Sv-ARBEC (80,000 cells/membrane) were seeded on a 0.1mg/mL rat tail collagen type I-coated tissue culture inserts (pore size-1 μ m; surface area 0.9 cm²,

Falcon) in 1 ml of growth medium. The bottom chamber of the insert assembly contained 2 ml of growth medium supplemented with the immortalized neonatal rat astrocytes-conditioned medium in a 1:1 (v/v) ratio. Equimolar amounts (5.6 μ M) of positive (FC5 constructs) or negative controls (A20.1, a *Clostridium difficile* toxin A binding V_HH; and EG2, an EGFR binding V_HH), and Fc-ABP from Example 1 were tested for their ability to cross the rat or human *in vitro* BBB model. Following exposure of equimolar amounts of the sdAb to the luminal side of the BBB, samples were taken after 15, 30 and 60min from the abluminal side. The sdAb content of each sample was then quantified by mass spectrometry (multiple reaction monitoring – isotype labeled internal standards; MRM – ILIS) (Fig. 4A, 4B, 4C).

- 10 **MRM-ILIS:** The methods are all as described in Haqqani et al. (2013). Briefly, to develop the SRM (selected reaction monitoring also known as multiple reaction monitoring (MRM)) assay for V_HH, each V_HH was first analyzed by nanoLC-MS/MS using data-dependent acquisition to identify all ionizable peptides. For each peptide, the 3 to 5 most intense fragment ions were chosen. An initial SRM assay was developed to monitor these fragments at attomole amounts
- 15 of the digest (about 100-300 amol). Fragments that showed reproducible intensity ratios at low amounts (i.e., had Pearson $r^2 \geq 0.95$ compared to higher amounts) were considered stable and were chosen for the final SRM assay. To further optimize the assay, elution times for each peptide were also included, with care taken to not choose peptides that have close m/z (mass-to-charge ratio) and elution times.
- 20 A typical multiplexed SRM analysis of V_HH in cell media or body fluids (serum or cerebrospinal fluid (CSF)) involved spiking known amount of ILIS (0.1-10 nM) followed by injecting 100-400 ng of CSF or cultured media proteins (0.3-1 μ L) or about 50-100 ng of serum proteins (1-3 nanoliters) into the nanoLC-MS system. The precursor m/z of each target peptide ion was selected in the ion trap (and the remaining unrelated ions were discarded) at the specified
- 25 elution time for the target, followed by collision induced dissociation (CID) fragmentation, and selection of only the desired fragment ions in the ion trap for monitoring by the detector. For quantification analysis, raw files generated by the LTQ (ThermoFisher) were converted to the standard mass spectrometry data format mzXML and intensities were extracted using an in-house software called Q-MRM (Quantitative-MRM; see Haqqani et al. 2013), which is a
- 30 modified version of MatchRx software. For each V_HH, extracted-ion chromatograms were generated for each of its fragment ion that consisted of combined intensities within 0.25 Da of the fragment m/z over the entire elution time. To obtain a final intensity value for each fragment, all intensities within 0.5 min of the expected retention times were summed. A V_HH was defined as detectable in a sample if the fragments of at least one of its peptides showed
- 35 the expected intensity ratios, i.e., the final intensity values showed a strong Pearson

correlation $r \geq 0.95$ and $p < 0.05$ compared with the final intensities values of its corresponding pure V_HH .

Samples containing mixtures of V_HH (media, serum, CSF) were reduced, alkylated and trypsin-digested as previously described (Haqqani et al., 2012; Gergov et al., 2003). The digests
5 (tryptic peptides) were acidified with acetic acid (5% final concentration) and analyzed on a reversed-phase nanoAcquity UPLC (Waters, Milford, MA) coupled to LTQ XL ETD or LTQ Orbitrap ETD mass spectrometer (ThermoFisher, Waltham, MA). The desired aliquot of the sample was injected and loaded onto a 300 μm I.D. \times 0.5 mm 3 μm PepMaps C18 trap (ThermoFisher) then eluted onto a 100 μm I.D. \times 10 cm 1.7 μm BEH130C18 nanoLC column
10 (Waters) using a gradient from 0% - 20% acetonitrile (in 0.1% formic) in 1 minute, 20% - 46% in 16 min, and 46% - 95% in 1 min at a flow rate of 400 nL/min. The eluted peptides were ionized into the mass spectrometer by electrospray ionization (ESI) for MS/MS and SRM analysis using CID for fragmentation of the peptide ions. The CID was performed with helium as collision gas at normalized collision energy of 35% and 30 ms of activation time. Ion
15 injection times into linear ion trap were adjusted by the instrument using an automatic gain control (AGC) target value of 6×10^3 and a maximum accumulation time of 200 ms.

Determination of the apparent permeability coefficient: Quantified values can be directly plotted or the P_{app} (apparent permeability coefficient) values can be determined with the given formula [Q_r/dt = cumulative amount in the receiver compartment versus time; A = area of the cell
20 monolayer; C_0 = initial concentration of the dosing solution] and plotted. The P_{app} value is commonly used to determine the ability of a molecule to cross the BBB. P_{app} values are a measure of the specific permeability of the compound across brain endothelial monolayer.

The specific peptides used for detection and quantification of FC5 constructs are shown in Table 1 below.

25

30

TABLE 1

Type	Tryptic MRM peptide	Protein
FC5 peptide	ITWGGDNTFYNSVK + ILIS	FC5-mFc-ABP
mFc peptide	NTEPVLDSDGSYFMYSK	FC5-mFc-ABP, host IgGs
ABP peptide	ASAQASLASK	FC5-mFc-ABP
IGF1R5-H2 peptide	GLEWVATIDWGDGGTR	IGF1R5-H2-mFc-ABP
IGF1R5-H2 peptide	AEDTAVYYCAMAR	IGF1R5-H2-mFc-ABP
A β	LVFFAEDVGSNK	Host APP, A β 1-42 or A β 1-40
Albumin	APQVSTPTLVEAAR	Host albumin

- 5 The samples were also analyzed by Western blot analysis using Fc-specific antibody (FIG. 4D, done in triplicate) as described in Example 2. FC5-mFc-ABP crossed the blood brain barrier as effectively as FC5-mFc, whereas Fc-ABP without the BBB carrier moiety FC5 did not traverse across the brain endothelial cell monolayer. As expected, control single domain antibodies EG2 and A20.1, or control full IgG (anti-HEL) did not cross the blood brain barrier.
- 10 Similar results were obtained with humanized FC5(H3)-hFc-ABP fusion protein (FIG. 4C and FIG. 4D). Similar results were obtained with IGF1R5-mFc-ABP ABP (Fig 17 A and B).

Example 5: BBB transmigration and Pharmacokinetics of FC5-Fc-L-ABP fusion molecules in vivo

- The ability of the constructs of Example 2 to transmigrate the blood brain barrier into the brain, specifically into the cerebrospinal fluid (CSF), was evaluated *in vivo*, as well as to quantify the construct presence in CSF and serum. FC5-mFc-ABP was administered intravenously into rats via tail vein at the indicated doses (2.5, 6.25, 12.5, and 25 mg/kg). Serum and CSF were serially collected. FC5-Fc-ABP levels were quantified using nanoLC- MRM method.
- 15

The technique used for multiple sampling of cisterna magna CSF was developed at NRC by modification of previously described methods (Huang et al., 1995; Kornhuber et al., 1986)). All animals were purchased from Charles River Laboratories International, Inc. (Wilmington, MA, USA). Animals were housed in groups of three in a 12 h light/dark cycle at a temperature of 24°C, a relative humidity of 50 ± 5%, and were allowed free access to food and water. All animal procedures were approved by the NRC's Animal Care Committee and were in compliance with the Canadian Council of Animal Care guidelines. Male Wistar rats aged 8-10 weeks (weight range, 230–250 g) were used in all studies.

In all experiments, test antibodies (FC5 Fc-fusions) were administered intravenously into tail vein in equimolar doses (7 mg/kg). CSF sample collections were made from cisterna magna by needle puncture up to five times over 96 hours. For sample collection rats were briefly and lightly anesthetized with 3% isoflurane, placed in a stereotaxic frame with the head rotated downward at a 45° angle. A 2-cm midline incision between the ears beginning at the occipital crest was made and muscles separated to expose dura mater covering cisternae magna. A 27G butterfly needle (QiuckMedical, Cat# SV27EL) with tubing attached to 1 ml syringe was used to puncture dura and aspirate the ~20 µl of CSF. The CSF was then transferred into the sample glass vial (Waters, Cat#186000384c) and placed in -80°C freezer until further analysis.

Blood samples were collected from the tail vein in a commercially available tube (BD microtainer, Cat# 365956). After clotting at room temperature for 15-30 minutes, the clot was removed by centrifuging at 1100 rcf (3422rpm) for 10 min; serum was then transferred into a clean glass vial (Waters, Cat#186000384c), frozen on dry ice and stored at -80°C until further analysis. At the end of collection, rats were sacrificed by cardiac puncture. Blood and CSF PK analyses were performed using WinLin 6.0 program.

Serum and CSF samples were analyzed by mass spectrometry and nanoLC-SRM based quantification as described in Example 4 using peptide signatures shown in Table 1.

CSF collection is a delicate procedure during which CSF can be easily contaminated with blood. Since the amounts of V_HH s were expected to be much smaller in the CSF (<0.1%) than blood, even a slight contamination with blood could seriously compromise the value of an individual CSF sample. It was therefore necessary to develop stringent exclusion criteria for blood-contaminated CSF samples. To evaluate blood-CSF albumin ratio, a nanoLC-SRM method was developed for quantifying albumin levels in plasma and CSF. An albumin peptide APQVSTPTLVEAAR was selected based on its unique retention time and m/z value (Mol Pharm) in order to have minimum interference with other peptide peaks in the multiplex assay.

The intensity of the peptide was quantified in both CSF and plasma samples using SRM as described above. The albumin ratio was calculated as follows for each rat:

$$\text{Albumin Ratio} = \text{Intensity per nL of plasma analyzed} / \text{Intensity per nL of CSF analyzed}$$

A ratio of 1500 and below was considered as blood contaminated.

- 5 As shown in Fig 5, FC5-mFc-ABP appeared in the CSF in a time- and dose-dependent manner with C_{max} between 12 and 24 h, indicating transport of ABP by FC5 into brain and CSF compartments *in vivo* (A). Serum PK parameters (FIG 5 and Table 2 below) show that alpha- and beta- half-life of FC5-mFc-ABP is similar to that of a full IgG (a benchmark antibody containing rat Fc) and is substantially higher than that of ABP or FC5 or FC5-ABP without Fc.

10

TABLE 2

Dose range: 2.5 mg/kg, 6 mg/kg, 12 mg/kg, 25 mg/kg,

mAb				FC5mFc-ABP (FC5 domain)			
Parameter	Estimate	Units	CV (%)	Parameter	Estimate	Units	CV (%)
V1	114.6	mL/kg	2.3	V1	144.5	mL/kg	2.2
V2	74.4	mL/kg	6.5	V2	218.4	mL/kg	27.2
CL	0.664	mL/(kg*hr)	1.9	CL	1.056	mL/(kg*hr)	26.5
CLd	2.682	mL/(kg*hr)	41.8	CLd	3.789	mL/(kg*hr)	10.6
Alpha T _{1/2}	11.2055	hr	38.0	Alpha T _{1/2}	14.36	hr	14.3
Beta T _{1/2}	205.279	hr	2.2	Beta T _{1/2}	263.8	hr	41.3
Vss	189.0	mL/kg	1.4	Vss	362.9	mL/kg	16.3

- **Serum half life (both distribution phase and terminal) are similar between 'benchmark mAb' and FC5mFc-cargo**

Example 6: Delivery of FC5-ABP construct to the brain in non-rodent larger animal

- 15 Serum and CSF PK profile of FC5-mFc-ABP was assessed in beagle dog. FC5-mFc-ABP was administered by intravenous injection to 10-12-year old beagle dogs and serum and CSF were serially collected and analyzed by nanoLC-MRM (left panel) and by Western blot using Fc-specific antibody (FIG. 6B) as described above in Example 5. Asterisks indicates blood-contaminated sample (not shown in MRM analyses). As can be seen, FC5-mFc-ABP appeared in the CSF in a time-dependent manner indicating transport of ABP by FC5 across

dog blood brain barrier in vivo, confirming the translational nature of the BBB carrier. The PK parameters and CSF exposure were analyzed by WinNonlin software and are shown in Table 3 below.

5

TABLE 3

Parameter	Unit	Dog 1		Dog 2	
		Mean Estimate	SD	Mean Estimate	SD
t _{1/2}	h	96	21	89	21
AUC 0-t	ng/ml*h	8.80E+06	1.99E+06	8.84E+06	2.16E+06
AUC 0-inf_obs	ng/ml*h	1.01E+07	2.21E+06	1.04E+07	2.76E+06
Cl_obs	ml/h/kg	1.54	0.36	1.53	0.48
Vss_obs	ml/h/kg	193	7	205	10
Alpha half life	h	19	10	13	8.5
Beta half life	h	114	37	166	151
$\frac{AUC_{0-t,csf}}{AUC_{0-t,serum}}$	%	0.88	0.09	0.52	0.04

Example 7: BBB permeability

BBB permeability and CSF appearance of FC5 fused with human Fc (hFc) and chemically linked to ABP (FC5-hFc-ABP) *in vivo* (rat model). FC5-hFc was linked with ABP-cystamide using a heterobifunctional cross-linker sulfo-SMCC (sulfosuccinimidyl 4-[N-maleimidomethyl] cyclohexane-1-carboxylate) according manufacturer's instructions (ThermoFisher Scientific).
5 Chemically conjugated molecule was administered intravenously into rats via tail vein at 6.25 mg/kg and serum and CSF samples were collected at 4 and 24 hrs and analyzed as described in Fig. 5. FC5-hFc-ABP appears in the CSF in a time-dependent manner, however Fc-ABP without the BBB carrier does not, confirming FC5-mediated transport of ABP across the blood
10 brain barrier, and as illustrated in FIG. 7.

Example 8: Delivery of BBB-Fc-L-ABP construct to the brain

The ability of the FC5-Fc-ABP constructs of Example 2 to transmigrate the blood brain barrier and penetrate the brain parenchyma *in vivo* was assessed in mice.

FC5-mFc-L-ABP was administered by intravenous injection via tail vein at either 15 mg/kg to
15 wild type (WT) and AD-transgenic (AD-Tg, B6.Cg-Tg, Jackson Lab) mice (Fig. 8A and 8B) or at 7.5, 15 and 30 mg/kg to AD-Tg mice, and circulated for 4 and 24h. Mice were then thoroughly perfused with 10 ml of heparinized (100U/ml) saline at a rate of 1 ml/min via the left common carotid artery to facilitate specific perfusion of the brain. Brains were then removed, and hippocampal and cortical tissues were dissected and immediately frozen and stored at -
20 80°C until use. Frozen tissue was homogenized in ice-cold homogenization buffer containing 50 mM Tris-HCl pH 8, 150 mM NaCl and protease inhibitor cocktail (Sigma-Aldrich, Oakville, ON) using Dounce homogenizer (10-12 stroke at 4°C). Samples were then sonicated three times for 10 s each at 4°C and insoluble material was removed (10,000 xg for 10 min at 4°C). The supernatant was analyzed for protein content, and about 0.5 µg of protein was used for
25 SRM analysis (Fig. 8A) using methods described in Example 4 and peptide signatures shown in Table 1. Samples were also analysed by Western blot using mFc-specific antibodies (FIG. 8B and 8C). Specific "signature" peptides belonging to all the three components of the fusion molecule (FC5, Fc and ABP) were detected by MRM in both cortex and hippocampus, indicating that FC5 carrier successfully delivered ABP to the target areas of the brain (only
30 data from FC5 peptide is shown in Fig 8A). Measured levels ranged between 750-1400 ng/g brain tissue at different time points, compared to ~50 ng/g tissue typically measured for control single-domain antibody A20.1 fused to Fc, or Fc fragment alone. This was further confirmed by Western blot analysis probing for Fc and ABP in tissue extracts (8B and 8C). No protein signal of the fusion molecule was detected in animals receiving just saline by Western blot. There

was a dose-dependent increase in FC5-mFc-ABP levels detected by Western blot in the target regions of the brain (FIG. 8C). These results clearly indicate that FC5 successfully delivers ABP to the target regions of the brain (i.e., the hippocampus and cortex) in wild type (WT) and AD-Tg mice.

5 Example 9: Clearance of A β from mouse brain

To evaluate the efficacy of ABP on the amyloid burden in Tg mice, the results of treatment with ABP alone or FC5-ABP construct were compared.

Comparison between treatment with ABP alone (FIG.9A) or ABP fused with BBB carrier FC5 (FIG. 9B and 9C). Two different AD Tg mouse models, triple transgenic (3X Tg-AD, sv129/
10 C57BL6 mice harboring PS1M146V, APP_{Swe} and tauP301L transgenes, Dr. F.M. LaFerla, University of California) and double transgenic (B6.Cg-Tg, harboring PSEN1dE9 and APP_{Swe} transgenes, Jackson Lab) were used; mice were dosed subcutaneously (sc) with 300 nmol/kg of free ABP every second day over a 3-month or a 2-month period, respectively. At the end of the treatment period, A β levels in the brain were measured by ELISA using a commercial
15 assay kit (InVitrogen, KHB3544) according to manufacturer's assay procedure. The treatment with ABP alone resulted in 25-50% reduction in brain A β after 2-3 months of multiple treatments (every second day) (9A). The FC5-mFc-ABP construct was injected intravenously into double-transgenic AD mice (B6.Cg-Tg, 15 mg/kg; equivalent of 220 nmol/kg) and brain A β levels were measured by both ELISA and nanoLC-MRM 24 h after injection as described
20 above in Example 8. Unexpectedly, about 50% amyloid reduction was observed within 24 hr of treatment with FC5-mFc-ABP (Fig 9B), indicating that efficient brain delivery of ABP by FC5 dramatically increased the efficacy of ABP in reducing brain A β levels. CSF analysis also indicated a significant decrease in A β ₁₋₄₂ levels within 24 hrs following FC5-mFc-ABP treatment (FIG. 9C). The A β peptide sequence detected by MRM (SEQ: LVFFAEDVGSNK,
25 Table 1 /ELISA analyses is remote/different from the A β epitope recognized by ABP (therefore, not interfering with its quantification by either ELISA or MRM).

Example 10: Introduction of Fc component into FC5-ABP construct enhances its serum half-life

FC5-ABP (FC5 SEQ ID NO 17; and ABP SEQ ID NO 36) and FC5-hFc-ABP (FC5 SEQ ID NO 17; hFc 1x7 SEQ ID NO 40 and ABP SEQ ID NO 36) constructs were produced in CHO cells
30 as described in Example 2. Serum PK was determined as described in Example 5. FC5-ABP and FC5-Fc-ABP constructs were administered intravenously into rat tail vein at 15mg/kg. Serum was serially collected and FC5-ABP and FC5-Fc-ABP levels were quantified by direct ELISA using FC5- and ABP-specific antibodies. Serum samples were diluted (1:5,000) in phosphate-buffered saline (PBS) and applied to Maxisorb plates and incubated overnight at

4°C. ELISA plates were washed 3 X 100 µl PBS and blocked with 1% BSA in TBST for 30 min at room temperature (RT). Blocking solution was removed and the plates were incubated with HRP-conjugated FC5 monoclonal antibody (90 min). Following incubation and wash, 100µl SureBlue reagent was added and incubated in the dark at RT for 10-15 min. At the end of
5 reaction, 100µl 1M HCl was added and the developed colour was read at 450 nm in a plate reader. As shown in Fig 10, FC5-ABP without Fc was very rapidly cleared in the serum (within an hr) compared to FC5-ABP construct containing Fc component (FC5-Fc-ABP). This assay was also repeated with ABP-specific antibody with similar results. After sample application, ELISA plates were incubated first with ABP rabbit polyclonal antibody (90 min), followed by
10 HRP-conjugated rabbit secondary antibody (30 min) and the bound antibody was detected as described above (data not shown).

Example 11: Clearance of Aβ from rat brain

AD-Tg rats were dosed with either saline or FC5-mFc-ABP via tail vein every week over a period of four weeks (loading dose of 30mg/kg and subsequent four weekly doses of 15
15 mg/kg). CSF levels of FC5-mFc-ABP and Aβ were analyzed by nanoLC MRM. Before and after four weeks of treatment, brain Aβ levels were determined by PET scan using a specific Aβ-binding agent [18F] NAV4694. FC5-mFc-ABP reduced CSF Aβ level in rats within 24 hrs. An inverse relationship between the CSF levels of FC5-mFc-ABP and Aβ was observed, as in Tg mice, suggesting target engagement and rapid clearance of Aβ by ABP delivered to the
20 brain and CSF by FC5 (FIG. 11A and FIG. 11B). This was further corroborated by PET scan which clearly indicated a significant reduction (30-50%) of rat brain Aβ levels following four weeks of treatment with FC5-mFc-ABP (FIG. 11C).

Example 12: Increased hippocampal volume and improved neuronal connectivity

In the experiment described in Example 11, saline- and FC5-mFc-ABP- treated Tg mice were
25 subjected volumetric and functional Magnetic Resonance Imaging (MRI) before after the treatment. Volumetric MRI (Fig. 12A) showed an increased hippocampal volume in ABP-treated Tg mice compared to saline-treated controls suggesting that ABP treatment arrested hippocampal atrophy. Functional MRI (Fig. 12B) showed improved connectivity in anterior cingulate cortex in ABP-treated Tg mice compared to saline-treated controls suggesting
30 restoration of neuronal connectivity. This data confirms the significance, efficacy and superior therapeutic advantages now provided.

Example 13: FC5-mFc2a-ABP treatment shows decreased levels of CSF Aβ in dogs
As described in Example 6, serum and CSF PK profile of FC5-mFc-ABP was assessed in beagle dog with two dose (15 mg/kg and 30 mg/kg. In addition to measuring serum and CSF

levels of FC5-mFc2a-ABP by nanoLC-MRM, CSF levels of A β was also measured by nanoLC-MRM as described in Example 9. As can be seen, FC5-mFc-ABP appeared in the CSF in a dose- and time-dependent manner (Fig 13B). Most importantly, as seen Tg mice and Tg rat, there was a significant decrease in CSF A β level that was inversely proportional to CSF FC5-mFc2a-ABP levels.

Example 14: Generation of ABP fusion molecule with a different BBB carrier

To assess the versatility of ABP fusion molecule, ABP was successfully fused with another humanized BBB carrier IGF1R5 (H2). As shown in FIG. 12, the bi-functionality of the molecule was retained, ABP's ability to bind A β oligomer (ELISA and overlay assays) and also IGF1R5's ability deliver ABP across BBB model *in vitro* (data not shown). This clearly indicates that ABP can be fused to different BBB-crossing single-domain antibodies to be delivered to the brain.

Example 15: A β oligomer binding by different BBB-crossing single-domain antibody-Fc-ABP constructs

Various FC5-Fc-ABP constructs with modified ABPs (site-specific mutations or removal of C-terminus portion of the molecule as indicated by SEQ ID Nos shown in Table1. and Fig 15) are provided. As shown in Fig. 15, all constructs retained similar potency in binding A β oligomers by ELISA method.

Example 16: Production of FC5-hFc1X7-L-ABP with specific mutations to improve stability and bio-manufacturability.

FC5-hFc1X7-L-ABP carrying specific mutations (ABP, SEQ ID NO 35; ABP, SEQ ID NO 36) were produced in CHO cells and separated on SDS-PGE under reducing (R) and non-reducing (NR) conditions and stained with Coomassie, blue as described in Fig 2. Separated protein transferred to nitrocellulose membrane and immunoblotted with FC5-specific, hFc-specific and ABP-specific antibodies. In another set, A β -binding of ABP in the fusion molecule was also tested by overlay assay. Bound A β was detected with A β -specific antibody 6E10. As can be seen, systematic modification of ABP with specific mutations (as shown here, for example, ABP SEQ ID NO: 35 and, ABP SEQ ID NO: 36) substantially enhanced the stability of the molecule generated, as is clearly indicated herein, for example, by a single protein band under reducing and non-reducing conditions (compare with other ABP constructs of Fig 2A, wherein double protein bands can be seen). This substantial enhancement in the stability of the fusion molecule advantageously facilitates the bio-manufacturability of a homogeneous molecule.

Example 17: BBB permeability of various FC5-Fc-L-ABP constructs and IGF1R5-Fc-ABP construct *in vitro*.

BBB- crossing was assessed in *in vitro* rat BBB model as described in Fig 4 and molecules crossing the blood brain barrier were detected by nanoLC-MRM method. All ABP variants fused to humanized FC5 and IGF1R carriers crossed the BBB effectively. As expected, A20.1, a non-BBB permeable sdAb did not cross BBB, and likewise, ABP fused to A20.1 did not permeate the BBB (FIG. 17A). In Fig 17B, it is shown that “finger-print” peptides for all the three components of the fusion molecule, FC5, Fc and ABP were detected by nanoLC-MRM.

The embodiments and examples described herein are illustrative and are not meant to limit the scope of the invention as claimed. Variations of the foregoing embodiments, including alternatives, modifications and equivalents, are intended by the inventors to be encompassed by the claims. Furthermore, the discussed combination of features might not be necessary for the inventive solution.

Example 18: Humanized FC5-Fc-ABP construct [FC5(H3)-hFc-ABP (with ABP SEQ ID NO: 35 and ABP SEQ ID NO: 36)] crosses *in vitro* rat blood brain barrier intact. Blood brain barrier-crossing of humanized FC5-ABP fusion molecules were assessed as described in Example 4. Fusion molecules crossing the blood brain barrier were analyzed by Western blot and ELISA methods. Immunoblots were probed with hFc-specific-, and ABP-specific antibodies. Both the antibodies recognized the molecule crossing the BBB (bottom chamber) and the molecular size was identical to that of FC5-ABP fusion construct that was applied to *in vitro* BBB (top chamber) indicating that the molecule that crossed the BBB remained intact. This was substantiated by sandwich ELISA assay where in the BBB-crossed molecule was captured with FC5-specific antibody and the captured molecule was detected with ABP antibody.

Example 19: Humanized FC5-Fc-ABP construct [FC5(H3)-hFc-L-ABP (with ABP SEQ ID NO: 35 and ABP SEQ ID NO: 36)] is transported across the BBB *in vivo* and delivered to the brain intact by FC5. Blood brain barrier-crossing and brain delivery of humanized FC5-ABP fusion molecule in mice was assessed as described in Example 8. Four hrs following intravenous administration of the molecule and intracardiac perfusion, brain was removed and the cortices were extracted in RIPA buffer and the presence of injected FC5-ABP fusion molecule was detected by Western blot probed with ABP-specific antibody and by sandwich ELISA by capturing the molecule with FC5-specific antibody and detecting with ABP-specific antibody. Western blot revealed the presence of full-length FC5-ABP construct in the cortex. This was substantiated by sandwich ELISA which revealed the intact nature of the molecule as indicated by the ability to capture the molecule by FC5-specific antibody and detect the captured molecule with ABP-specific antibody.

Example 20: Ex-vivo (A) and in vivo (B) binding of humanized FC5-Fc-ABP construct [FC5(H3)-hFc-ABP (ABP SEQ ID NO 36)] (target engagement)

Immunohistochemistry was carried out as described in Example 3. Brain sections from AD-transgenic mice were incubated with FC5(H3)-hFc1X7-ABP (ABP, SEQ ID NO: 36) and the bound fusion molecule was visualized with HRP-conjugated FC5-specific antibody. As a negative control, sections were incubated with either no construct, or FC5-hFc construct without fused ABP. Briefly, Formalin-fixed 40µm free-floating sections containing cortex and hippocampus from APP/PS1 transgenic mouse were subjected to antigen retrieval in 10mM sodium citrate buffer (pH 9) at 80°C for 30 minutes then cooled to room temperature. Sections were rinsed in PBS, treated with 3% H₂O₂ in PBS for 30 minutes to block endogenous peroxidase, then rinsed again. Following a one hour incubation in Dako serum free protein block containing 0.3% triton X-100, sections were incubated with FC5(H3)-hFc1x7-ABP or the molar equivalent of FC5-hFc1x7 in Dako diluent containing 0.3% triton X-100 for 90 minutes at room temperature. After thoroughly rinsing in PBS, the sections were incubated with anti-FC5-HRP in Dako diluent for 60 minutes at room temperature, rinsed again, and then developed using Vector Impact DAB following the kit directions. The sections were placed on Superfrost plus slides, allowed to air dry overnight, then rehydrated, counterstained with methyl green, dipped in acetone/0.05% acetic acid (v/v) and dehydrated, cleared and coverslipped with Permount. Selective binding (black spots) was seen with FC5(H3)-hFc1X7-ABP construct but not with FC5(H3)-hFc1X7 without ABP, indicating ABP-dependent binding of Aβ deposits (target engagement) in the brain. No binding was seen in brain sections from wild type mice that does not produce amyloid deposits (data not shown).

Similar binding of Aβ deposits were detected following intra-hippocampal injection of FC5(H3)-hFc-ABP construct into AD transgenic mice (B). Four hours after intra-hippocampal injection, Tg mice were perfused and the brains were removed and sectioned. FC5(H3)-hFc-ABP binding to Aβ deposits (shiny white dots) were visualised with ABP-specific polyclonal antibody. Brain sections were first incubated with ABP-specific monoclonal antibody followed by Alexa-647 conjugated anti rabbit-Fc secondary antibody

Example 21: Target engagement by humanized FC5-Fc-ABP construct (FC5(H3)-hFc-ABP (SEQ ID NO: 46) in vivo. Fluorophore-labelled (FIG.18A) or naïve FC5-ABP (FIG 18B) fusion molecule was microinjected into the hippocampal region of wild-type (Wt) and AD-Tg (Tg) mice. 30 min after microinjection of fluorophore-labelled FC5-ABP fusion molecule, brains were removed, sectioned and observed under fluorescence microscope. As shown in 21A, injected molecule bound to Aβ deposits as confirmed by its co-localization with Aβ-specific antibody. In a parallel study, four hrs after intra-hippocampal injection of naïve FC5-ABP fusion

molecule, hippocampal formation from injected (ips) and non-injected (con) regions were collected and homogenized in Tris-buffered saline. Hippocampal extracts were subjected to sandwich ELISA with FC5 antibody as capturing antibody and either ABP or A β -specific antibody as detection antibody. As shown in 21B, microinjected FC5-ABP molecule remained intact and ABP was able to engage and bind the target A β in vivo as indicated by the presence of A β in the pulled-down complex detected by A β -specific antibody.

Example 22: PK/PD comparison between non-humanized and humanized FC5-Fc-L-ABP constructs

FC5-mFc2a-ABP or FC5(H3)-hFc1x7-ABP was administered intravenously into rats via tail vein injection at 15 mg/kg as described for Fig 5. Serum and CSF were serially collected. FC5-Fc-ABP levels were quantified using nanoLC- MRM method. As shown in Fig 22 A, serum and CSF PK profile were very similar for non-humanized and humanized constructs. FC5-mFc2a-ABP or FC5(H3)-hFc1x7-ABP was administered intravenously into Tg mice via tail vein injection at 15 mg/kg as described for Fig 9B. FC5-Fc-ABP and A β levels in the CSF were measured by nanoLC-MRM as described in Fig 9B. As shown in Fig 22 B, the levels of non-humanized and humanized FC5-Fc-ABP in the CSF were similar, and most importantly, changes (decrease) in CSF A β levels were also very similar, indicating that humanization of FC5-Fc-ABP construct did not affect the PK and PD profile of the fusion construct.

SEQUENCES

SEQ ID NO:	Sequence	Description
1	GFKITHYTMG	CDR1 FC5
2	RITWGGDNTFYNSVK	CDR2 FC5
3	GSTSTATPLRVDY	CDR3 FC5
4	EYPSNFYA	CDR1 IGF1R-3
5	VSRDGLTT	CDR2 IGF1R-3
6	AIVITGVWNKVDVNSRSYHY	CDR3 IGF1R-3
7	GGTVSPTA	CDR1 IGF1R-4
8	ITWSRGTT	CDR2 IGF1R-4
9	AASTFLRLPEESAYTY	CDR3 IGF1R-4
10	GRTIDNYA	CDR1 IGF1R-5
11	IDWGDGGX; where X is A or T	CDR2 IGF1R-5
12	AMARQSRVNLDVARYDY	CDR3 IGF1R-5
13	X ₁ VQLVX ₂ SGGGLVQPGGSLRLSCAASGFKITHYTMGWX ₃ RQAPGKX ₄ X ₅ EX ₆ VSRLTWGGDNTFYNSVKGRFTISRDNKNTX ₇ YLMNSLRAEDTAVYYCAAGSTSTATPLRVDYWGQGLTVTVSS, where X ₁ =D or E, X ₂ =A or E, X ₃ =F or V, X ₄ =E or G, X ₅ =R or L, X ₆ =F or W, X ₇ =L or V;	Humanized FC5 consensus
14	DVQLQASGGGLVQAGGSLRLSCAASGFKITHYTMGWFRQAPGKERE FVSRLTWGGDNTFYNSVKGRFTISRDNKNTVYLMNSLKPEDTADY YCAAGSTSTATPLRVDYWGKGTQTVTVSS	FC5
15	EVQLVESGGGLVQPGGSLRLSCAASGFKITHYTMGWVRQAPGKGLE	FC5-H1

	WVSRITWGGDNFTFYSNSVKGRFTISRDN SKNTLYLQMNSLRAEDTAV YYCAAGSTSTATPLRVDYWGQGLTVTVSS	
16	EVQLVESGGGLVQP GGSRLS CAASGFKITHYTMGWWRQAPGKGLE WVSRITWGGDNFTFYSNSVKGRFTISRDN SKNTVYLQMNSLRAEDTAV YYCAAGSTSTATPLRVDYWGQGLTVTVSS	FC5-H2
17	EVQLVESGGGLVQP GGSRLS CAASGFKITHYTMGWFRQAPGKGLEF VSRITWGGDNFTFYSNSVKGRFTISRDN SKNTVYLQMNSLRAEDTAVYY CAAGSTSTATPLRVDYWGQGLTVTVSS	FC5-H3
18	X ₁ VX ₂ LX ₃ ESGGGLVQX ₄ GGSLRLSCX ₅ ASEYPSNFYAMSWX ₆ RQAPGK X ₇ X ₈ EX ₉ VX ₁₀ GVS RDGLTTL YADSVKGRFTX ₁₁ SRDNX ₁₂ KNTX ₁₃ X ₁₄ LQM NSX ₁₅ X ₁₆ AEDTAVYYCAIVITGVWVNKVDVNSRSYHYWGQGT X ₁₇ VTVSS , where X ₁ is E or Q; X ₂ is K or Q; X ₃ is V or E; X ₄ is A or P; X ₅ is V or A; X ₆ is F or V; X ₇ is E or G; X ₈ is R or L; X ₉ is F or W; X ₁₀ is A or S; X ₁₁ is M or I; X ₁₂ is A or S; X ₁₃ is V or L; X ₁₄ is D or Y; X ₁₅ is V or L; X ₁₆ is K or R; and X ₁₇ is Q or L,	IGF1R-3 Consensus
19	QVKLEESGGGLVQAGGSLRLSCVASEYPSNFYAMSWFRQAPGKERE FVAGVSRDGLTTL YADSVKGRFTMSRDNAKNTVDLQMNSVKAEDTAV YYCAIVITGVWVNKVDVNSRSYHYWGQGTQVTVSS	IGF1R-3
20	EVQLVESGGGLVQP GGSRLS CAASEYPSNFYAMSWFRQAPGKERE FVSGVSRDGLTTL YADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVY YCAIVITGVWVNKVDVNSRSYHYWGQGLTVTVSS	IGF1R-3-H5
21	X ₁ VX ₂ LX ₃ ESGGGLVQX ₄ GGSLRLSCX ₅ X ₆ SGGTVSPTAMGWX ₇ RQAPG KX ₈ X ₉ EX ₁₀ VX ₁₁ HITWSRGTTTRX ₁₂ ASSVKX ₁₃ RFTISRDX ₁₄ X ₁₅ KNTX ₁₆ YLQ MNSLX ₁₇ X ₁₈ EDTAVYYCAASTFLRILPEESAYTYWGQGT X ₁₉ VTVSS, where X ₁ is E or Q; X ₂ is K or Q; X ₃ is V or E; X ₄ is A or P; X ₅ is A or E; X ₆ is V or A; X ₇ is V or F; X ₈ is G or E; X ₉ is L or R; X ₁₀ is F or W; X ₁₁ is G or S; X ₁₂ is V or Y; X ₁₃ is D or G; X ₁₄ is N or S; X ₁₅ is A or S; X ₁₆ is L or V; X ₁₇ is K or R; X ₁₈ is A or S; and X ₁₉ is L or Q,	IGF1R-4 Consensus
22	QVKLEESGGGLVQAGGSLRLSCEVSGGTVSPTAMGWFRQAPGKERE FVGHITWSRGTTTRVASSVKDRFTISRDS AKNTVYLQMNSLKS EDTAVY YCAASTFLRILPEESAYTYWGQGTQVTVSS	IGF1R-4
23	QVQLVESGGGLVQP GGSRLS CAVSGGTVSPTAMGWFRQAPGKGLE FVGHITWSRGTTTRYASSVKGRFTISRDN SKNTVYLQMNSLRAEDTAVY YCAASTFLRILPEESAYTYWGQGLTVTVSS	IGF1R-4-H3
24	X ₁ VX ₂ LX ₃ ESGGGLVQX ₄ GGSLRLS CAASGRTIDNYAMAWX ₅ RQAPGKX X ₆ X ₇ EX ₈ VX ₉ TIDWGDGGX ₁₀ RYANSVKGRFTISRDNX ₁₁ KX ₁₂ TX ₁₃ YLQMNX X ₁₄ LX ₁₅ X ₁₆ EDTAVYX ₁₇ CAMARQSRVNLDVARYDYWGQGT X ₁₈ VTVSS, where X ₁ is E or Q; X ₂ is K or Q; X ₃ is V or E; X ₄ is A or P; X ₅ is V or S; X ₆ is D or G; X ₇ is L or R; X ₈ is F or W; X ₉ is A or S; X ₁₀ is A or T; X ₁₁ is A or S; X ₁₂ is G or N; X ₁₃ is M or L; X ₁₄ is N or R; X ₁₅ is E or R; X ₁₆ is P or A; X ₁₇ is S or Y; and X ₁₈ is Q or L,	IGF1R-5 Consensus
25	QVKLEESGGGLVQAGGSLRLS CAASGRTIDNYAMAWSRQAPG KDREFVATIDWGDGGARYANSVKGRFTISRDN AKGTMYLQMN NLEPEDTAVYSCAMARQSRVNLDVARYDYWGQGTQVTVSS	IGF1R-5
26	QVQLVESGGGLVQP GGSRLS CAASGRTIDNYAMAWWRQAPG KGLEWATIDWGDGGTRYANSVKGRFTISRDN SKNTMYLQMN SLRAEDTAVYYCAMARQSRVNLDVARYDYWGQGT LTVTVSS	IGF1R-5-H2
27	SGKTEYMAFPKPFESSSIGAEKPRNKKLP EEEVESSRTPWLYEQEG EVEKPKFIKTGFSVSVEKSTSSNRKNQLDTNGRRRQFDEESLESFSSMP DPVDPTTVTKTFKTRKASQAASLASKDKTPKSKSKKRNSTQLKSRVKN ITHARRILQQSNRNACNEAPETGSDFSMFEA	PK-4
28	FSSMPDPVDPTTVTKTFKTRKASQAASLASKDKTPKSKSK	P4 peptide
29	KDKTPKSKSKKRNSTQLKSRVKNITHARRILQQSNRNACN	P5 peptide

30	KTFKTRKASQAASLASKDKTPKSKSKKRNSTQLKSRVKNI	P4-5 peptide (ABP)
31	X ₁ TFX ₂ TX ₃ X ₄ ASAQAASLASKDKTPKSKSKKX ₅ X ₆ STQLX ₇ SX ₈ VX ₉ NI here X ₁ = G or A, X ₂ = G or V, X ₃ = G or A, X ₄ = G or A, X ₅ = G or V, X ₆ = G or V, X ₇ = G or V, X ₈ = G or A, X ₉ = G or A (SEQ ID NO: 31)	ABP consensus
32	KTFKTRKASQAASLASKDKTPKSKSKKRGSTQLKSRVKNI	[ABP(G)]
33	KTFKTRKASQAASLASKDKTPKSKSKKGGSTQLKSRVKNI	[ABP(GG)]
34	KTFKTRGASQAASLASKDKTPKSKSKKRGSTQLKSRVKNI	[ABP(RG-G)]
35	KTFKTTGGASQAASLASKDKTPKSKSKKRGSTQLKSRVKNI	[ABP(GG-G)]
36	GTFGTGGASQAASLASKDKTPKSKSKKGGSTQLKSRVKNI	[ABP(6G)]
37	KTFKTRKASQAASLASKDKTPKSKSKKGGSTVKNI	[ABP(GGspv)]
38	KTFKTRKASQAASLASKDKTPKSKSKKRG	[ABP(trc)]
39	ASEPRGPTIKPCPPCKCPAPNLLGGPSVFIFPPKIKDVLMISSLPIVTCV VVDVSEDDPDVQISWVFNVEVHTAQQTTHREDYNSTLRVVSALPIQH QDWMSGKEFKCKVNNKDLPAPIERTISKPKGSRAPQVYVLPPEEE MTKKQVTLTCMVTDFMPEDIYVEWTNNGKTELNYKNTPEVLDSGGSY FMYSKLRVEKKNWVERNSYSCSVVHEGLHNHHTTKSFSRTPG	Mouse Fc2a
40	AEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEGPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDE LTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGGSFF LYSKLTVDKSRWQQGNVFSVMSVHEALHNHYTQKSLSLSPG	Human Fc1x7
41	AEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEGPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDE LTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGGSFF LYSKLTVDKSRWQQGNVFSVMSVHEALHNHYTQKSLSLSPG	Human Fc1X0
42	EVQLQASGGGLVQAGGSLRLSCAASGFKITHYTMGWFRQAPGKERE FVSRITWGGDNTFYNSVKGRFTISRDNKNTVYLQMNSLKPEDTADY YCAAGSTSTATPLRVDYWGQGTQVTVSSASEPRGPTIKPCPPCKCPA PNLLGGPSVFIFPPKIKDVLMISSLPIVTCVVVDVSEDDPDVQISWVFN NVEVHTAQQTTHREDYNSTLRVVSALPIQHQDWMSGKEFKCKVNNKD LPAPIERTISKPKGSRAPQVYVLPPEEEMTKKQVTLTCMVTDFMPE DIYVEWTNNGKTELNYKNTPEVLDSGGSYFMYSKLRVEKKNWVERNS YSCSVVHEGLHNHHTTKSFSRTPGTGGGSGGGGSKTFKTRKASQA ASLASKDKTPKSKSKKRNSTQLKSRVKNI	FC5-mFc2a-ABP
43	EVQLVESGGGLVQPGGSLRLSCAASGFKITHYTMGWFRQAPGKGLEF VSRITWGGDNTFYNSVKGRFTISRDNKNTVYLQMNSLRAEDTAVYY CAAGSTSTATPLRVDYWGQGTQVTVSSASEPRGPTIKPCPPCKCPA PNLLGGPSVFIFPPKIKDVLMISSLPIVTCVVVDVSEDDPDVQISWVFN EVHTAQQTTHREDYNSTLRVVSALPIQHQDWMSGKEFKCKVNNKDL APIERTISKPKGSRAPQVYVLPPEEEMTKKQVTLTCMVTDFMPEDIY VEWTNNGKTELNYKNTPEVLDSGGSYFMYSKLRVEKKNWVERNSYS CSVSVVHEGLHNHHTTKSFSRTPGTGGGSGGGGSKTFKTRKASQA ASLASKDKTPKSKSKKRNSTQLKSRVKNI	FC5-H3-mFc2a-L-ABP
44	EVQLVESGGGLVQPGGSLRLSCAASGFKITHYTMGWFRQAPGKGLEF VSRITWGGDNTFYNSVKGRFTISRDNKNTVYLQMNSLRAEDTAVYY CAAGSTSTATPLRVDYWGQGTQVTVSSAEPKSSDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEGPEVKFNWYVDG VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIA VEWESNGQPENNYKTTTPVLDSGGSFFLYSKLTVDKSRWQQGNVFS CSVMSVHEALHNHYTQKSLSLSPGTGGGSGGGGSKTFKTRKASQA SLASKDKTPKSKSKKRNSTQLKSRVKNI	FC5-H3-hFc1X7-L-ABP
45	EVQLVESGGGLVQPGGSLRLSCAASGFKITHYTMGWFRQAPGKGLEF	FC5-H3-

	VSRITWGGDNTFYNSVKGRTISRDN SKNTVYLQMNSLRAEDTAVYY CAAGSTSTATPLRVDYWGQGLTVTVSSAEPKSSDKTHTCPPCPAPEL LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEGPEVKFNWYVDGV EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIA VEWESNGQPENNYKTTPVLDSGSSFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGT GGGSGGGG SKTFKTRKASAAQASLA SKDKTPKSKSKKRGSTQLKSRVKNI	hFc1X7-L- ABP(G)
46	EVQLVESGGGLVQPGGSLRLSCAASGFKITHYTMGWFRQAPGKGLEF VSRITWGGDNTFYNSVKGRTISRDN SKNTVYLQMNSLRAEDTAVYY CAAGSTSTATPLRVDYWGQGLTVTVSSAEPKSSDKTHTCPPCPAPEL LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEGPEVKFNWYVDGV EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVK GFYPSDIAVEWESNGQPENNYKTTPVLDSGSSFFLYSKLTVDKSRW QQGNVFS CSVMHEALHNHYTQKSLSLSPGT GGGSGGGG SKTFKTGG ASAQASLASKDKTPKSKSKKRGSTQLKSRVKNI	FC5-H3- hFc1X7-L- ABP(GG-G)
47	EVQLVESGGGLVQPGGSLRLSCAASGFKITHYTMGWFRQAPGKGLEF VSRITWGGDNTFYNSVKGRTISRDN SKNTVYLQMNSLRAEDTAVYY CAAGSTSTATPLRVDYWGQGLTVTVSSAEPKSSDKTHTCPPCPAPEL LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEGPEVKFNWYVDGV EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIA VEWESNGQPENNYKTTPVLDSGSSFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGT GGGSGGGG SGTFGTGGASAAQASL ASKDKTPKSKSKKGGSTQLKSRVKNI	FC5-H3- hFc1X7-L- ABP(6G)
48	EVQLVESGGGLVQPGGSLRLSCAASGFKITHYTMGWFRQAPGKGLEF VSRITWGGDNTFYNSVKGRTISRDN SKNTVYLQMNSLRAEDTAVYY CAAGSTSTATPLRVDYWGQGLTVTVSS GGGSGGGG SKTFKTGGAS AAQASLASKDKTPKSKSKKRGSTQLKSRVKNI GGGSGGGG SAEPKSS DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH EGPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWL NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSGSSFFLYSKL TVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPG	FC5(H3)-L- ABP(GG-G)-L hFc1X7
49	EVQLVESGGGLVQPGGSLRLSCAASGFKITHYTMGWFRQAPGKGLEF VSRITWGGDNTFYNSVKGRTISRDN SKNTVYLQMNSLRAEDTAVYY CAAGSTSTATPLRVDYWGQGLTVTVSSAEPKSCDKTHTCPPCPAPEL LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEGPEVKFNWYVDG VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDI AVEWESNGQPENNYKTTPVLDSGSSFFLYSKLTVDKSRWQQGNVFS SCSVMHEGLHNHYTQKSLSLSPGT GGGSGGGG SKTFKTGGASAAQAS LASKDKTPKSKSKKRGSTQLKSRVKNI	FC5-H3- hFc1X0-L- ABP(GG-G)
50	QVQLVESGGGLVQPGGSLRLSCAASGRTIDNYAMAWVRQAPGKGLE WWATIDWGDGGTRYANSVKGRTISRDN SKNTMYLQMNSLRAEDTAV YYCAMARQSRVNLDAVYDYWGQGLTVTVSSAEPKSSDKTHTCPPC PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEGPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFY PSDIAVEWESNGQPENNYKTTPVLDSGSSFFLYSKLTVDKSRWQQG NVFSCSVMHEALHNHYTQKSLSLSPGT GGGSGGGG SKTFKTGGASA QASLASKDKTPKSKSKKRGSTQLKSRVKNI	IGF1R5-H2- hFc1X7-L- ABP(GG-G)
51	KTFKTGGASAAQASLASKDKTPKSKSKKRGSTQLKSRVKNI GGGSGGGG G SAEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEGPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLT VLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR DELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSGDS	ABP(GG-G)-L- hFc1X7- IGF1R5-H2

	FFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGQVQL VESGGGLVQPGGSLRLSCAASGRTIDNYAMAWVRQAPGKGLEWVATI DWGDGGTRYANSVKGRFTISRDN SKNTMYLQMNSLRAEDTAVYYCA MARQSRVNL DVARYDYWGQGTLVTVSS	
52	AEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEGPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDE LTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFF LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP GGGGSG GGGSK TFKTGGASAQASLASKDKTPKSKSKRGSTQLKSRVKNI GGG SGGGGS QVQLVESGGGLVQPGGSLRLSCAASGRTIDNYAMAWVRQA PGKGLEWVATIDWGDGGTRYANSVKGRFTISRDN SKNTMYLQMNSLR AEDTAVYYCAMARQSRVNL DVARYDYWGQGTLVTVSS	hFc1X7- L- ABP(GG-G)-L- IGF1R5-H2
53	EVQLVESGGGLVQPGGSLRLSCAASGFKITHYTMGWFRQAPGKGLEF VSRITWGGDNTFYNSVKGRTISRDN SKNTVYLQMNSLRAEDTAVYY CAAGSTSTATPLRVDYWGQGTLVTVSSAEPKSSDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEGPEVKFNWYVDGV EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIA VEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGT X₁GX₂X₃GX₄X₅GX₆ ,GTFGTGGASAQ ASLASKDKTPKSKSKGGSTQLKSRVKNI where X ₁ = A or G., X ₂ = A or G, X ₃ = S or T, X ₄ = G or V, X ₅ = A or V, X ₆ = S or T	FC5-H3- hFc1X7- L(consensus)- ABP(6G)

5

10 REFERENCES

All patents, patent applications and publications referred to herein and throughout the application are hereby incorporated by reference.

Abbott NJ (2013) Blood-brain barrier structure and function and the challenges for CNS drug delivery. J Inherit Metab Dis. 36(3):437-49.

- Arbabi-Ghahroudi M, Desmyter A, Wyns L, Hamers R, and Muyldermans S (1997) Selection and identification of single domain antibody fragments from camel heavy-chain antibodies, FEBS Lett 414, 521-526
- Barageb SH, Sonawane KD (2015) Amyloid cascade hypothesis: Pathogenesis and
5 therapeutic strategies in Alzheimer's disease. *Neuropeptides* doi: 0.10 16/j .npep.20 15 .06.008
- Bard F et. al. (2000) Peripherally administered antibodies against amyloid beta-peptide enter the central nervous system and reduce pathology in a mouse model of Alzheimer disease *Nat Med.* 6, 916-919.
- Bell A, Wang ZJ, Arbabi-Ghahroudi M, Chang TA, Durocher Y, Trojahn U, Baardsnes J,
10 Jaramillo ML, Li S, Baral TN, O'Connor-McCourt M, Mackenzie R, and Zhang J. (2010) Differential tumor-targeting abilities of three single-domain antibody formats. *Cancer Lett.* 289, 81-90.
- Caram-Salas N, Boileau E, Farrington GK, Garber E, Brunette E, Abulrob A, Stanimirovic D. In vitro and in vivo methods for assessing FcRn-mediated reverse transcytosis across the
15 blood-brain barrier. *Methods Mol Biol.* 2011;763: 383-401.
- Chakravarthy B, Michel Menard, Leslie Brown, Melissa Hewitt, Trevor Atkinson, and James Whitfield. (2013) A synthetic peptide corresponding to a region of the human pericentriolar material 1 (PCM-1) protein binds 13-amyloid (A/31-42) oligomers. *J. Neurochem.* 126, 415-424.
- 20 Chakravarthy B, Shingo Ito, Trevor Atkinson, Chantal Gaudet, Michel Menard, Leslie Brown, James Whitfield. (2014) Evidence that a synthetic amyloid-13 oligomer-binding peptide (ABP) targets amyloid-13 deposits in transgenic mouse brain and human Alzheimer's disease brain. *Biochem. Biophys. Res. Commun.* 445: 656-660.
- Chothia C., and Lesk A.M. (1987) Canonical structures for the hypervariable regions of
25 immunoglobulins. *J. Mol. Biol.* 196, 901-917.
- Davies J., and L. Riechmann. (1996), Affinity improvement of single antibody VH domains: residues in all three hypervariable regions affect antigen binding. *Immunotechnology* 2, 169-179
- DeLaGarza, V.W. (2003) Pharmacologic treatment of Alzheimer's disease: an update. *Am Fam Physician.* 68, 1365-72.
30

- Dumoulin, M., Conrath, K., Van Meirhaighe, A., Meersman, F., Heremans, K., Frenken, L.G., Muyldermans, S., Wyns, L., and Matagne, A. (2002) Single-domain antibody fragments with high conformational stability. *Protein Sci* 11, 500-515.
- Durocher Y, Perret S, Kamen A. (2002) High-level and high-throughput recombinant protein
5 production by transient transfection of suspension-growing human 293-EBNA1 cells. *Nucleic Acids Res.* 30 (2):E9.
- Eisenberg, D., Schwarz, E., Komaromy, M., and Wall, R. (1984) Analysis of membrane and surface protein sequences with the hydrophobic moment plot. *J. Mol. Biol.* 179, 125-142
- 10 Farrington GK, Caram-Salas N, Haqqani AS, Brunette E, Eldredge J, Pepinsky B, Antognetti G, Baumann E, Ding W, Garber E, Jiang S, Delaney C, Boileau E, Sisk WP, Stanimirovic DB. (2014) A novel platform for engineering blood-brain barrier-crossing bispecific biologics. *FASEB J.* 28, 4764-4778.
- Garberg, P., Ball, M., Borg, N., Cecchelli, R., Fenart, L., Hurst, R. D., Lindmark, T., Mabondzo, A., Nilsson, J. E., Raub, T. J., Stanimirovic, D., Terasaki, T., Oberg, J.-O., and Osterberg, T.
15 (2005) In vitro models for the blood-brain barrier. *Toxicol. In Vitro* 19, 299–334
- Gonzales NR, DePascalis R, Schlom J, Kashmiri SVS (2005) Minimizing the Immunogenicity of Antibodies for Clinical Application. *Tumor Biol* 26, 31-43
- 20 Gottesman MM and Pastan I (1993) Biochemistry of multidrug resistance mediated by the multidrug transporter. *Ann. Rev. Biochem.* 62, 385-427
- Goure WF, Krafft GA, Jerecic J, Hefti F. (2014) Alzheimer's Research & Therapy Targeting the proper amyloid-beta neuronal toxins: a path forward for Alzheimer's disease. *Immunotherapeutics*, 6, 42-56.
- 25 Gergov M, Ojanpera I, Vuori E. (2003) Simultaneous screening for 238 drugs in blood by liquid chromatography–ionspray tandem mass spectrometry with multiple-reaction monitoring. *Journal of Chromatography B*, 795, 41–53
- Hardy J, Bogdanovic N, Winblad B, Portelius E, Andreassen N, Cedazo-Minguez A, Zetterberg
30 H. (2014) Pathways to Alzheimer's disease. *J Intern Med.* 275, 296-303.

- Hamers-Casterman, C., Atarhouch, T., Muyldermans, S., Robinson, G., Hamers, C., Songa, E.B., Bendahman, N., and Hamers, R. (1993) Naturally occurring antibodies devoid of light chains. *Nature* 363, 446-448.
- 5 Haqqani, A. S., Caram-Salas, N., Ding, W., Brunette, E., Delaney, C. E., Baumann, E., Boileau, E., and Stanimirovic, D. (2013) Multiplexed evaluation of serum and CSF pharmacokinetics of brain-targeting single-domain antibodies using a NanoLC-SRM-ILIS method. *Mol. Pharm.* 10, 1542–1556
- Huang YL, Sa'jjo' A, Suneson A, Hansson HA. (1995) A new approach for multiple sampling of cisternal cerebrospinal fluid in rodents with minimal trauma and inflammation. *J Neurosci Meth*;63:13-22.
- 10 Hussack G., Hirama T., Ding W., MacKenzie R., and Tanha J. (2011) Engineered Single-Domain Antibodies with High Protease Resistance and Thermal Stability *PLoS ONE* 6, e28218.
- 15 Hussack G, Arbabi-Ghahroudi M, van Faassen H, Songer JG, Ng KK, MacKenzie R, Tanha J (2011b) Neutralization of *Clostridium difficile* toxin A with single-domain antibodies targeting the cell receptor binding domain. *J Biol Chem.* 286(11): 8961-76.
- 20 Iqbal U., Trojahn U., Albaghdadi H., Zhang J., O'Connor M., Stanimirovic D., Tomanek B., Sutherland G., and Abulrob A. (2010) Kinetic analysis of novel mono- and multivalent VHH-fragments and their application for molecular imaging of brain tumours. *Br. J. Pharmacol.* 160, 1016-1028.
- Jaspers, L., Schon, O., Famm, K., and Winter, G. (2004) Aggregation-resistant domain antibodies selected on phage by heat denaturation. *Nat. Biotechnol.* 22, 1161-1165.
- 25 Ji W and Ha I (2010) Drug Development for Alzheimer's Disease: Recent Progress *Experimental Neurobiology* 19, 120-131.
- Jones PT, Dear PH, Foote J, Neuberger MS, Winter G (1986) Replacing the complementarity-determining regions in a human antibody with those from a mouse. *Nature* 321, 522-525.
- 30 Kabat EA, Wu TT. Identical V region amino acid sequences and segments of sequences in antibodies of different specificities. Relative contributions of VH and VL genes, minigenes, and complementarity-determining regions to binding of antibody-combining sites. *J Immunol.* 1991;147:1709-19.

- Kim, D.Y., Kandalafi, H., Ding, W., Ryan, S., van Fassen, H., Hiram, T., Foote, S.J., MacKenzie, R., and Tanha, J. (2012) Disulfide linkage engineering for improving biophysical properties of human VH domains PEDS advance access August 30, 2012, 1-9.
- 5 Kornhuber ME, Kornhuber J, Cimniak U. (1986) A method for repeated CSF sampling in the freely moving rat. *J Neurosci Meth* 1986;17:63-68.
- Lannfelt L, Relkin, NR, Siemers ER (2014) Amyloid- β -directed immunotherapy for Alzheimer's disease. *J Intern Med*. 275, 284-295.
- 10 Li S, Zheng W, Kuolee R, Hiram, T, Henry M, Makvandi-Nejad S, Fjallman T, Chen W, Zhang J. Pentabody-mediated antigen delivery induces antigen-specific mucosal immune response. *Mol Immunol* 2009;46:1718-26.
- Mangialasche F, Solomon A, Winblad B, Mecocci P, Kivipelto M (2010) Alzheimer's disease: clinical trials and drug development. *Lancet Neurol* 9, 702- 716
- 15 Monson A, and Weiner HL (2003) Immunotherapeutic approaches to Alzheimer's disease *Science*. 302, 834-838.
- Morrone CD, Mingzhe Liu M, Black SE, McLaurin JA (2015) Interaction between therapeutic interventions for Alzheimer's disease and physiological A β clearance mechanisms. *Frontiers in Aging Neuroscience*. 7, Article 64, 1-16.
- 20 Musiek ES, Holtzman DM (2015) Three dimensions of the amyloid hypothesis: time, space and "wingmen". *Nature Neurosci*. 18, 800-806.
- Nicaise M, Valeio-Lepiniec M, Minard P, Desmadril M. (2004) Affinity transfer by CDR grafting on a nonimmunoglobulin scaffold. *Protein Sci*. 13(7): 1882-1891.
- 25 Nuttall, S.D., Krishnan, U.V., Doughty, L., Pearson, K., Ryan, M.T., Hoogenraad, N.J., Hattarki, M., Carmichael, J.A., Irving, R.A., and Hudson, P.J. (2003) Isolation and characterization of an IgNAR variable domain specific for the human mitochondrial translocase receptor Tom70. *Eur. J. Biochem*. 270, 3543-3554.
- Padlan EA (1991) A possible procedure for reducing the immunogenicity of antibody variable domains while preserving their ligand-binding properties. *Mol Immunol* 28, 489-498.
- Pardridge, W.M., *Adv. Drug Delivery Reviews*, 15, 5-36 (1995)

- Pardridge, W. M. Drug and gene delivery to the brain: the vascular route, *Neuron*. 2002, 36, 555-558.
- Queen C, Schneider WP, Selick HE, Payne PW, Landolfi NF, Duncan JF, Avdalovic NM, Levitt M, Junghans RP, Waldmann TA (1989) A humanized antibody that binds to the interleukin 2
5 receptor. *Proc Natl Acad Sci USA* 86, 10029-10033.
- Rafii MS, Aisen PS. (2015) Advances in Alzheimer's disease drug development. *BMC Med*. 13:62.
- Riechmann L, Clark M, Waldmann H, Winter G (1988) Reshaping human antibodies for
10 therapy. *Nature* 332, 323-327.
- Samuels B.L., *J. Clin. Pharmacol.* (1993) Modulation of vinblastine resistance with cyclosporine: a phase I study. *Ther.* 54, 421-429
- Savonenko AV, Melnikova T, Hiatt A, LiT, Worley PF, Troncoso JC, Wong PC, Price DL.
15 (2012) Alzheimer's therapeutics: translation of preclinical science to clinical drug development. *Neuropsychopharmacology*. 37, 261-277
- Selkoe DJ, and Hardy J (2016) The amyloid hypothesis of Alzheimer's disease at 25 years *EMBO Molecular Medicine*. 8, 595-608
- Sengupta U, Nilson AN, Kayed R (2016) The Role of Amyloid- β Oligomers in Toxicity,
20 Propagation, and Immunotherapy. *EBioMedicine*. 6:42-49
- Sevigny J et al., (2016) The antibody aducanumab reduces A β plaques in Alzheimer's disease. *Nature*. 537, 50-56
- Shields RL, Namenuk AK, Hong K, Meng YG, Rae J, Briggs J, Xie D, Lai J, Stadlen A, Li
25 B, Fox JA, Presta LG (2001) High resolution mapping of the binding site on human IgG1 for Fc gamma RI, Fc gamma RII, Fc gamma RIII, and FcRn and design of IgG1 variants with improved binding to the Fc gamma R. *J Biol Chem*. 276, 6591-6604.
- Tempest PR, Bremner P, Lambert M, Taylor G, Furze JM, Carr FJ, Harris WJ (1991)
30 Reshaping a human monoclonal antibody to inhibit human respiratory syncytial virus infection in vivo. *Biotechnology* 9, 266-271.

To R, Hirama T, Arbabi-Ghahroudi M, MacKenzie R, Wang P, Xu P, Ni F, and Tanha J. (2005) Isolation of Monomeric Human VHS by a Phage Selection. J. Biol. Chem. 280, 41395-41403.

Tsurushita N, Hinton, RP, Kumar S (2005) Design of humanized antibodies: From anti-Tac to Zenapax. Methods 36, 69-83.

- 5 Watanabe, T. (1995) Comparative Study on Reversal Efficacy of SDZ PSC 833, Cyclosporin a and Verapamil on Multidrug Resistance in Vitro and in Vivo Acta Oncol., 34, 235-241

WO 95/04069

WO/2004/076670

WO2003/046560

- 10 WO 2002/057445

WO 2011/127580

WO 2007/036021

WO 2006/133566

CLAIMS:

1. A compound comprising a fusion protein, comprising an antibody or fragment thereof that transmigrates the blood brain barrier (BBB) a polypeptide that binds β -amyloid.

2. The compound of claim 1 further comprising a Fc fragment.

5 3. The compound of claim 1 or 2, wherein the polypeptide that binds β -amyloid comprises a sequence

X_1 TFX₂TX₃X₄ASAQASLASKDKTPKSKSKX₅X₆STQLX₇SX₈VX₉NI (SEQ ID NO: 31)

wherein X_1 = G or A, X_2 = G or V, X_3 = G or A, X_4 = G or A, X_5 = G or V, X_6 = G or V, X_7 = G or V, X_8 = G or A, X_9 = G or A.

10 3. The compound of claim 2, wherein the polypeptide that binds β -amyloid comprises a sequence selected from the group consisting of:

SGKTEYMAFPKPFESSSSIGAEKPRNKKLPEEEEVESSRTPWLYEQEGEVEKPFIKTGFS
VSVEKSTSSNRKNQLDTNGRRRQFDEESLESFSSMPDPVDPTTVTKTFKTRKASAQAS
LASKDKTPKSKSKRNSTQLKSRVKNIHARRILQQSNRNACNEAPETGSDFSMFEA
15 (SEQ ID NO:27);

FSSMPDPVDPTTVTKTFKTRKASAQASLASKDKTPKSKSK (SEQ ID NO:28);

KDKTPKSKSKRNSTQLKSRVKNIHARRILQQSNRNACN (SEQ ID NO:29);

KTFKTRKASAQASLASKDKTPKSKSKRNSTQLKSRVKNI (SEQ ID NO:30);

KTFKTRKASAQASLASKDKTPKSKSKKRGSTQLKSRVKNI (SEQ ID NO:32);

20 KTFKTRKASAQASLASKDKTPKSKSKGGSTQLKSRVKNI (SEQ ID NO:33);

KTFKTRGASAQASLASKDKTPKSKSKKRGSTQLKSRVKNI (SEQ ID NO:34);

KTFKTGGASAQASLASKDKTPKSKSKKRGSTQLKSRVKNI (SEQ ID NO:35);

GTFGTGGASAQASLASKDKTPKSKSKGGSTQLKSRVKNI (SEQ ID NO:36);

KTFKTRKASAQASLASKDKTPKSKSKGGSTVKNI (SEQ ID NO:37);

25 KTFKTRKASAQASLASKDKTPKSKSKKRG (SEQ ID NO:38); and

a sequence substantially identical to any of the above sequences.

4. The compound of any one of claims 1 to 3, wherein the antibody or fragment thereof comprises a sequence selected from the group consisting of:

5 an antibody or fragment thereof comprising a complementarity determining region (CDR) 1 sequence of GFKITHYTMG (SEQ ID NO:1), a CDR2 sequence of RITWGGDNTFYNSVSKG (SEQ ID NO:2), a CDR3 sequence of GSTSTATPLRVDY (SEQ ID NO:3);

10 an antibody or fragment thereof comprising CDR1 sequence of EYPSNFYA (SEQ ID NO:4), a CDR2 sequence of VSRDGLTT (SEQ ID NO:5), a CDR3 sequence of AIVITGVWNKVDVNSRSYHY (SEQ ID NO:6);

an antibody or fragment thereof comprising CDR1 sequence of GGTVSPTA (SEQ ID NO:7), a CDR2 sequence of ITWSRGTT (SEQ ID NO:8), a CDR3 sequence of AASTFLRILPEESAYTY (SEQ ID NO:9); and

15 an antibody or fragment thereof comprising CDR1 sequence of GRTIDNYA (SEQ ID NO:10), a CDR2 sequence of IDWGDGGX; where X is A or T (SEQ ID NO:11), a CDR3 sequence of AMARQSRVNLDVARYDY (SEQ ID NO:12).

5. The compound of claim 4, wherein the antibody or fragment thereof is humanized.

6. The compound of any one of claims 1 to 5, wherein the antibody or fragment thereof comprises a sequence selected from the group consisting of:

20 X1VQLVX2SGGGLVQPGGSLRLSCAASGFKITHYTMGWX3RQAPGKX4X5EX6VSRITWGGDNTFYNSVSKGRFTISRDNKNTX7YLQMNSLRAEDTAVYYCAAGSTSTATPLRVDYWGQGTLLTVSS (SEQ ID NO:13), where X1=D or E, X2=A or E, X3=F or V, X4=E or G, X5=R or L, X6=F or W, X7=L or V;

25 X1VX2LX3ESGGGLVQX4GGSLRLSCX5ASEYPSNFYAMSWX6RQAPGKX7X8EX9VX10GVSRDGLTTLTLYADSVKGRFTX11SRDNX12KNTX13X14LQMNSX15X16AEDTAVYYCAIVI TGVWNKVDVNSRSYHYWGQGTX17TVSS, (SEQ ID NO:18), where X1 is E or Q; X2 is K or Q; X3 is V or E; X4 is A or P; X5 is V or A; X6 is F or V; X7 is E or G; X8 is R or L; X9 is F or W; X10 is A or S; X11 is M or I; X12 is A or S; X13 is V or L; X14 is D or Y; X15 is V or L; X16 is K or R; and X17 is Q or L;

5 X1VX2LX3ESGGGLVQX4GGSLRLSCX5X6SGGTVSPTAMGWX7RQAPGKX8X9EX1
 0VX11HITWSRGTTTRX12ASSVKX13RFTISRDX14X15KNTX16YLQMNSLX17X18EDT
 AVYYC AASTFLRILPEESAYTYWGQGT X19VTVSS, (SEQ ID NO:21), where X1 is E
 or Q; X2 is K or Q; X3 is V or E; X4 is A or P; X5 is A or E; X6 is V or A; X7 is V or F;
 X8 is G or E; X9 is L or R; X10 is F or W; X11 is G or S; X12 is V or Y; X13 is D or G;
 X14 is N or S; X15 is A or S; X16 is L or V; X17 is K or R; X18 is A or S; and X19 is L or
 Q; and

10 X1VX2LX3ESGGGLVQX4GGSLRLSCAASGRTIDNYAMAWX5RQAPGKX6X7EX8VX9
 TIDWGDGGX10RYANSVKGRFTISRDNX11KX12TX13YLQMNX14LX15X16EDTAVYX
 17CAMARQSRVNLDVARYDYWGQGT X18VTVSS (SEQ ID NO: 24), where X1 is E or
 Q; X2 is K or Q; X3 is V or E; X4 is A or P; X5 is V or S; X6 is D or G; X7 is L or R; X8
 is F or W; X9 is A or S; X10 is A or T; X11 is A or S; X12 is G or N; X13 is M or L; X14
 is N or R; X15 is E or R; X16 is P or A; X17 is S or Y; and X18 is Q or L.

7. The compound of any one of claims 1 to 6, wherein the antibody or fragment thereof
 15 comprises a sequence selected from any one of:

DVQLQASGGGLVQAGGSLRLSCAASGFKITHYTMGWFRQAPGKEREFSRITWGGD
 NTFYSNSVKGRFTISRDNKNTVYLQMNSLKPEDTADYYCAAGSTSTATPLRVDYWG
 KGTQVTVSS (SEQ ID NO:14);

20 EVQLVESGGGLVQPGGSLRLSCAASGFKITHYTMGWRQAPGKGLEWSRITWGGD
 NTFYSNSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCAAGSTSTATPLRVDYWG
 QGTLVTVSS (SEQ ID NO: 15)

EVQLVESGGGLVQPGGSLRLSCAASGFKITHYTMGWRQAPGKGLEWSRITWGGD
 NTFYSNSVKGRFTISRDNKNTVYLQMNSLRAEDTAVYYCAAGSTSTATPLRVDYWG
 QGTLVTVSS (SEQ ID NO:16);

25 EVQLVESGGGLVQPGGSLRLSCAASGFKITHYTMGWFRQAPGKGLEFSRITWGGD
 NTFYSNSVKGRFTISRDNKNTVYLQMNSLRAEDTAVYYCAAGSTSTATPLRVDYWG
 QGTLVTVSS (SEQ ID NO:17);

30 QVKLEESGGGLVQAGGSLRLSCVASEYPSNFYAMSWFRQAPGKEREFSRITWGGD
 TTYADSVKGRFTISRDNKNTVDLQMNSVKAEDTAVYYCAIVITGVWNKVDVNSRS
 YHYWGQGTQVTVSS (SEQ ID NO: 19);

EVQLVESGGGLVQPGGSLRLSCAASEYPSNIFYAMSWFRQAPGKEREFSVSGVSRDGL
TTLYADSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCAIVITGVWNKVDVNSRSY
HYWGQGTQVTVSS (SEQ ID NO: 20);

5 QVKLEESGGGLVQAGGSLRLSCEVSGGTVSPTAMGWFRQAPGKEREFGHITWSR
GTTRVASSVKDRFTISRDSAKNTVYLQMNSLKSEDTAVYYCAASTFLRILPEESAYTY
WGQGTQVTVSS (SEQ ID NO: 22);

QVQLVESGGGLVQPGGSLRLSCAVSGGTVSPTAMGWFRQAPGKLEFGHITWSR
GTTRYASSVKGRFTISRDNKNTVYLQMNSLRAEDTAVYYCAASTFLRILPEESAYTY
WGQGTQVTVSS (SEQ ID NO 23)

10 QVKLEESGGGLVQAGGSLRLSCAASGRTIDNYAMAWSRQAPGKDREFVATIDWGDG
GARYANSVKGRFTISRDNAGTMYLQMNNLEPEDTAVYSCAMARQSRVNLDVARYD
YWGQGTQVTVSS (SEQ ID NO:25);

QVQLVESGGGLVQPGGSLRLSCAASGRTIDNYAMAWVRQAPGKLEWWATIDWGD
GGTRYANSVKGRFTISRDNKNTMYLQMNSLRAEDTAVYYCAMARQSRVNLDVARY
15 DYWGQGTQVTVSS (SEQ ID NO:26); and

a sequence substantially identical to any of the above sequences.

8. The compound of any one of claims 1 and 7, wherein the antibody or fragment thereof is a single-domain antibody (sdAb).

9. The compound of any one of claims 1 to 8, wherein the Fc fragment is mouse Fc2a or
20 human Fc1.

10. The compound of claim 9, wherein the Fc fragment comprises SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41.

11. The compound of any one of claims 1 to claim 10, wherein the fusion protein forms a dimer.

25 12. The compound of claim any one of claims 10 to 11, wherein the fusion protein comprises an antibody or fragment thereof, an Fc fragment, and the polypeptide that binds β -amyloid.

13. The compound of claim 12, wherein the fusion protein comprises the antibody or fragment thereof linked to the N-terminus of the Fc fragment, and the polypeptide that binds β -amyloid is linked to the C-terminus of the Fc fragment.

14. The compound of claim 12, wherein the antibody or fragment thereof is linked to the C-terminus of the Fc fragment and the polypeptide that binds β -amyloid linked to the N-terminus of the Fc fragment.
15. The compound of claim 13 or 14, wherein the fusion protein further comprises a linker.
- 5 16. The compound of claim 15, wherein the linker is an independently selected linker sequence linking the antibody or fragment thereof and/or linking the polypeptide that binds β -amyloid to the Fc.
17. The compound of claim 16 wherein the linker sequence is GGGSGGGGS, or any suitable linker and in SEQ ID NO: 53.
- 10 18. The compound of claim 13, wherein the fusion protein comprises an antibody or fragment thereof selected from the group consisting of SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:26 ; a polypeptide that binds b-amyloid selected from the group consisting of SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID
- 15 NO:36, SEQ ID NO:37, SEQ ID NO:38 ; and an Fc fragment selected from the group consisting of SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41.
19. The compound of claim 18, wherein the fusion protein comprises any one of SEQ ID NO: 42 to SEQ ID NO: 53, and a sequence substantially identical to any of these sequences, and/or dimers thereof.
- 20 20. The compound of claim19, wherein the fusion protein comprises a linker sequence that may be substituted for any suitable linker sequence.
21. The compound of any one of claims 1 to 20, wherein the compound comprises dimers of the fusion protein.
22. The compound of any one of claims 1 to 20, wherein the antibody or fragment thereof
- 25 transmigrates the blood-brain barrier.
23. A nucleic acid molecule encoding a compound of any one of claims 1 to 20.
24. A vector comprising the nucleic acid molecule of claim 23.
25. A composition comprising a compound of any one of claims 1 to 22 and a pharmaceutically-acceptable carrier, diluent, or excipient.

26. A kit comprising the pharmaceutical composition of claim 25.
27. A composition according to claim 25 for treating Alzheimer's disease in a patient.
28. A method of treating Alzheimer's diseases, comprising administering a compound of any one of claims 1 to 22 or the composition of claim 25 to a subject in need thereof.
- 5 29. A method of reducing toxic β -amyloid levels in the brain of a subject having increased levels of brain amyloid beta comprising the steps of repeated parenteral administration of a sufficient amount of a pharmaceutical composition of claim 25 to a subject.
30. A method of claim 29, wherein parenteral administration is subcutaneous or intravenous administration.
- 10 31. A method of claim 29 or 30 wherein toxic β -amyloid levels are reduced, after repeated parenteral administration of the composition of claim 25, in the brains of subjects having increased brain levels of amyloid beta.
32. A method according to claim 31 wherein toxic β -amyloid are reduced within four weeks of repeated parenteral administration of the composition of claim 22.
- 15 33. A method according to claim 29, wherein toxic β -amyloid levels in the cerebrospinal fluid (CSF) of subjects having increased CSF is reduced after parenteral administration of the composition of claims 25.
34. A method according to claim 30, wherein toxic β -amyloid levels in the cerebrospinal fluid (CSF) of subjects having increased CSF is reduced within 24 hours of a single parenteral
20 administration of the composition of claim 25.
35. A method of reducing toxic β -amyloid levels in the brains of subjects having increased levels of brain amyloid beta comprising the administration of a compound of any one of claims 1 to 22.
36. An isolated polypeptide comprising an amino acid sequence
- 25 $X_1\text{TFX}_2\text{TX}_3\text{X}_4\text{ASAQASLASKDKTPKSKSKX}_5\text{X}_6\text{STQLX}_7\text{SX}_8\text{VX}_9\text{NI}$ (SEQ ID NO: 31)
- wherein $X_1 = \text{G or A}$, $X_2 = \text{G or V}$, $X_3 = \text{G or A}$, $X_4 = \text{G or A}$, $X_5 = \text{G or V}$, $X_6 = \text{G or V}$, $X_7 = \text{G or V}$, $X_8 = \text{G or A}$, $X_9 = \text{G or A}$.

37. An isolated polypeptide according to claim 36, wherein the polypeptide sequence is selected from the group consisting of SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, and sequence substantially equivalent thereto.
- 5 38. An isolated polypeptide according to claim 36 or 37, fused to an antibody or fragment capable of transmutating the blood brain barrier.
39. A isolated polypeptide according to claim 36 or 37, further comprising an antibody or fragment capable of transmutating the blood brain barrier.
40. A isolated polypeptide according to claim 36 or 37, further comprising an Fc fragment.
- 10 41. A fusion protein comprising an antibody or fragment thereof selected from the group consisting of SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO: 25, SEQ ID NO:26 ; a polypeptide that binds b-amyloid selected from the group consisting of SEQ ID NO: 32, SEQ ID NO:33, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, 15 SEQ ID NO:38 ; ; and an Fc fragment selected from the group consisting of SEQ ID NO: SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41.
42. A fusion protein according to claim 41, wherein the fusion protein further comprises a linker peptide (for example GGGSGGGGS); wherein the fusion protein is a single chain polypeptide.
43. A fusion protein according to claim 42, wherein the fusion protein forms a dimeric 20 polypeptide.
44. A composition comprising the fusion protein of any one of claim 41 to 43, and a pharmacologically acceptable carrier.
45. A fusion protein of any one of claims 41 to 43, comprising an Fc with attenuated effector functions.
- 25 46. A nucleic acid, or vector comprising a nucleic acid, encoding a fusion protein of any one of claims 41 to 43 and 45.
47. A pharmaceutical composition comprising the isolated polypeptide of any one of claims 36 to 40, and a pharmaceutically acceptable diluent, carrier, vehicle or excipient for ameliorating the symptoms of Alzheimer's disease.

48. A pharmaceutical composition of claim 47 for use in reducing toxic β -amyloid levels in the brains of subjects having increased levels of brain amyloid beta.

49. A method of reducing toxic β -amyloid levels in the brains of subjects having increased levels of brain amyloid beta comprising the step of introducing a pharmaceutical composition of
5 claim 48 into a subject's body.

10

15

20

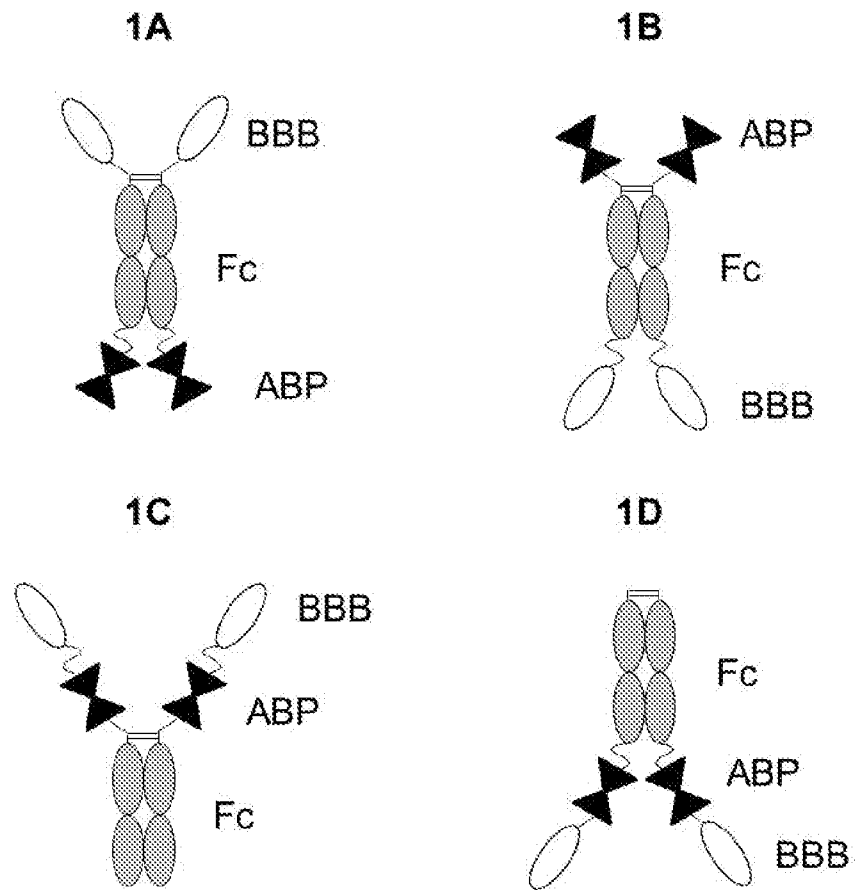


FIG. 1

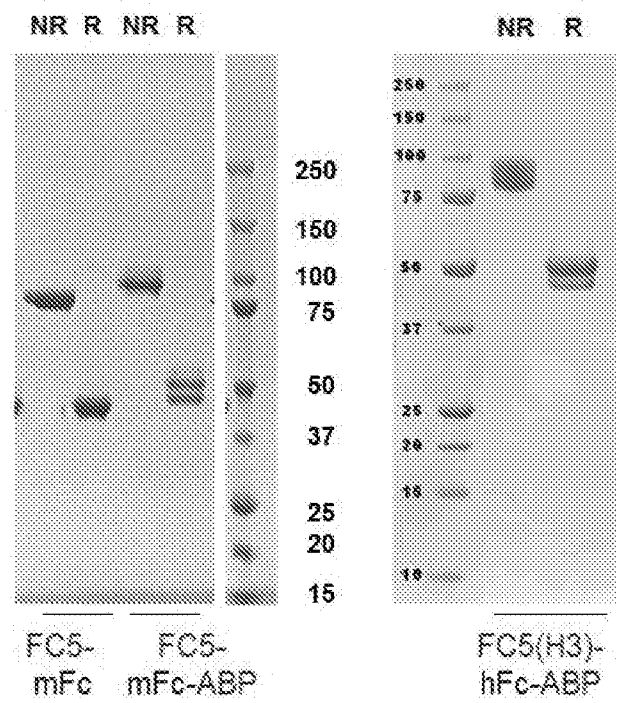


FIG. 2A

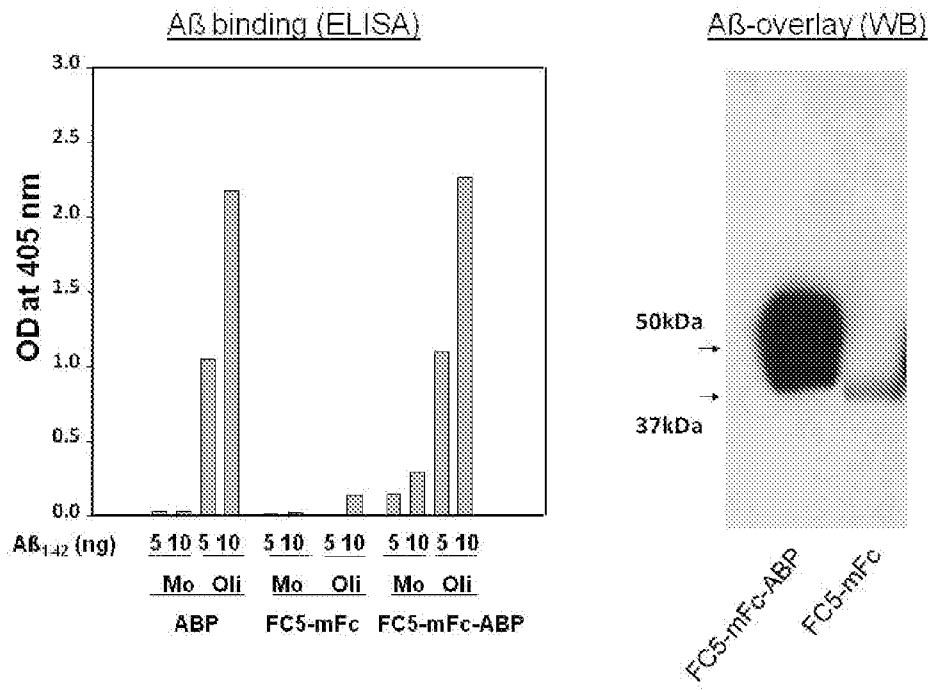


FIG. 2B

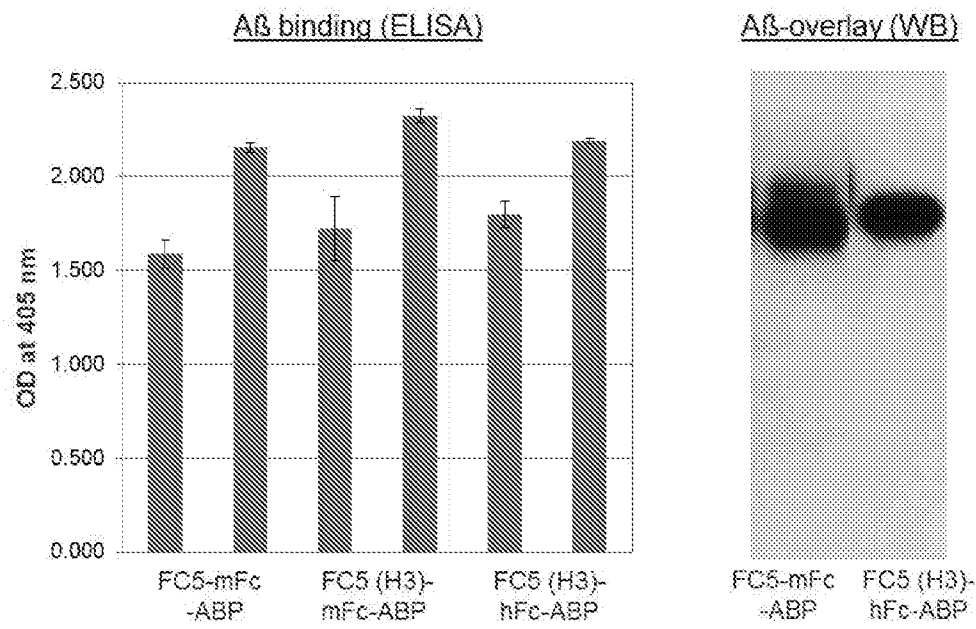


FIG. 2C

IR-800-FC5-mFc-ABP binding of brain A β

Wild type AD-Tg

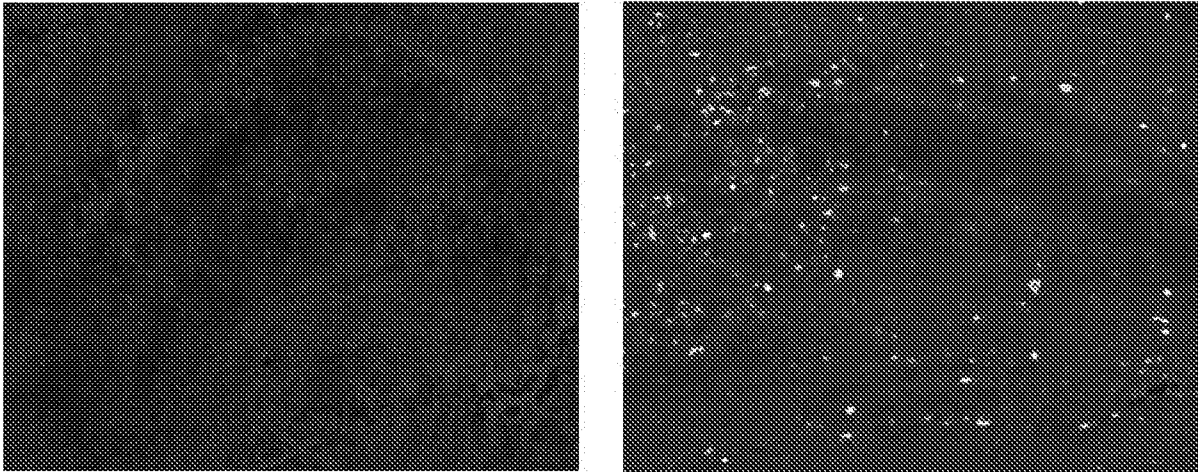


FIG. 3

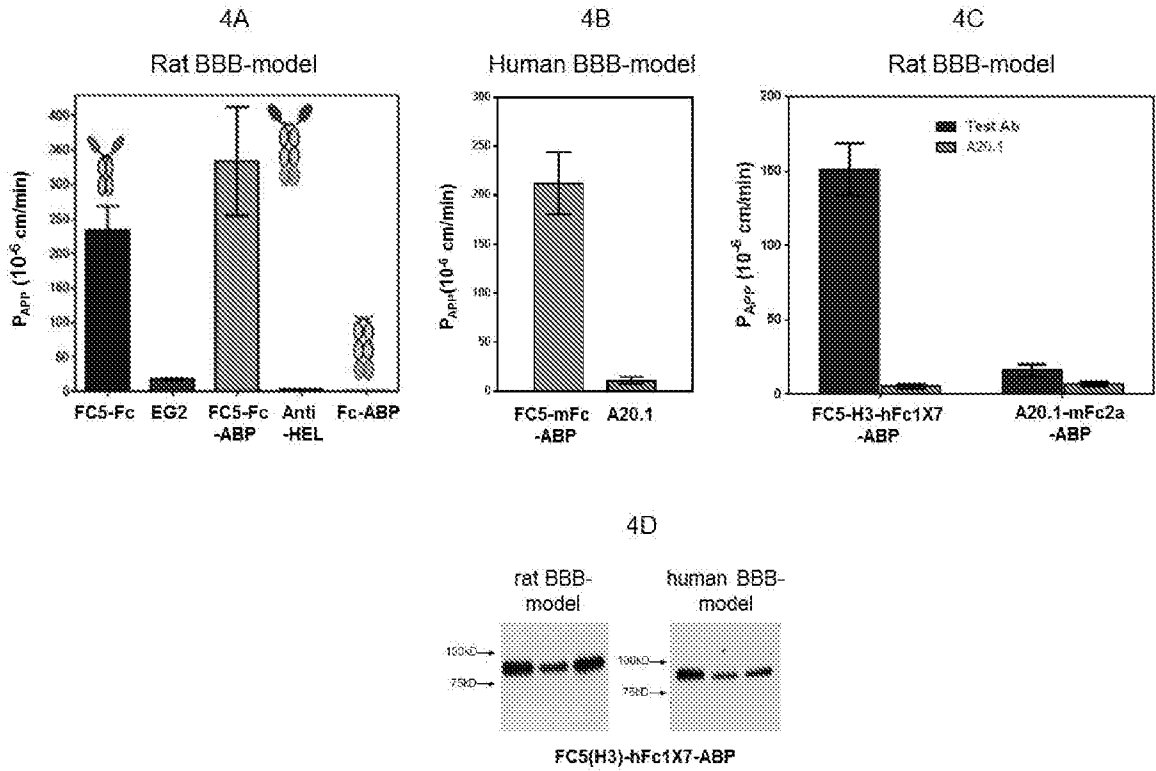


FIG. 4

Serum and CSF PK - CSF appearance of iv injected FC5-mFc-ABP
(rat)

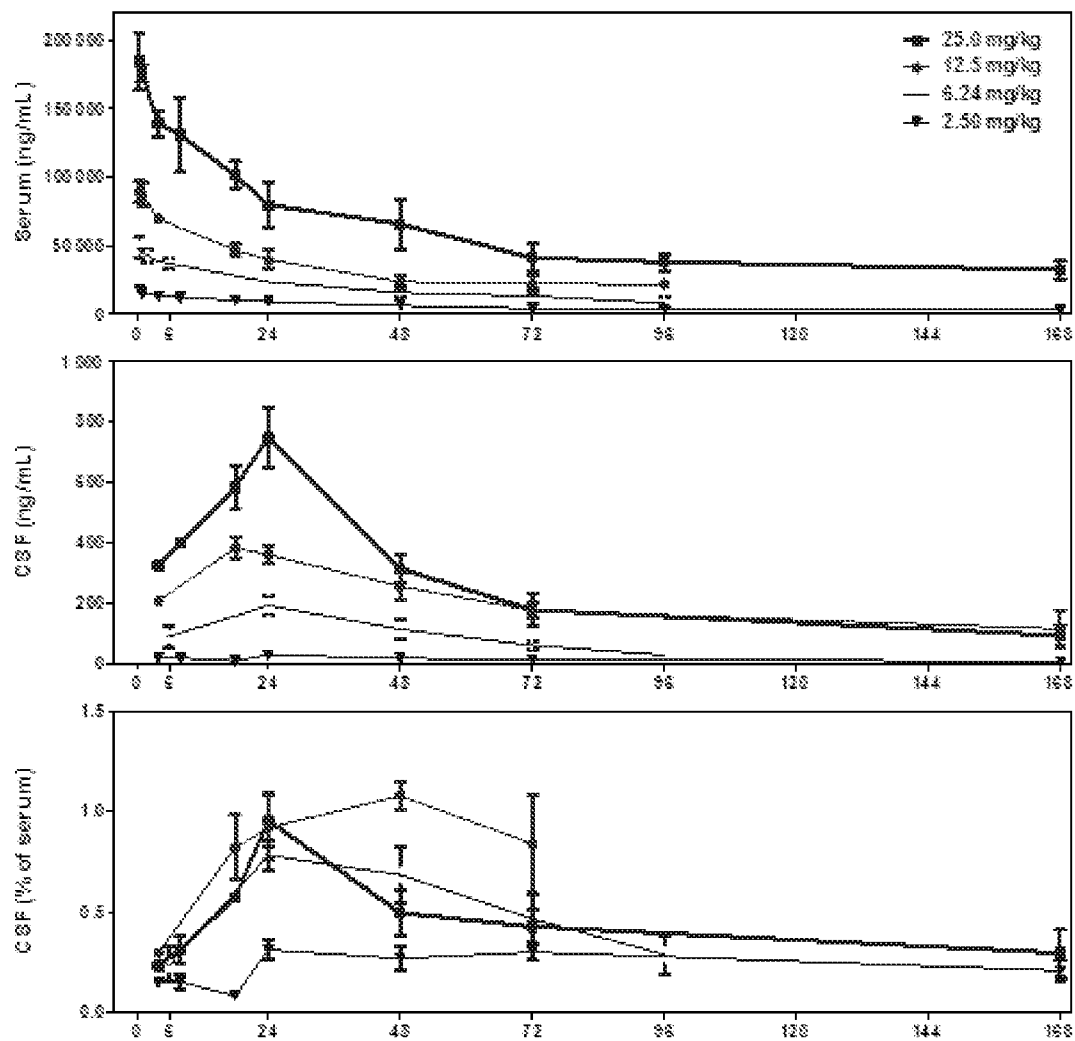


Fig. 5

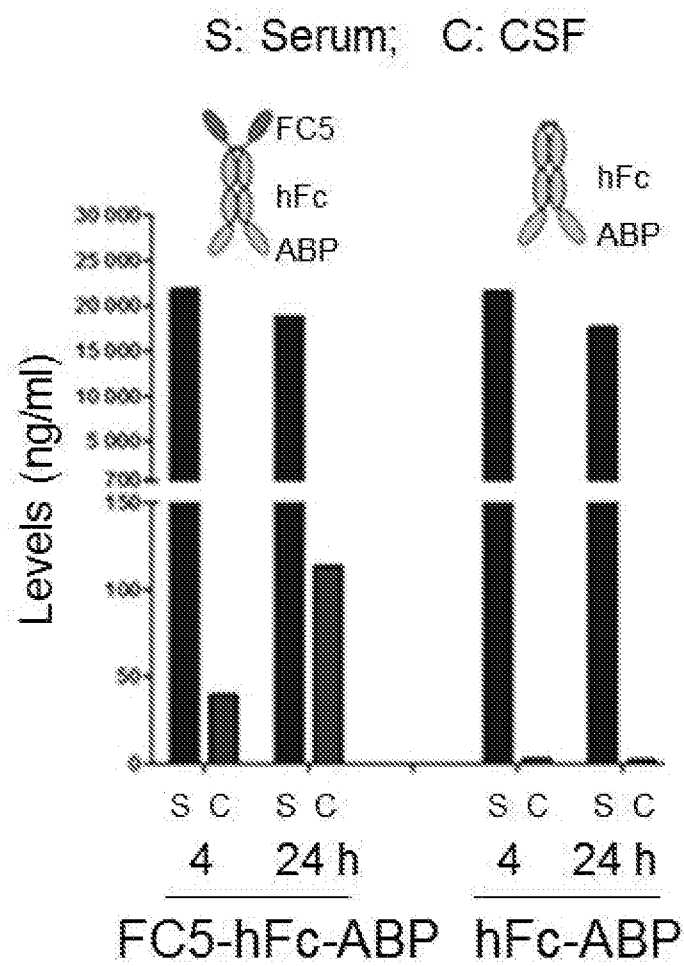


FIG. 7

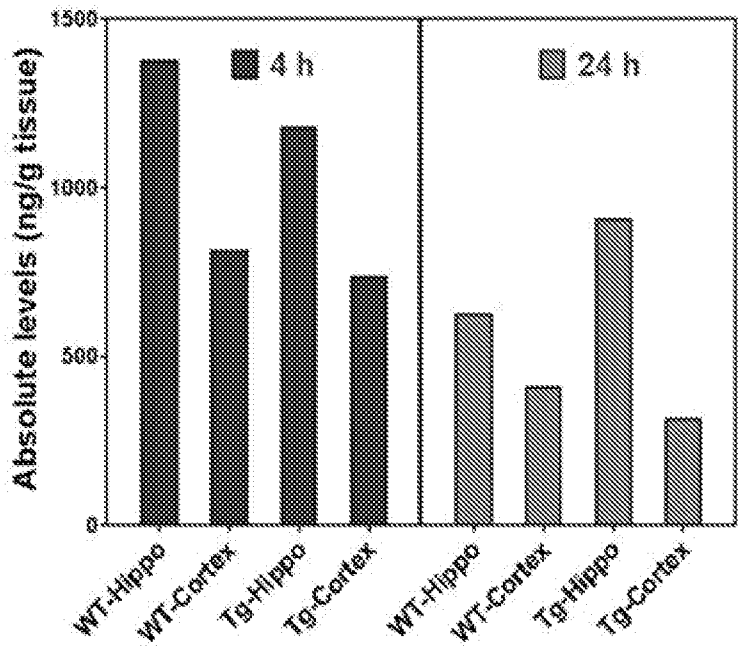


FIG. 8A

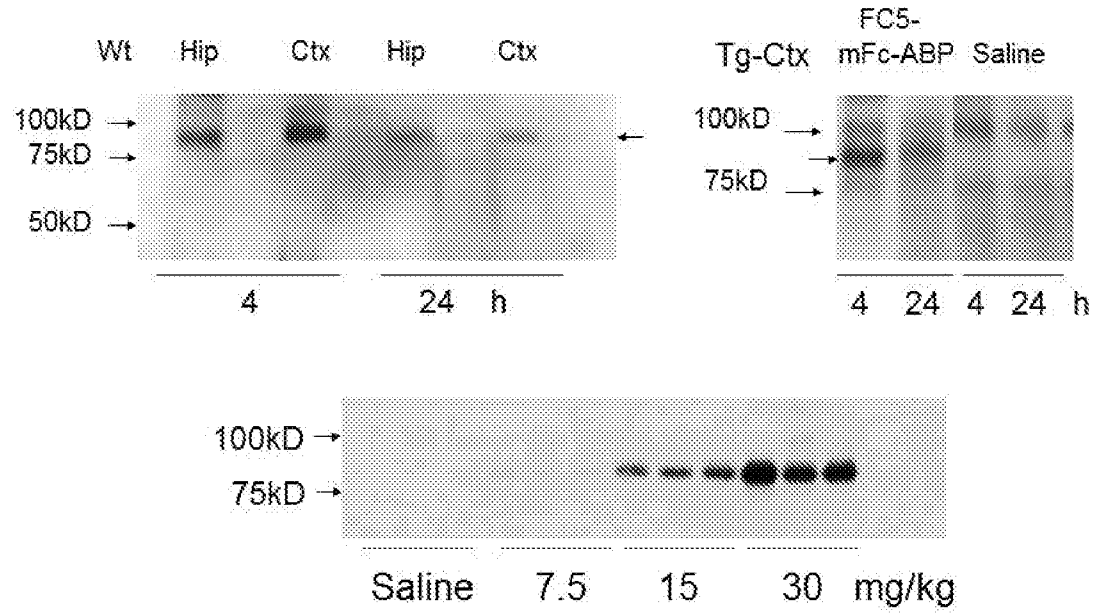


FIG. 8B

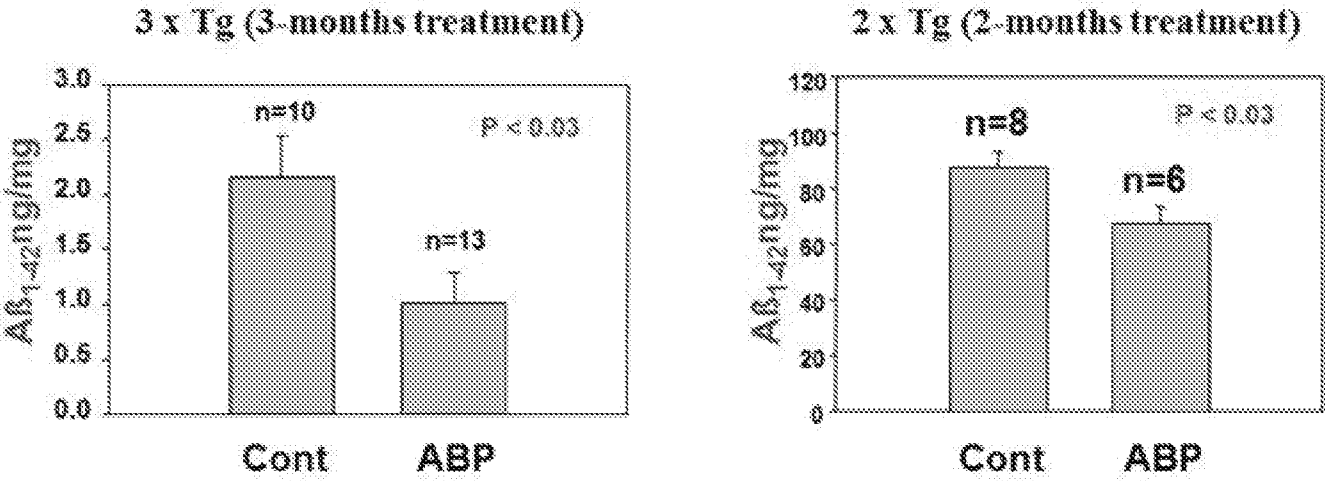


FIG. 9A

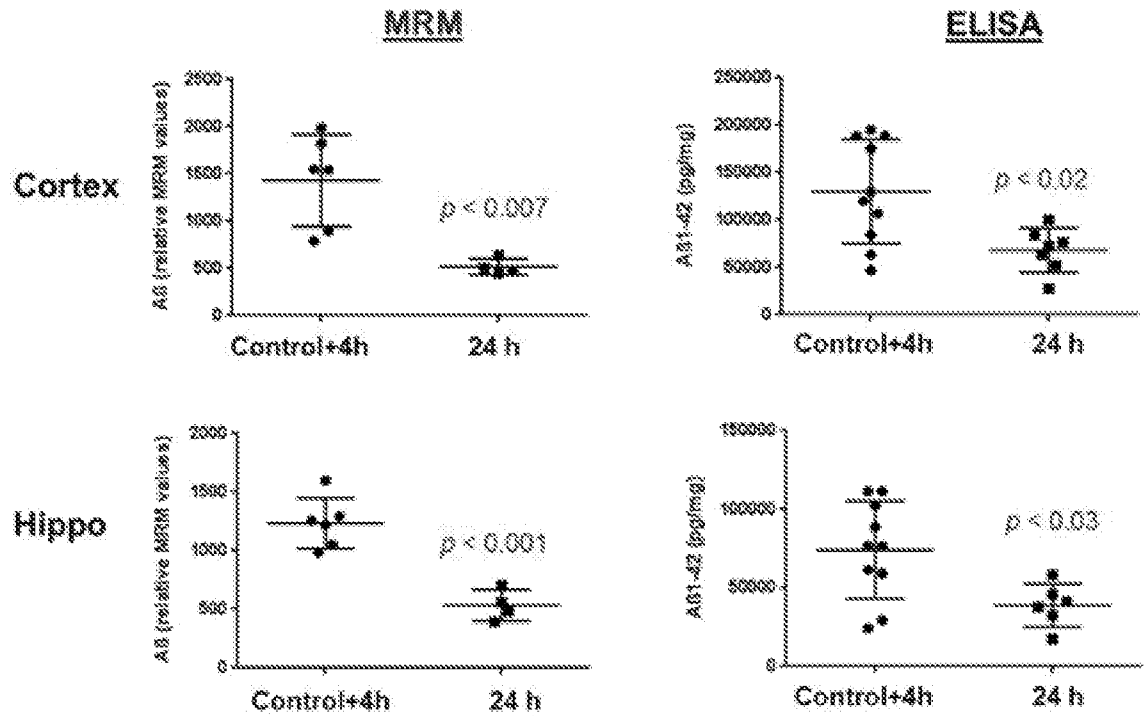


FIG. 9B

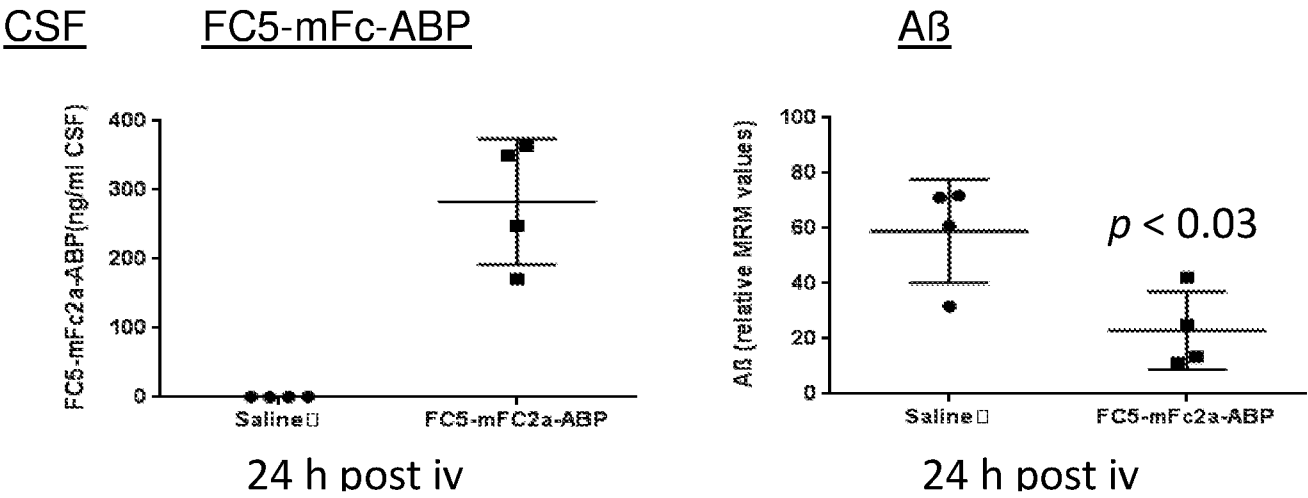
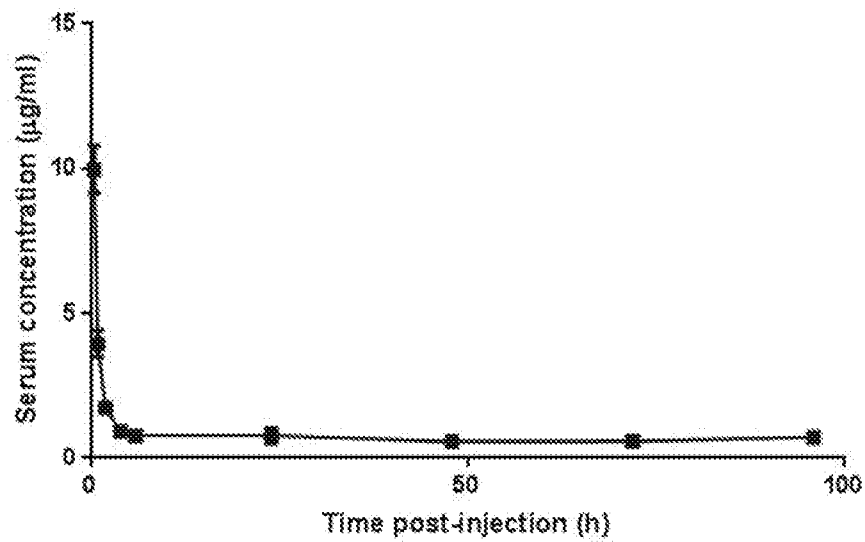


FIG. 9C

A FC5-ABP



B FC5-Fc-ABP

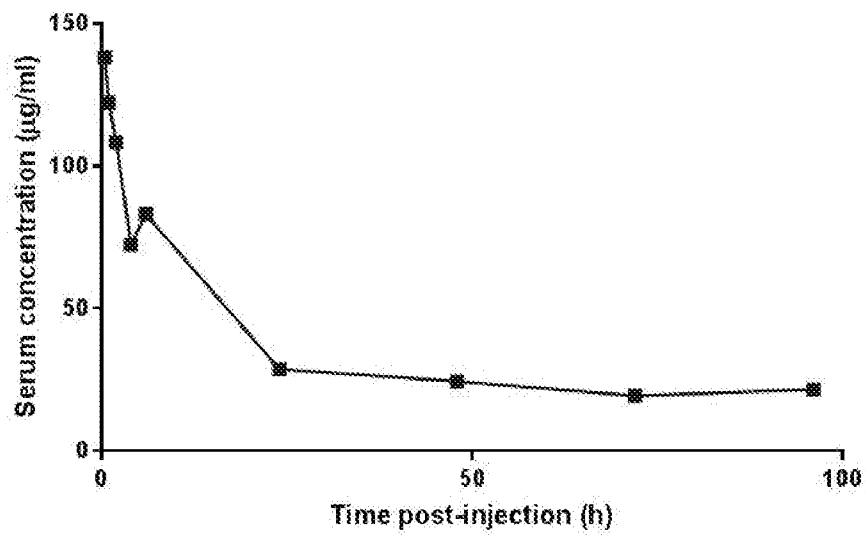


FIG. 10

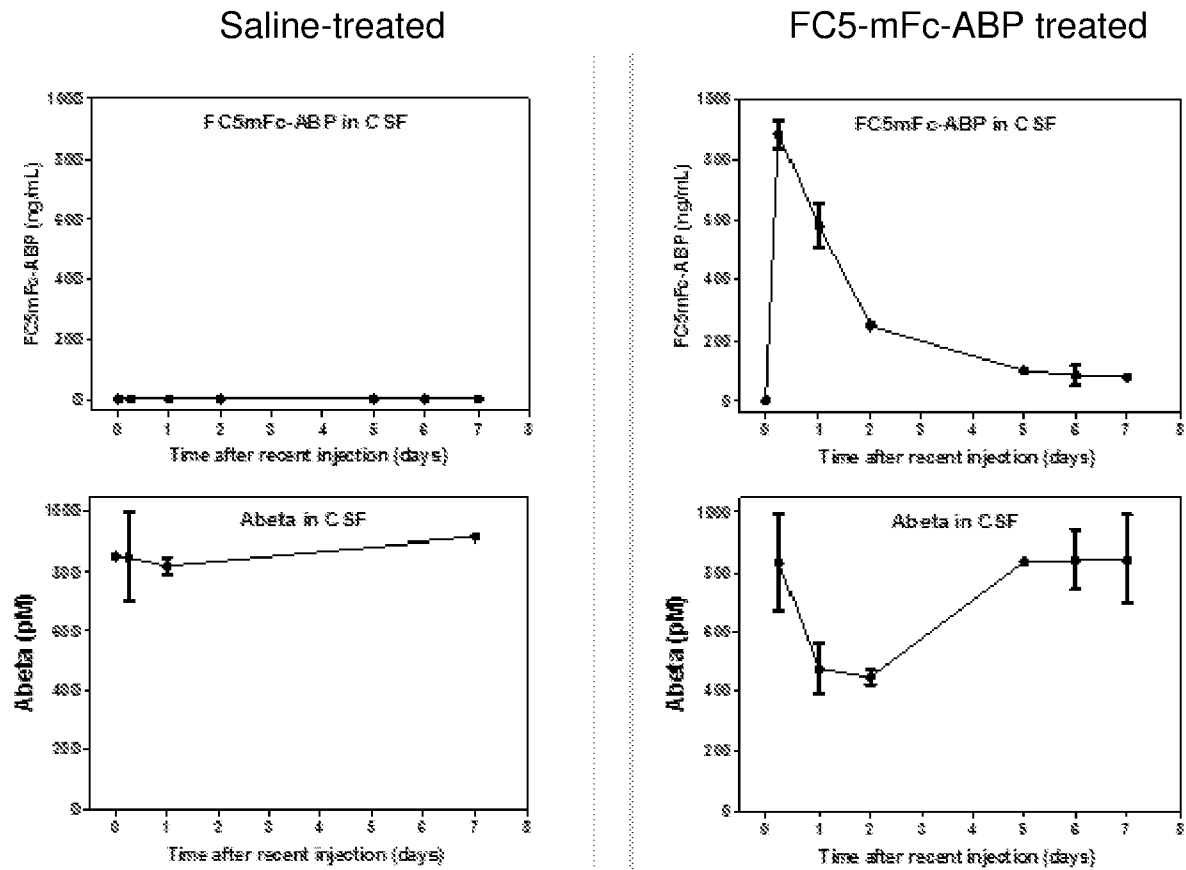


FIG. 11A

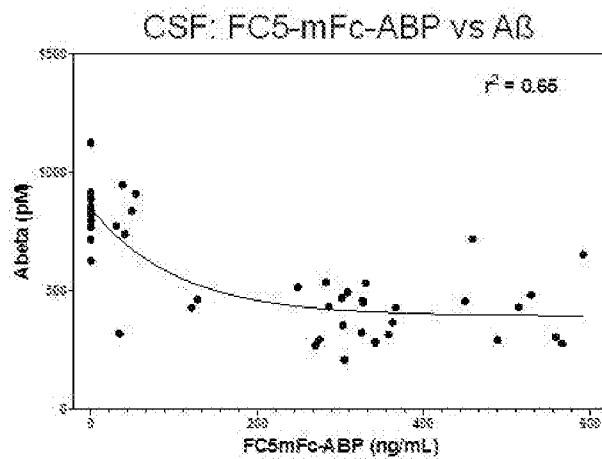


FIG. 11B

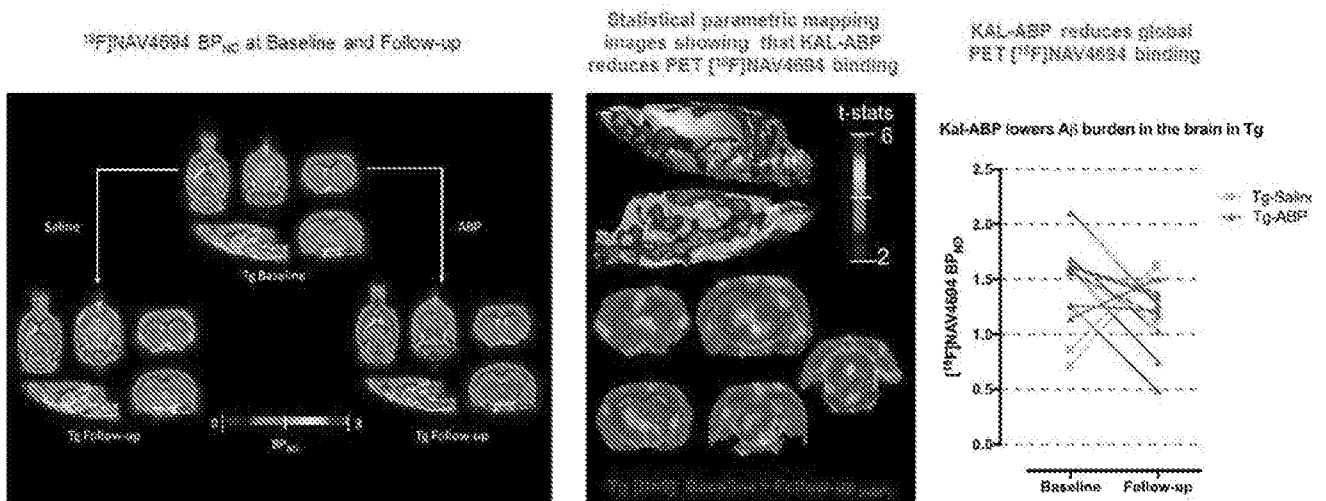


FIG. 11C

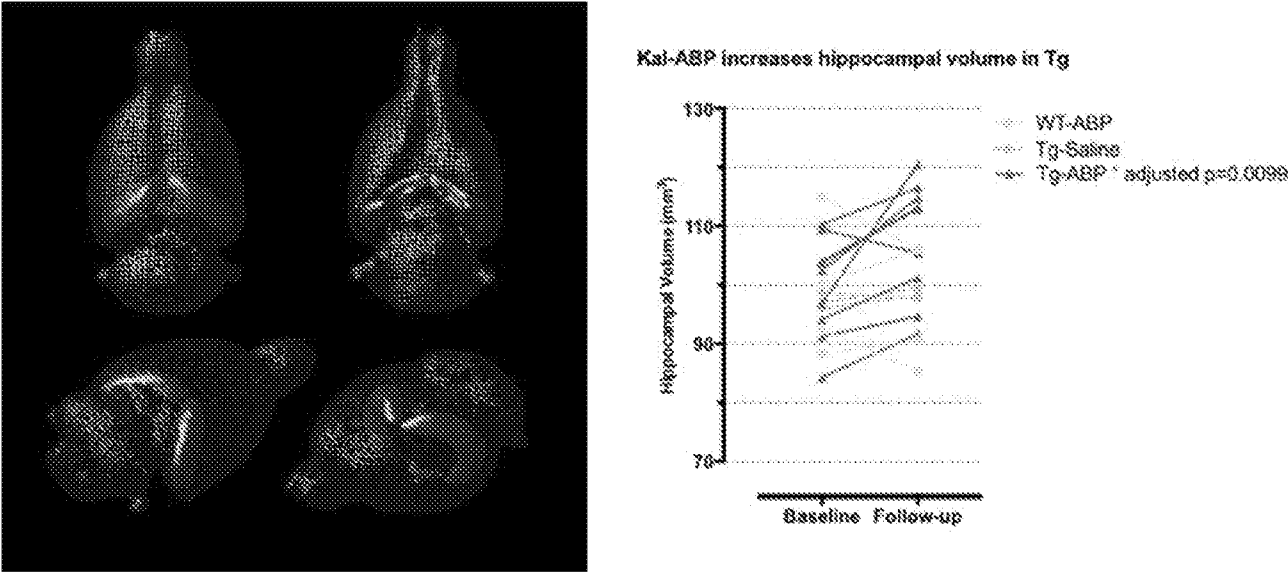


FIG. 12A

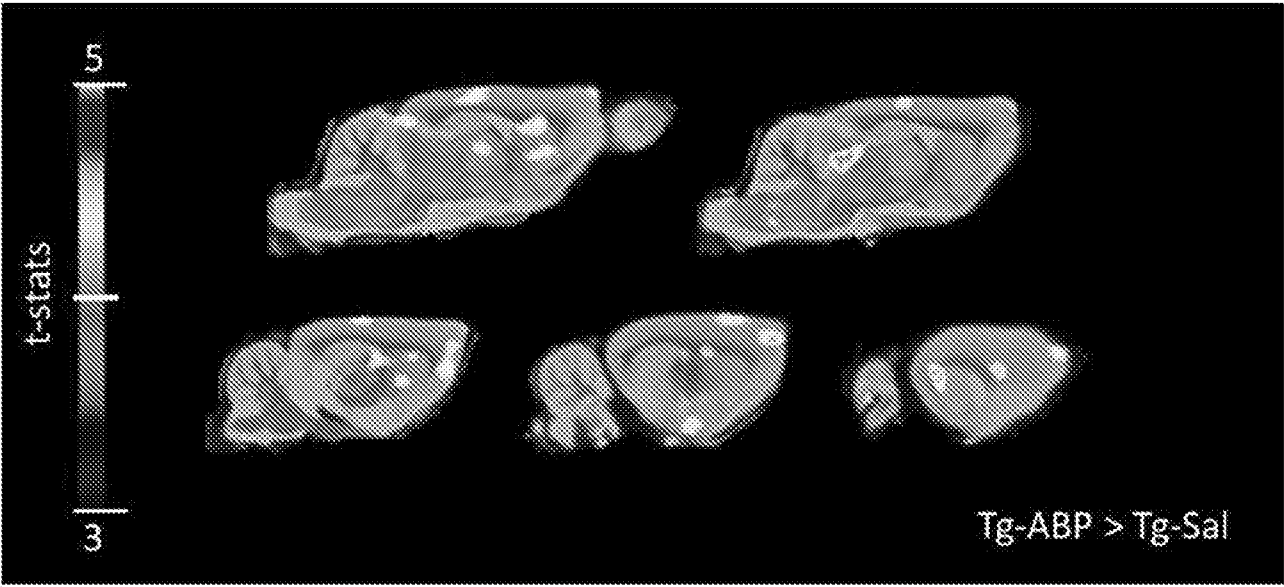


FIG. 12B

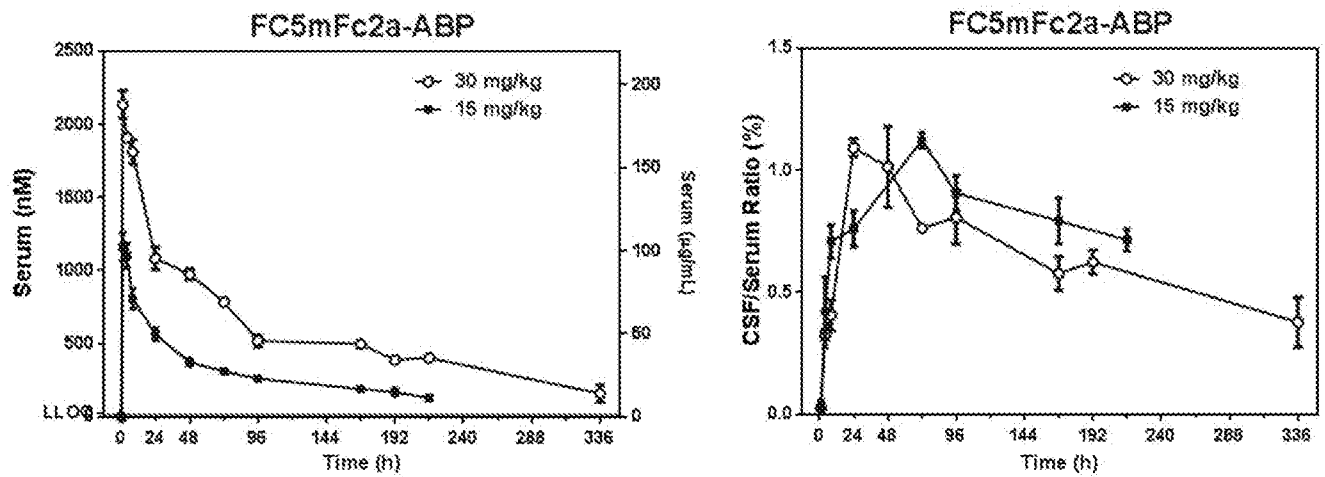


FIG. 13A

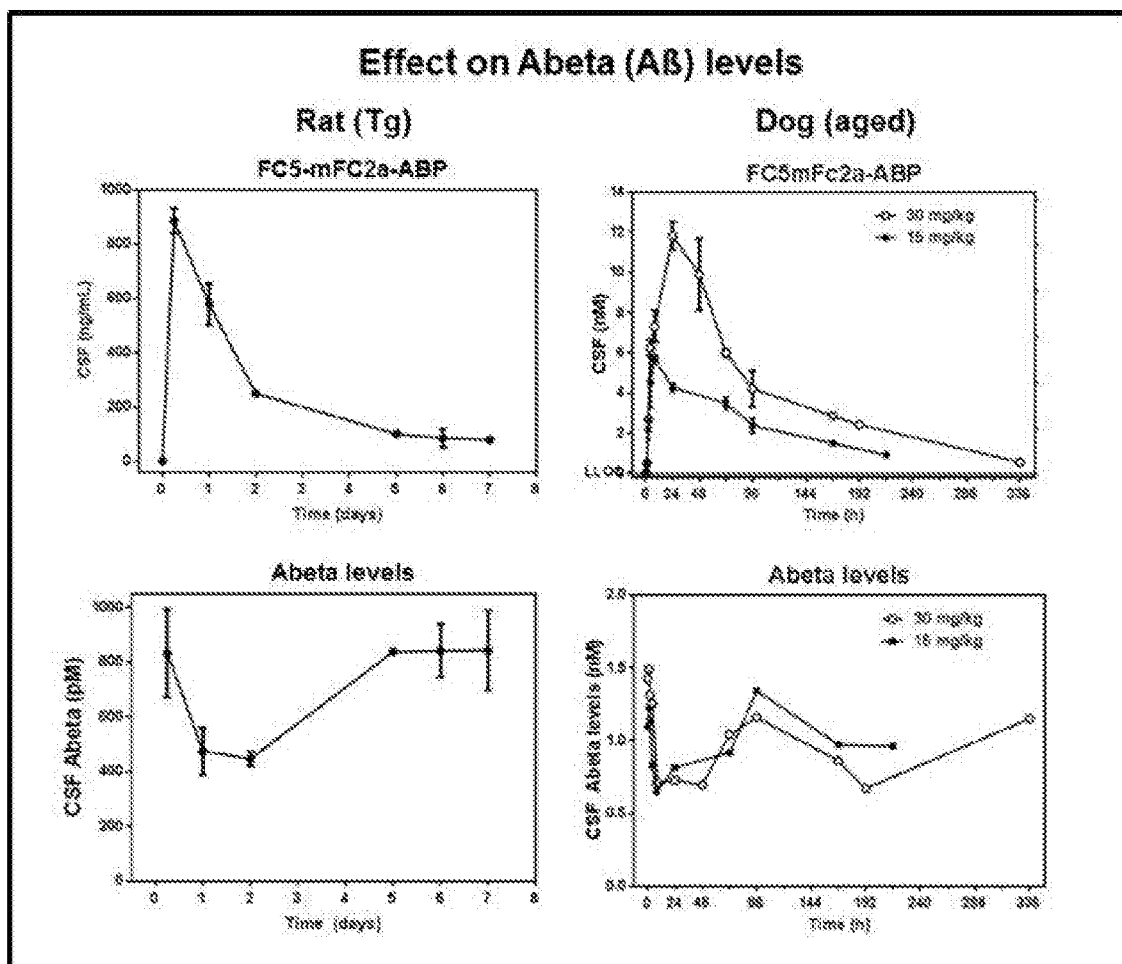


FIG. 13 B

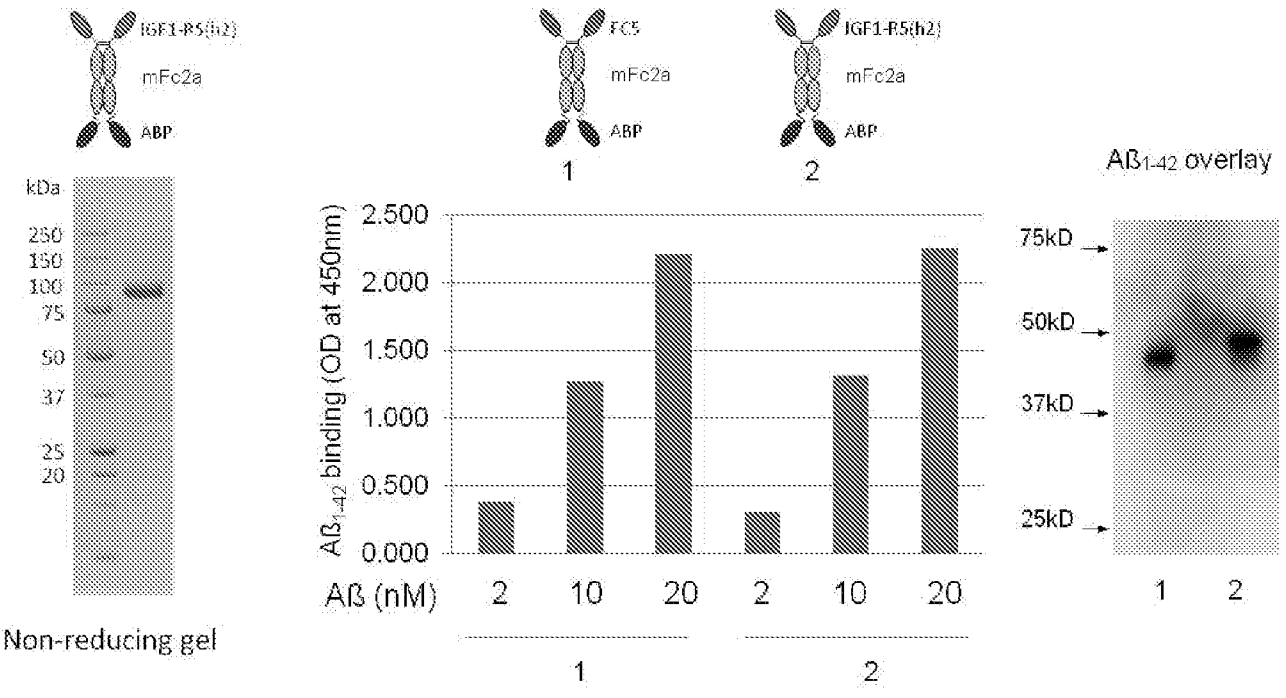


FIG. 14

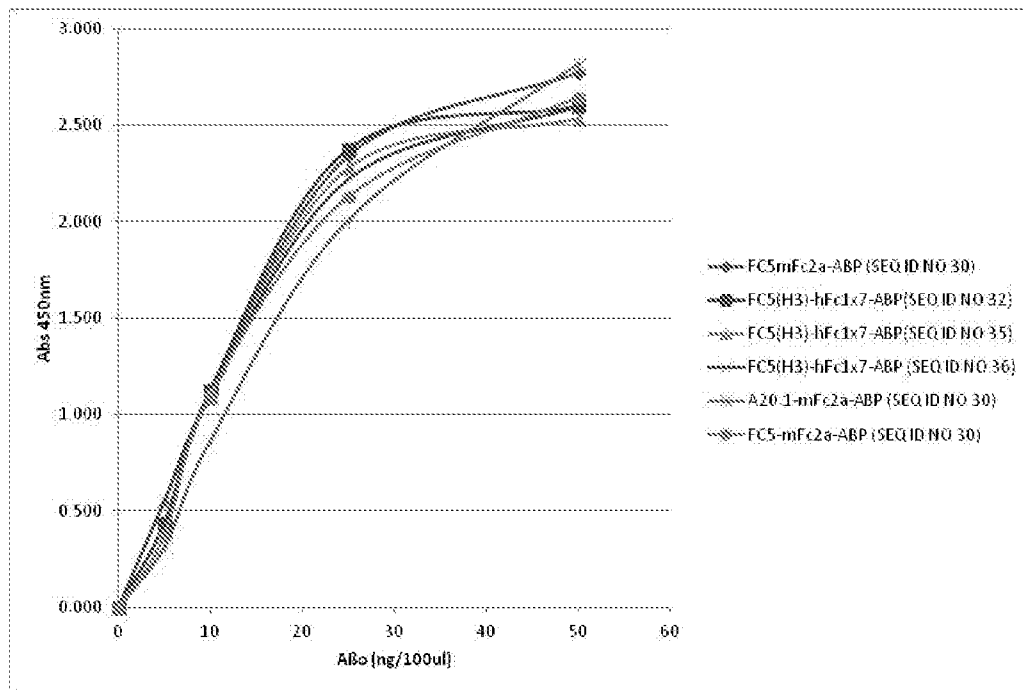


FIG. 15A

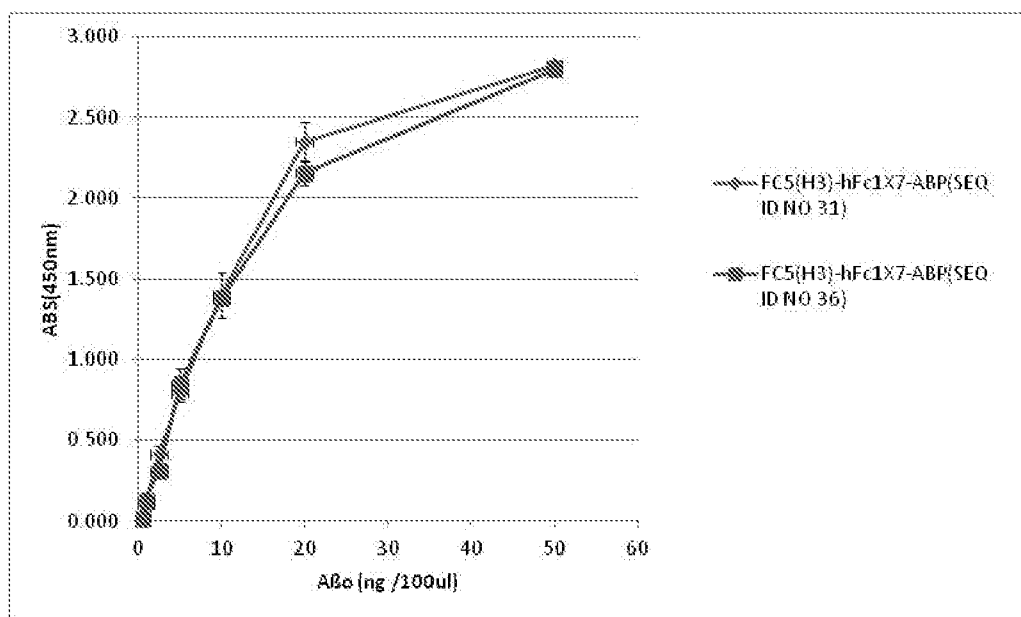


FIG. 15B

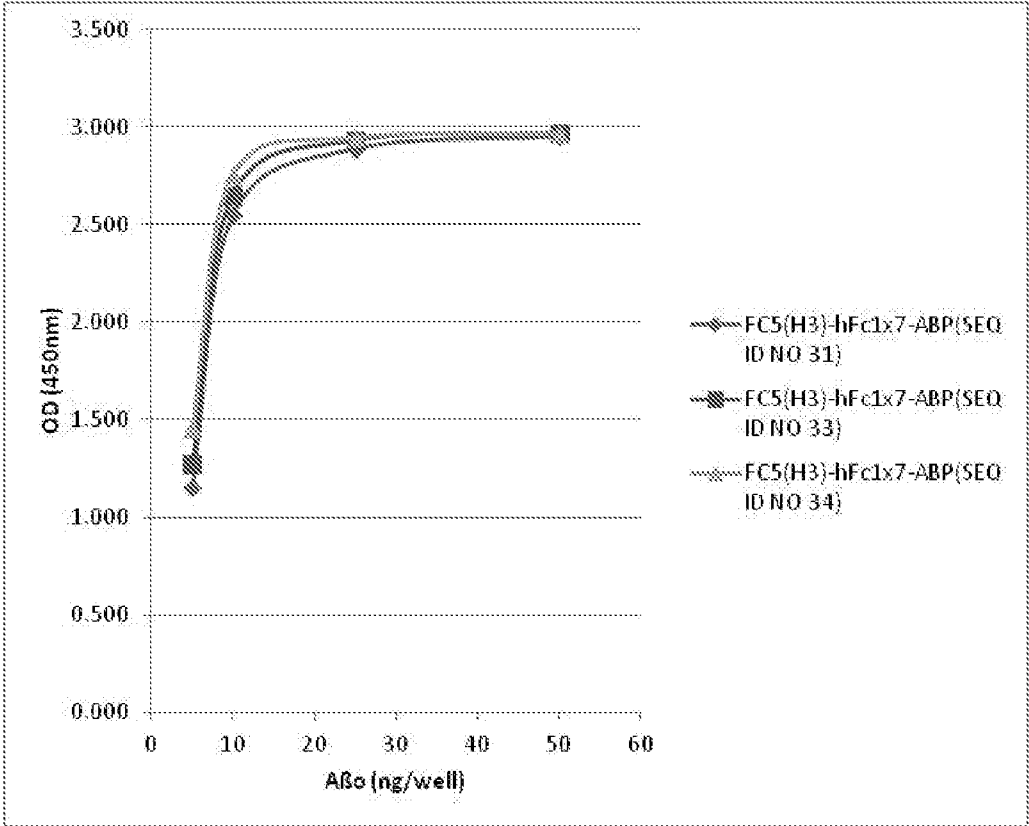
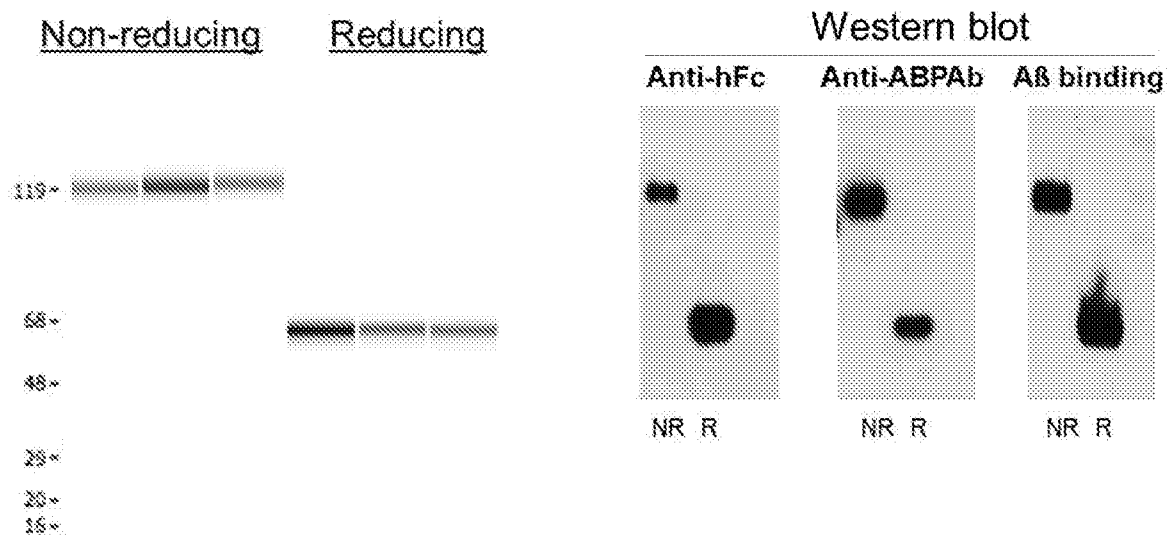


FIG. 15C

FC5(H3)-hFc1X7-ABP (ABP SEQ ID NO 35)



FC5(H3)-hFc1X7-ABP (ABP SEQ ID NO 36)

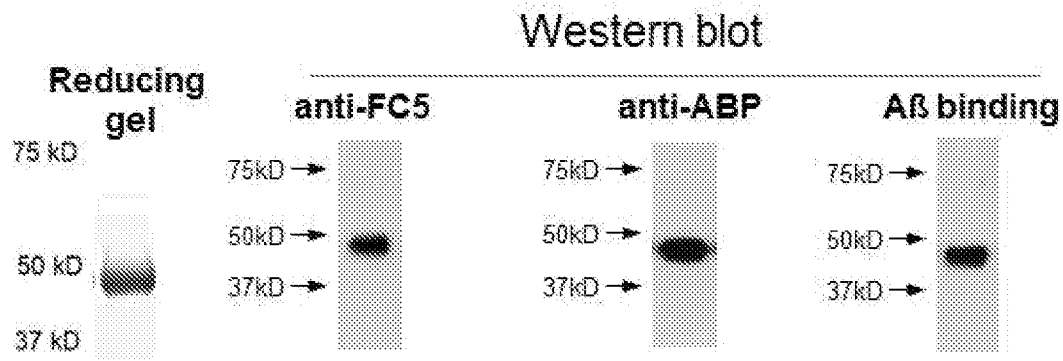


FIG. 16

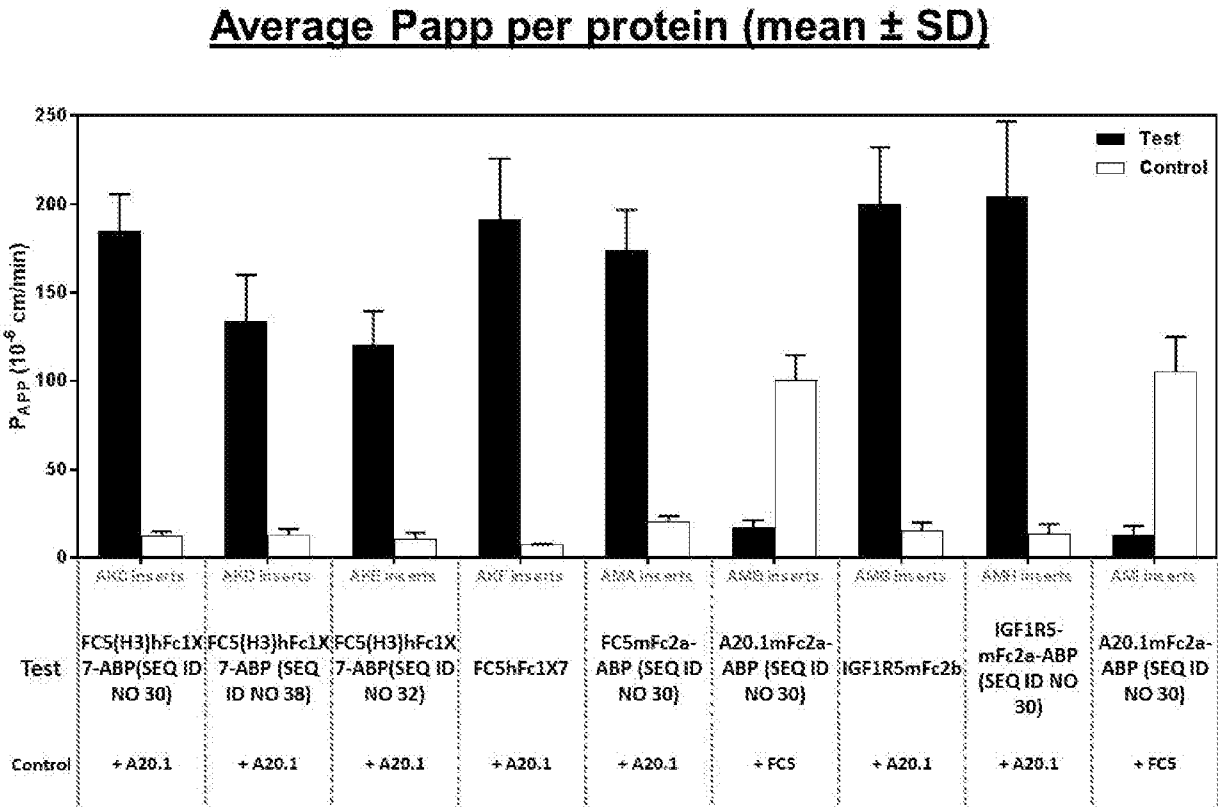


FIG. 17A

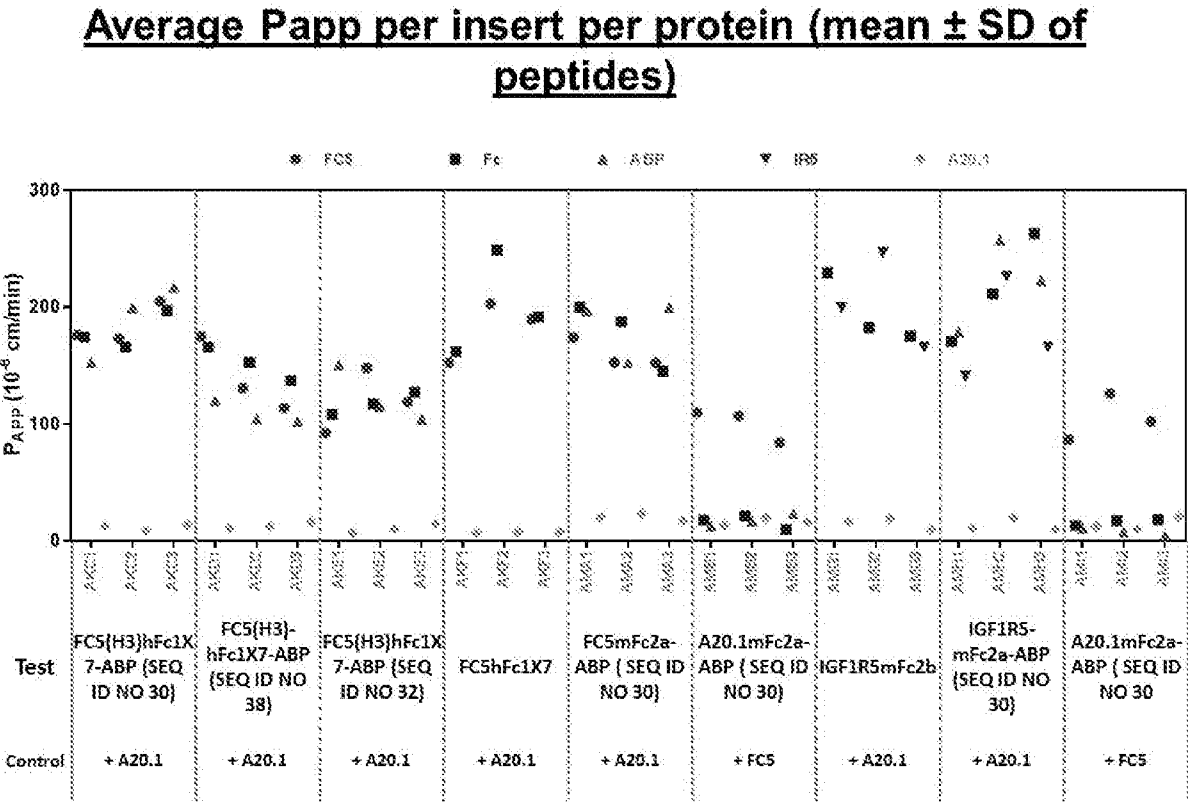
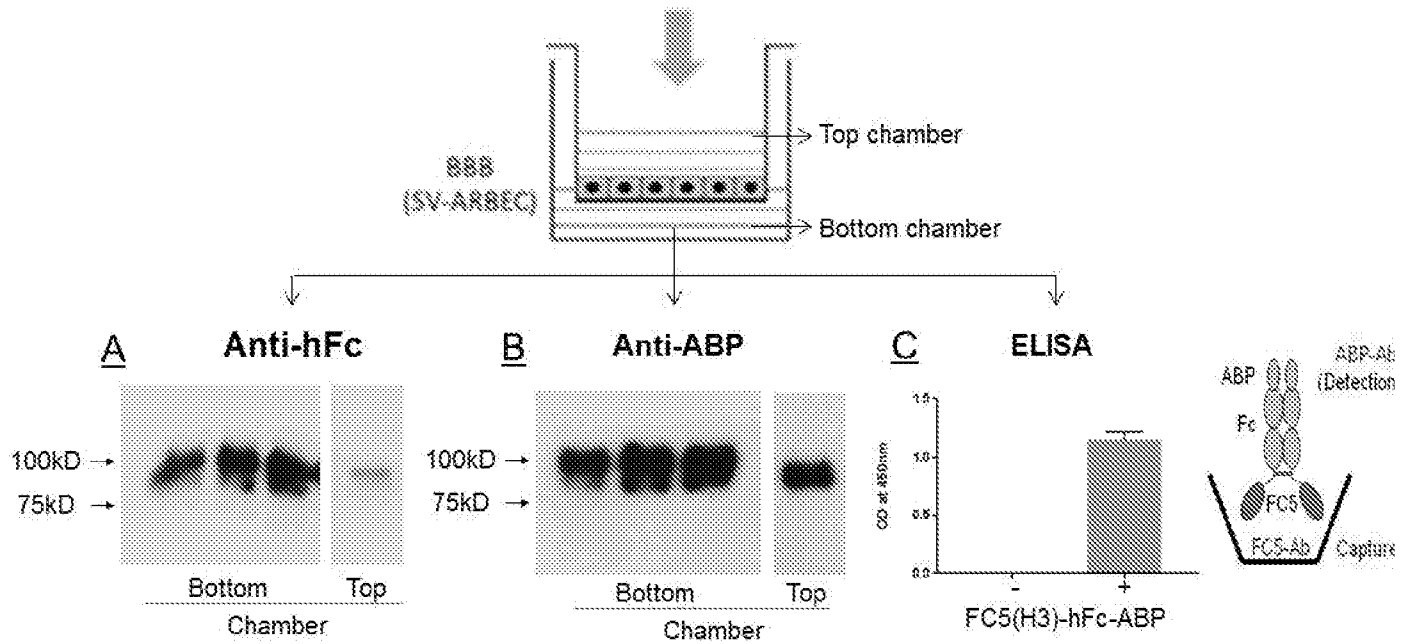


FIG. 17B

FC5(H3)-hFc-ABP crosses in vitro rat-BBB intact

1.

FC5(H3)-hFc-ABP (ABP SEQ ID NO 35)



2.

FC5(H3)-hFc-ABP (ABP SEQ ID NO 36)

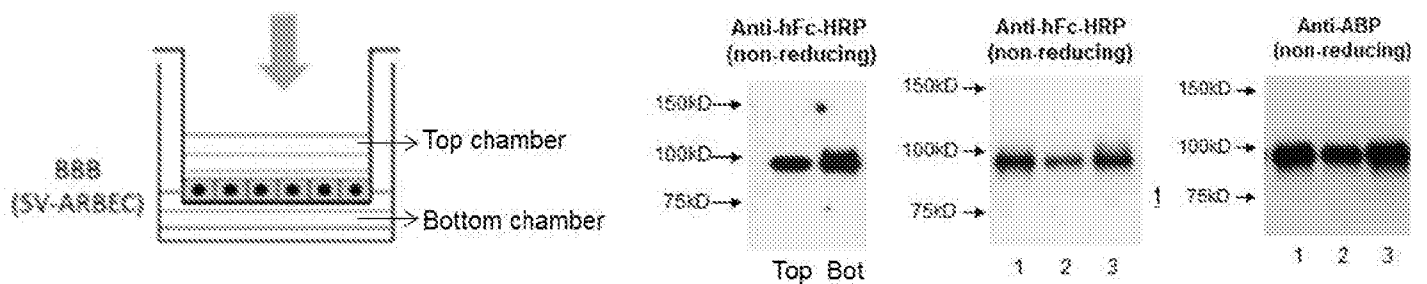


FIG. 18

FC5(H3)-hFc-ABP crosses in vivo BBB intact

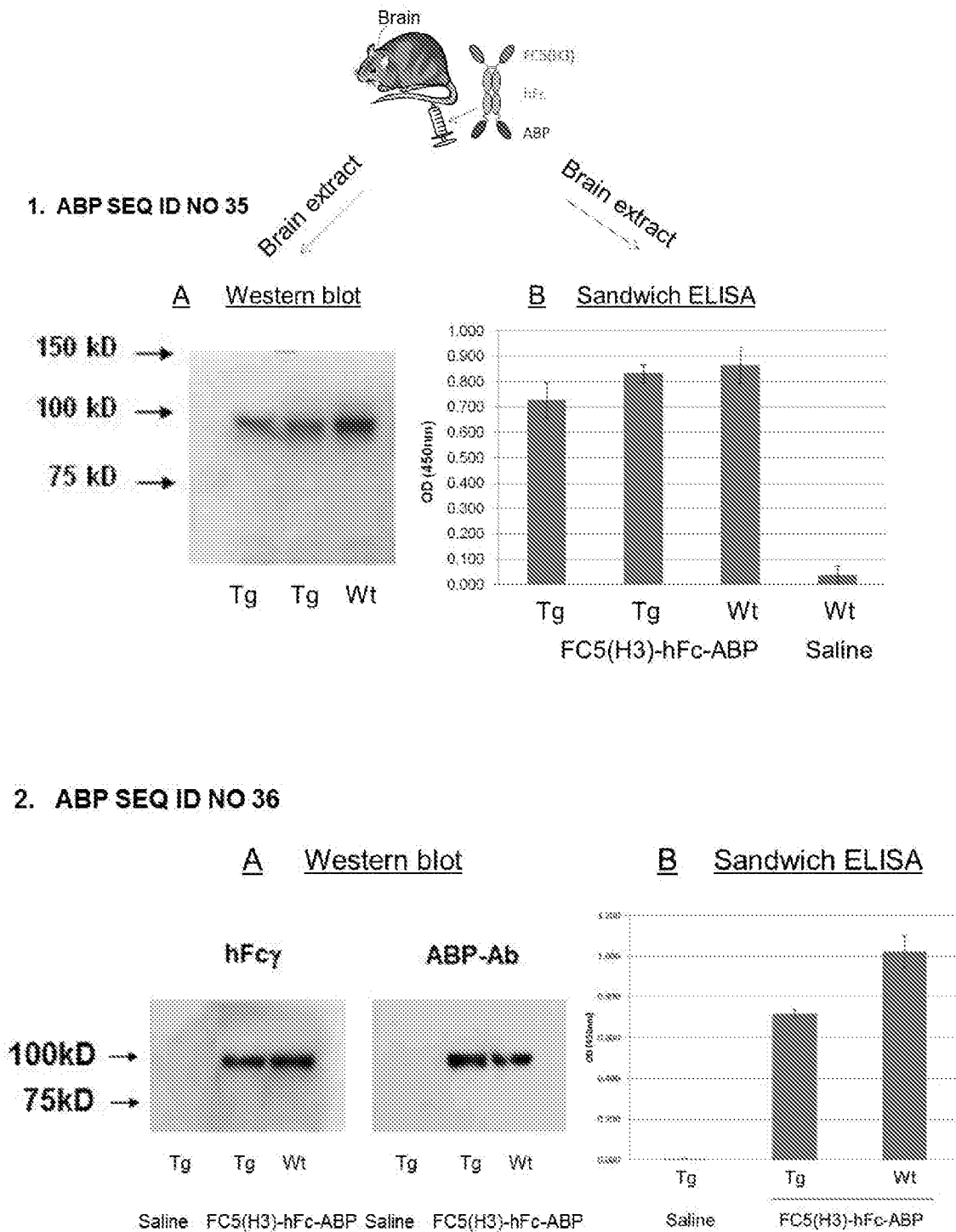
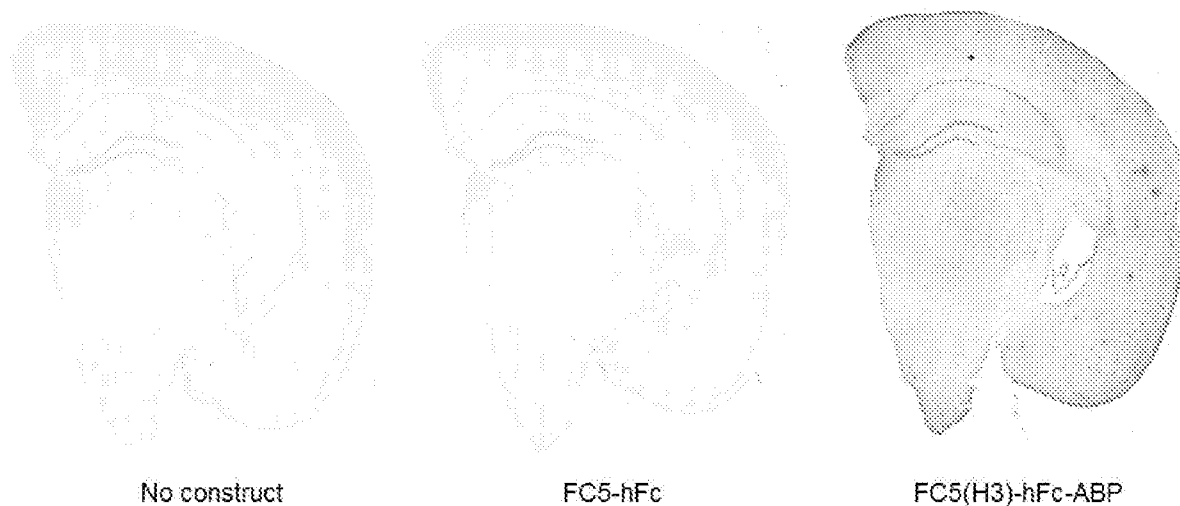


FIG. 19

A. Ex vivo binding of FC5(H3)-hFc-ABP (ABP SEQ ID NO 36)
(in vitro target engagement)



B. In vivo binding of FC5(H3)-hFc1x7-ABP (ABP SEQ ID NO 36)

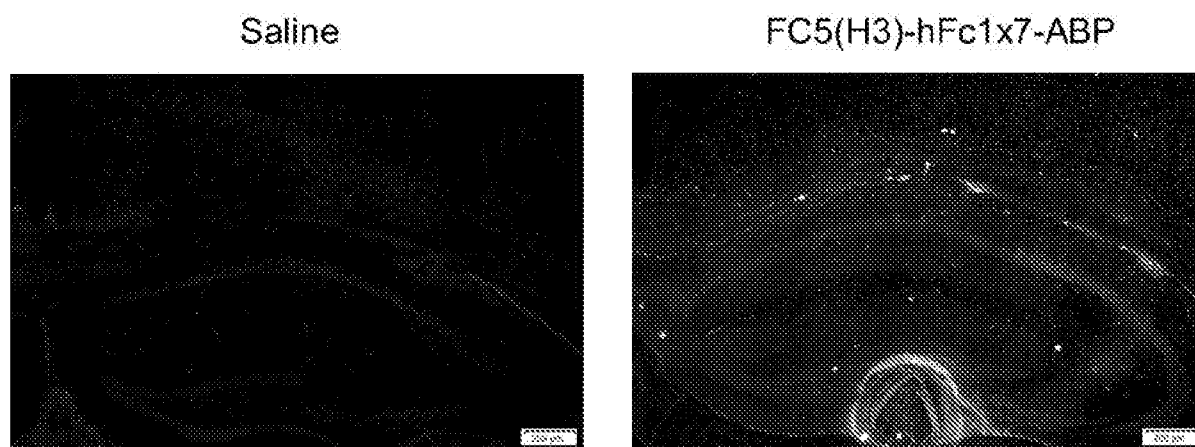


FIG. 20

In vivo target engagement by FC5(H3)-hFc-ABP

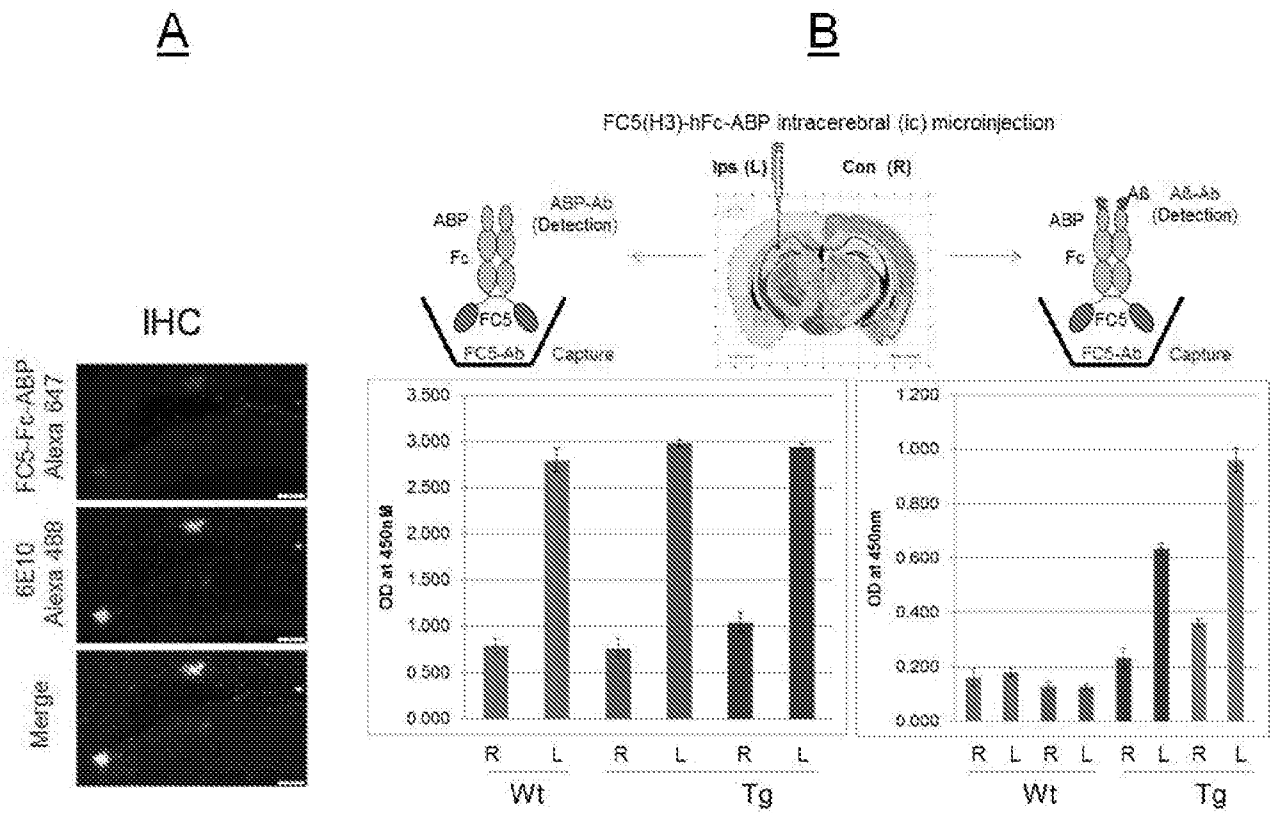


FIG. 21

Comparison between non-humanized and humanized FC5-Fc-ABP

A: PK studies in rats

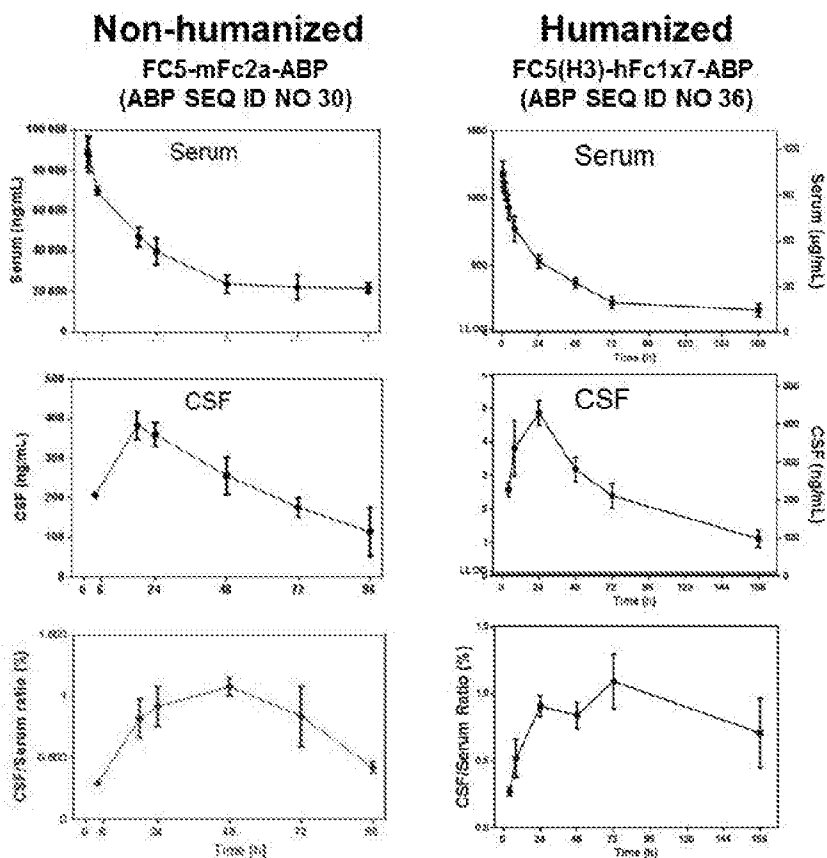


FIG. 22 A

B: PD studies in Tg mice

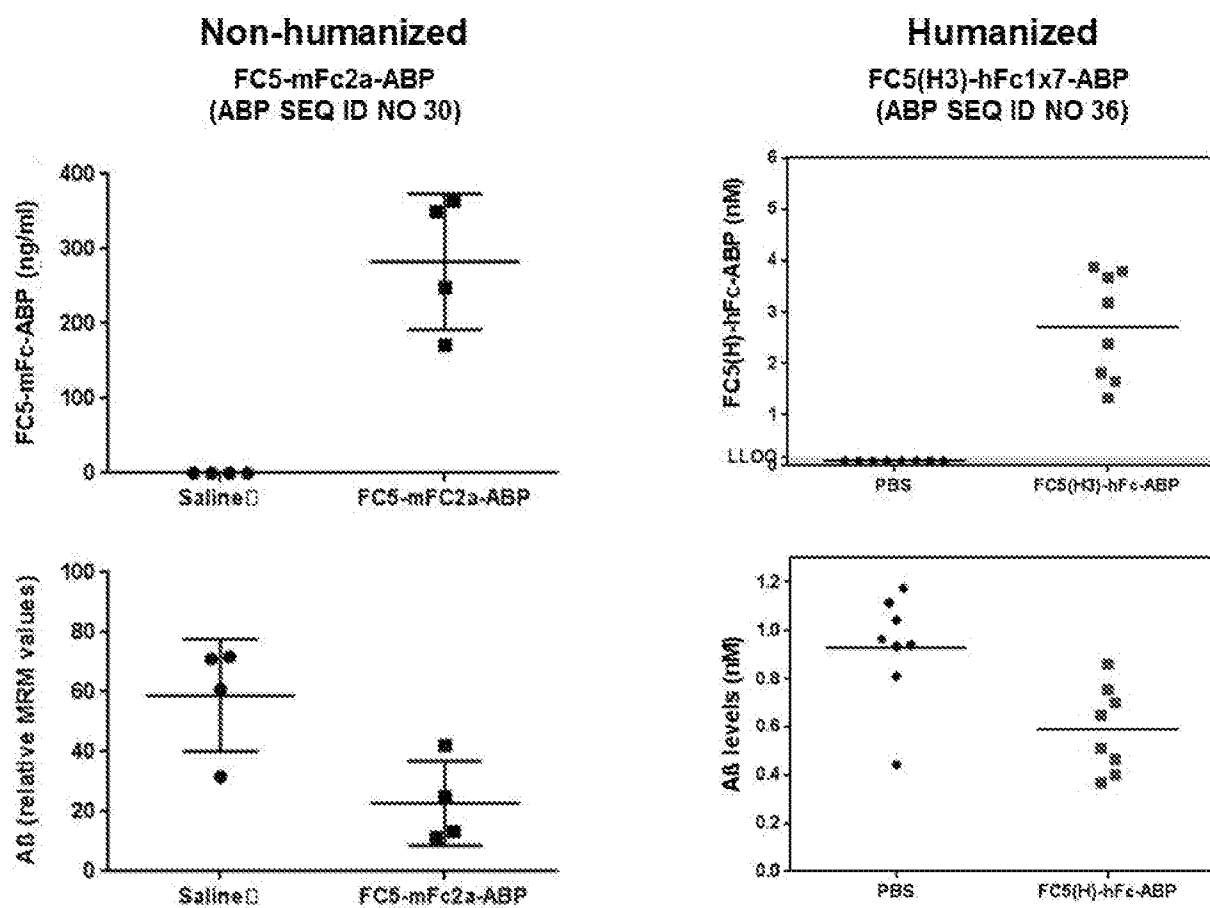


FIG. 22 B

INTERNATIONAL SEARCH REPORT

International application No.

PCT/IB2018/050576

A. CLASSIFICATION OF SUBJECT MATTER

IPC: **C07K 14/47** (2006.01), **A61K 47/68** (2017.01), **A61P 25/28** (2006.01), **C07K 14/00** (2006.01), **C07K 14/46** (2006.01), **C07K 16/00** (2006.01) (more IPCs on the last page)

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)

IPC: **C07K 14/47** (2006.01), **A61K 47/68** (2017.01), **A61P 25/28** (2006.01), **C07K 14/00** (2006.01), **C07K 14/46** (2006.01), **C07K 16/00** (2006.01), **C07K 16/46** (2006.01), **C07K 19/00** (2006.01), **C12N 15/62** (2006.01)

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

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

Databases: Google, Google Patent, Google Scholar, NCBI, Questel Orbit, Canadian Patent Database, GenomeQuest

Keywords: fusion, blood-brain barrier, amyloid, fusion protein, Alzheimer, amyloid

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	CHAKRAVARTHY, B et al., " <i>Brain delivery of an amyloid-β oligomer (AβO)-targeting peptide-therapeutic by a novel blood-brain barrier (BBB)-crossing domain antibody</i> ". Alzheimer's and Dementia, July 2017 (07-2017), Vol. 13(7 Suppl.), pp. P625, ISSN 1552-5260 [online] [retrieved on 30 April 2018 (30-04-2018)]. Retrieved from the Internet: < https://doi.org/10.1016/j.jalz.2017.06.703 >	1-49
X	SUMBRIA, RK et al., " <i>Disaggregation of amyloid plaque in brain of Alzheimer's Disease transgenic mice with daily subcutaneous administration of a tetravalent bispecific antibody that targets the transferrin receptor and the Abeta amyloid peptide</i> ". Mol. Pharmaceutics, 7 August 2013 (07-08-2013), Vol. 10, pp. 3507-3513, ISSN: 1543-8384 (print) 1543-8392 (electronic) (please see whole document)	1, 2, 8, 9 and 22-27
Y		

☒ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* "A" "E" "L" "O" "P"	Special categories of cited documents: document defining the general state of the art which is not considered to be of particular relevance earlier application or patent but published on or after the international filing date 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) document referring to an oral disclosure, use, exhibition or other means document published prior to the international filing date but later than the priority date claimed	"T" "X" "Y" "&"	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 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 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
30 April 2018 (30-04-2018)

Date of mailing of the international search report
04 May 2018 (04-05-2018)

Name and mailing address of the ISA/CA
Canadian Intellectual Property Office
Place du Portage I, C114 - 1st Floor, Box PCT
50 Victoria Street
Gatineau, Quebec K1A 0C9
Facsimile No.: 819-953-2476

Authorized officer

Brad Temple (819) 576-2089

INTERNATIONAL SEARCH REPORT

International application No.

PCT/IB2018/050576

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHAKRAVARTHY, B et al., " <i>A synthetic peptide corresponding to a region of the human pericentriolar material 1 (PCM-1) protein binds β-amyloid ($A\beta$1-42) oligomers</i> ". J Neurochem, August 2013 (08-2013), Vol. 126(3), pp. 415-424, ISSN 0022-3042 (Print), 1471-4159 (Electronic)	36 and 37
Y	(please see pages 657 and 659)	3-7, 10-21 and 38-48
A	PARDRIDGE, W.M., " <i>Re-engineering therapeutic antibodies for Alzheimer's disease as blood-brain barrier penetrating bi-specific antibodies</i> ". Expert Opinion on Biological Therapy, 2016, Vol. 16(12), pp. 1455-1468, ISSN 1471-2598 (print), 1744-7682 (electronic)	
A	CHAKRAVARTHY, B et al., " <i>Evidence that a synthetic amyloid-β oligomer-binding peptide (ABP) targets amyloid-β deposits in transgenic mouse brain and human Alzheimer's disease brain</i> ". Biochem Biophys Res Commun., 14 March 2014 (14-03-2014), Vol. 445(3), pp. 656-660, ISSN 0006-291X (print), 1090-2104 (electronic)	

INTERNATIONAL SEARCH REPORT

International application No.

PCT/IB2018/050576**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the 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. ☒ Claim Nos.: 28-35 and 49
because they relate to subject matter not required to be searched by this Authority, namely:

Claims 28-35 and 49 are directed to a method for treatment of the human or animal body by surgery or therapy, which the International Searching Authority is not required to search under PCT Rule 39.1(iv). However, this Authority has carried out a search based on the alleged effect or purpose/use of the product as determined from the methods of claims 28-35 and 49.

2. ☐ Claim 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. ☐ Claim 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:

Each polypeptide that binds β -amyloid forms a separate alleged invention. The alleged special technical feature that appears to unify the claims appears to be the combination of antibody or fragment thereof that transmigrates the blood-brain barrier and a polypeptide that binds β -amyloid. However, this combination is known from at least D1, which discloses a fusion protein comprising an antibody that transmigrates the blood-brain barrier and a polypeptide that binds β -amyloid. Therefore, the 11 polypeptides that bind β -amyloid indicated in the claims, as represented by SEQ ID NOs: 27-38, form separate alleged inventions.

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 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 claim 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 claim Nos.:

Remark on Protest

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

INTERNATIONAL SEARCH REPORT

International application No.

PCT/IB2018/050576

C07K 16/46 (2006.01), ***C07K 19/00*** (2006.01), ***C12N 15/62*** (2006.01)