Innovation, Sciences et Développement économique Canada
Office de la Propriété Intellectuelle du Canada

Innovation, Science and Economic Development Canada
Canadian Intellectual Property Office

CA 3030754 C 2022/04/26

(11)(21) 3 030 754

(12) BREVET CANADIEN
CANADIAN PATENT

(13) C

(86) Date de dépôt PCT/PCT Filing Date: 2017/07/12
(87) Date publication PCT/PCT Publication Date: 2018/01/25
(45) Date de délivrance/Issue Date: 2022/04/26
(85) Entrée phase nationale/National Entry: 2019/01/11
(86) N° demande PCT/PCT Application No.: US 2017/041799
(87) N° publication PCT/PCT Publication No.: 2018/017370
(30) Priorité/Priority: 2016/07/20 (US62/364,813)

(51) Cl.Int./Int.Cl. *A61K 39/395* (2006.01),
A61P 25/00 (2006.01)

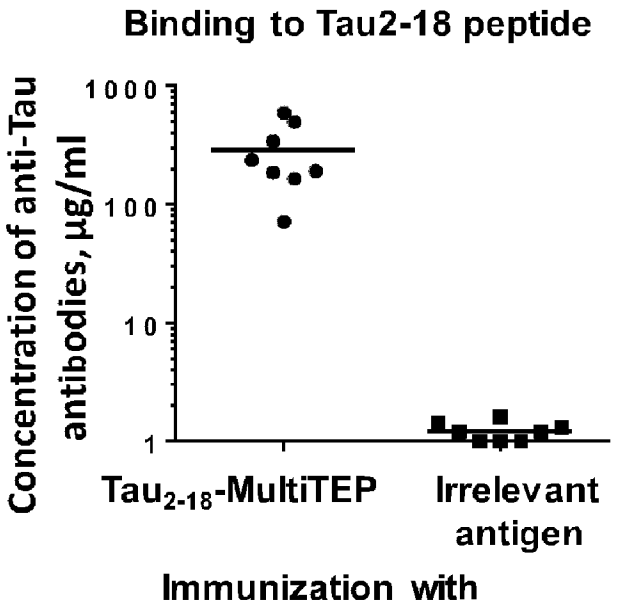
(72) Inventeurs/Inventors:
GHOCHIKYAN, ANAHIT, US;
AGADJANYAN, MICHAEL, US

(73) Propriétaires/Owners:
GHOCHIKYAN, ANAHIT, US;
AGADJANYAN, MICHAEL, US

(74) Agent: MARKS & CLERK

(54) Titre : ANTICORPS ANTI-TAU HUMANISES, COMPOSITIONS ET PROCEDES DE FABRICATION ET METHODES D'UTILISATION DANS LE TRAITEMENT, LE DIAGNOSTIC ET LA SURVEILLANCE DE TAUOPATHIES

(54) Title: HUMANIZED ANTI-TAU ANTIBODIES AND COMPOSITIONS FOR AND METHODS OF MAKING AND USING IN TREATMENT, DIAGNOSIS AND MONITORING OF TAUOPATHIES



(57) Abrégé/Abstract:
The disclosure provides methods of treating tauopathies (e.g. Alzheimer's disease, Amyotrophic Lateral Sclerosis, Frontotemporal Dementia with Parkinsonism linked to chromosome 17, Pick's Disease, Progressive Supranuclear Palsy, Dementia Pugilistica, Down's Syndrome and others) by administering humanized antibodies. The disclosure also provides the anti-tau humanized antibodies that bind the N-terminal region of tau and also bind to pathological tau aggregates, conformational epitopes and peptides mimicking these epitopes (mimotopes).

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
25 January 2018 (25.01.2018)

WIPO | PCT

(10) International Publication Number
WO 2018/017370 A1

(51) International Patent Classification:
A61K 39/00 (2006.01) *A61K 47/48* (2006.01)
A61K 39/39 (2006.01) *A61K 48/00* (2006.01)

(21) International Application Number:
PCT/US2017/041799

(22) International Filing Date:
12 July 2017 (12.07.2017)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/364,813 20 July 2016 (20.07.2016) US

(72) Inventor; and
(71) Applicant: **GHOCHIKYAN, Anahit** [US/US]; 16371 Gothard St. Suite H, Huntington Beach, CA 92647-3652 (US).

(72) Inventor: **AGADJANYAN, Michael**; 16371 Gothard St. Suite H, Huntington Beach, CA 92647-3652 (US).

(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.

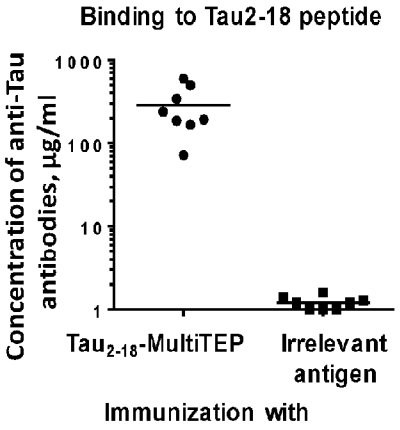
(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, 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).

Published:
— *with international search report (Art. 21(3))*
— *with sequence listing part of description (Rule 5.2(a))*

WO 2018/017370 A1

(54) Title: HUMANIZED ANTI-TAU ANTIBODIES AND COMPOSITIONS FOR AND METHODS OF MAKING AND USING IN TREATMENT, DIAGNOSIS AND MONITORING OF TAUOPATHIES

FIG. 1A



(57) Abstract: The disclosure provides methods of treating tauopathies (e.g. Alzheimer's disease, Amyotrophic Lateral Sclerosis, Frontotemporal Dementia with Parkinsonism linked to chromosome 17, Pick's Disease, Progressive Supranuclear Palsy, Dementia Pugilistica, Down's Syndrome and others) by administering humanized antibodies. The disclosure also provides the anti-tau humanized antibodies that bind the N-terminal region of tau and also bind to pathological tau aggregates, conformational epitopes and peptides mimicking these epitopes (mimotopes).

HUMANIZED ANTI-TAU ANTIBODIES AND COMPOSITIONS FOR AND METHODS OF MAKING AND USING IN TREATMENT, DIAGNOSIS AND MONITORING OF TAUOPATHIES

TECHNICAL FIELD

[0001] This patent application relates generally to antibodies that react with tau and methods using these antibodies in treatment of tauopathies, including Alzheimer's Disease.

BACKGROUND

[0002] Misfolding and aggregation of some specific proteins is a hallmark of a variety of neurodegenerative disorders, including but not limited to Alzheimer's Disease (AD) and frontotemporal dementia.

[0003] Attention and research has focused primarily on deposition of amyloid-β (Aβ) in senile plaques, although aggregation of pathological tau protein in neurofibrillary tangles also plays an important role in disease progression (Ballatore C. et al., Nat Rev Neurosci 2007, 8, 663-672).

[0004] Tau is a microtubule-associated protein. In AD, tau undergoes several changes to a pathological state. Tau can be abnormally folded and phosphorylated resulting in the generation of neurofibrillary tangles toxic to neurons. In AD, amyloid accumulation in the brain can occur decades before the beginning of symptoms such as memory loss and personality change. Current data suggest that Aβ pathology emerges prior to tau pathology, but may accelerate toxic neurofibrillary tangle formation. At best however, anti-Aβ immunotherapy only slightly decreases tau pathology and often does not affect the level of pathological tau at all. Moreover, pathological tau burden in the brains of patients with mild to moderate AD plays an important role in disease progression.

SUMMARY

[0005] The disclosure is directed to humanized antibodies that recognize and bind to an epitope in the N-terminal region of pathological neurotoxic forms of tau.

Date Reçue/Date Received 2020-05-13

[0005a] Accordingly, in one aspect of the present invention there is provided a pharmaceutical composition for treating tauopathy, the pharmaceutical composition comprising:

a. a pharmaceutically acceptable excipient; and

b. a humanized anti-tau antibody comprising one of:

(i) a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 23, a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 24, a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 25, a VL CDR1 comprising the amino acid sequence of SEQ ID NO: 26, a VL CDR2 comprising the amino acid sequence of SEQ ID NO: 27 and a VL CDR3 comprising the amino acid sequence of SEQ ID NO: 28;

(ii) a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 29, a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 30, a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 31, a VL CDR1 comprising the amino acid sequence of SEQ ID NO: 32, a VL CDR2 comprising the amino acid sequence of SEQ ID NO: 33, and a VL CDR3 comprising the amino acid sequence of SEQ ID NO: 34; and

(iii) a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 35, a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 36, a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 37, a VL CDR1 comprising the amino acid sequence of SEQ ID NO: 26, a VL CDR2 comprising the amino acid sequence of SEQ ID NO: 27, and a VL CDR3 comprising the amino acid sequence of SEQ ID NO: 28.

Date Reçue/Date Received 2020-05-13

[0005b] According to another aspect of the present invention there is provided a pharmaceutical composition for diagnosing or screening a subject for the presence of tauopathy, the pharmaceutical composition comprising:

a. a pharmaceutically acceptable excipient; and

b. a humanized anti-tau antibody comprising one of:

(i) a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 23, a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 24, a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 25, a VL CDR1 comprising the amino acid sequence of SEQ ID NO: 26, a VL CDR2 comprising the amino acid sequence of SEQ ID NO: 27 and a VL CDR3 comprising the amino acid sequence of SEQ ID NO: 28;

(ii) a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 29, a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 30, a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 31, a VL CDR1 comprising the amino acid sequence of SEQ ID NO: 32, a VL CDR2 comprising the amino acid sequence of SEQ ID NO: 33, and a VL CDR3 comprising the amino acid sequence of SEQ ID NO: 34; and

(iii) a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 35, a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 36, a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 37, a VL CDR1 comprising the amino acid sequence of SEQ ID NO: 26, a VL CDR2 comprising the amino acid sequence of SEQ ID NO: 27, and a VL CDR3 comprising the amino acid sequence of SEQ ID NO: 28.

Date Reçue/Date Received 2020-05-13

[0005c] According to yet another aspect of the present invention there is provided a use of a pharmaceutical composition for treating tauopathy, the pharmaceutical composition comprising:

a. a pharmaceutically acceptable excipient; and

b. a humanized anti-tau antibody comprising one of:

(i) a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 23, a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 24, a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 25, a VL CDR1 comprising the amino acid sequence of SEQ ID NO: 26, a VL CDR2 comprising the amino acid sequence of SEQ ID NO: 27 and a VL CDR3 comprising the amino acid sequence of SEQ ID NO: 28;

(ii) a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 29, a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 30, a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 31, a VL CDR1 comprising the amino acid sequence of SEQ ID NO: 32, a VL CDR2 comprising the amino acid sequence of SEQ ID NO: 33, and a VL CDR3 comprising the amino acid sequence of SEQ ID NO: 34; and

(iii) a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 35, a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 36, a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 37, a VL CDR1 comprising the amino acid sequence of SEQ ID NO: 26, a VL CDR2 comprising the amino acid sequence of SEQ ID NO: 27, and a VL CDR3 comprising the amino acid sequence of SEQ ID NO: 28.

Date Reçue/Date Received 2020-05-13

[0005d] According to still yet another aspect of the present invention there is provided a use of a pharmaceutical composition for diagnosing or screening a subject for the presence of tauopathy, the pharmaceutical composition comprising:

a. a pharmaceutically acceptable excipient; and

b. a humanized anti-tau antibody comprising one of:

(i) a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 23, a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 24, a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 25, a VL CDR1 comprising the amino acid sequence of SEQ ID NO: 26, a VL CDR2 comprising the amino acid sequence of SEQ ID NO: 27 and a VL CDR3 comprising the amino acid sequence of SEQ ID NO: 28;

(ii) a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 29, a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 30, a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 31, a VL CDR1 comprising the amino acid sequence of SEQ ID NO: 32, a VL CDR2 comprising the amino acid sequence of SEQ ID NO: 33, and a VL CDR3 comprising the amino acid sequence of SEQ ID NO: 34; and

(iii) a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 35, a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 36, a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 37, a VL CDR1 comprising the amino acid sequence of SEQ ID NO: 26, a VL CDR2 comprising the amino acid sequence of SEQ ID NO: 27, and a VL CDR3 comprising the amino acid sequence of SEQ ID NO: 28.

Date Reçue/Date Received 2020-05-13

[0005e] The humanized antibody may bind an epitope comprising the amino acids PRQEF or PRQEFE located within the following sequences: SEQ ID NO:1, SEQ ID NO:2 SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5 and SEQ ID NO:6.

[0005f] The humanized antibody may comprises one of:

(i) a heavy chain variable region comprising SEQ ID NO:38 and a light chain variable region comprising SEQ ID NO:39;

(ii) a heavy chain variable region comprising SEQ ID NO:40 and a light chain variable region comprising SEQ ID NO:41;

(iii) a heavy chain variable region comprising SEQ ID NO:42 and a light chain variable region comprising SEQ ID NO:43;

(iv) a heavy chain variable region comprising SEQ ID NO:44 and a light chain variable region comprising SEQ ID NO:45;

(v) a heavy chain variable region comprising SEQ ID NO:46 and a light chain variable region comprising SEQ ID NO:47;

(vi) a heavy chain variable region comprising SEQ ID NO:48 and a light chain variable region comprising SEQ ID NO:49; and

(vii) a heavy chain variable region comprising SEQ ID NO:50 and a light chain variable region comprising SEQ ID NO:51.

[0005g] The heavy chain constant region of the humanized antibody may comprise the heavy chain constant regions of the human immunoglobulins IgG1, IgG2, IgG3, or IgG4.

[0005h] The humanized antibody may:

(i) bind to pathologically aggregated tau,

(ii) reduce tau aggregates in a brain, or

(iii) inhibit transcellular propagation of pathological, aggregated tau.

[0005i] The composition may comprise cells or viruses expressing the humanized antibody. The viruses may be an adenovirus, an adeno-associated virus, or a lentivirus.

Date Reçue/Date Received 2021-03-24

[0006] These and other aspects of the present invention will become evident upon reference to the following detailed description and attached drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

[0007] Figure 1 shows humoral (Figure 1A and Figure 1B) and cellular (Figure 1C) responses in B6SJL immunized with tau_{2-18} fused with MultiTEP carrier protein. Mice were immunized biweekly (3 times) with 40 μg/mouse tau_{2-18}-MultiTEP vaccine or irrelevant control peptide formulated in Quil-A adjuvant. Figure 1A shows titers of antibodies specific to tau_{2-18} peptide determined in serially diluted individual sera. Lines indicate the average of titers in experimental (n=8) and control (n=8) groups of mice. Figure 1B shows the level of antibody response to full-length tau protein detected in anti-tau_{2-18} immune sera diluted 1:32000. Lines indicate the average of OD_{450} (n=8). Figure 1C shows the number of IFN-γ producing cells detected in the cultures of immune splenocytes. The number of IFNγ producing splenocytes was analysed by ELISPOT assay after ex vivo re-stimulation of cells with 10 μg/ml tau_{2-18} peptide and MultiTEP protein (Figure 1C) or tau_{2-18}-MultiTEP protein (data not shown). Error bars indicate average ± s.d. (n=4; $P \leq 0.001$).

[0008] Figure 2 shows photographs of brain sections stained with anti-tau_{2-18} antibody. Sera from mice immunized with tau_{2-18}-MultiTEP and control antisera from mice immunized with an irrelevant antigen were used to stain sections of Alzheimer's Disease (AD) brain and non-AD brain. Original magnifications: 10x, scale bar=100 μm; 20x, scale bar = 50 μm.

[0009] Figures 3 shows efficacy of anti-tau_{2-18} antibody detected in ex vivo model system. Figure 3A demonstrates inhibition of trans-cellular propagation of tau aggregates by anti-tau_{2-18} antibody. HEK293 cells transfected with RD(LM)-HA or mock transfected (NT) were co-cultured for 48 h with an equivalent number of HEK293f cells co-transfected with RD(ΔK)-CFP (ΔK-C) and RD(ΔK)-YFP (ΔK-Y) prior to FRET analysis. Increased FRET signal was detected in cells co-cultured with cells transfected with RD(LM)-HA. Addition of serial dilutions of purified anti-tau_{2-18} antibody decreased FRET signal due to inhibition of trans-cellular propagation of aggregated RD. Figure 3B demonstrates the binding of anti-tau_{2-18} antibodies to RD-(ΔK)-YFP aggregates. HEK293 cells were transfected with RD(ΔK)-YFP or were mock-transfected (NT). Anti-tau_{2-18} antibody was added to the culture medium for 48 h. Cells were fixed, permeabilized, and stained with an anti-mouse secondary antibody labeled with Alexa 546 and analyzed by confocal microscopy. Anti-tau_{2-18}/RDΔ(K)-YFP complexes were identified when RDΔ(K)-YFP is expressed but not in its absence (NT).

[0010] Figures 4 shows a graph (Figure 4A) of FRET analysis of anti-tau_{2-18} antibody used to block the ability of brain lysate from transgenic mice to induce aggregation of intracellular repeat domain (RD). Brain lysate was either untreated or treated with anti-tau_{2-18} antibody or irrelevant control IgG and added to HEK293 cells co-transfected with RD(ΔK)-CFP and RD(ΔK)-

Date Reçue/Date Received 2021-03-24

YFP prior to FRET analysis. Increased FRET signal was detected in wells with untreated brain lysate or treated with irrelevant control IgG brain lysate. Treatment of lysate with anti-tau_{2-18} antibody decreased FRET signal due to blocking the full-length tau in brain lysate and inhibition of induction of RD aggregation. Figures 4B, Figure 4C, Figure 4D and Figure 4E are confocal microscope images of exemplary binding of anti-tau_{2-18} antibody to brain lysate. Figure 4B shows non-transfected HEK293 cells stained with anti-tau_{2-18} antibody followed by secondary anti-mouse Ig conjugated with Alexa546. Figure 4C shows non-transfected HEK293 cells stained with brain lysate/anti-tau_{2-18} complexes followed by secondary anti-mouse immunoglobulin conjugated with Alexa546. Figure 4D shows RD-YFP-transfected HEK293 cells stained with brain lysate/anti-tau_{2-18} complexes followed by secondary anti-mouse immunoglobulin conjugated with Alexa546. Figure 4E shows YFP-transfected HEK293 cells stained with anti-tau_{2-18} antibody followed by secondary anti-mouse immunoglobulin conjugated with Alexa546.

[0011] Figure 5 shows photographs of brain sections stained with anti-tau_{2-18} monoclonal antibodies (2F6/E8, 2F6/H11, 4F3/H1, 1C9 and 2F6/G8) and control mouse IgG. Concentration of antibodies is 1 μg/ml. Original magnifications: 40x.

[0012] Figure 6A and Figure 6B depict amino acid sequence VH and VL chains of mouse monoclonal antibody 1C9 and a nucleotide sequence encoding the amino acid sequence. CDRs are bold and underlined.

[0013] Figure 7A and Figure 7B depict amino acid sequence VH and VL chains of mouse monoclonal antibody 2F6/H11 and a nucleotide sequence encoding the amino acid sequence. CDRs are bold and underlined.

[0014] Figure 8A and Figure 8B depicts amino acid sequence VH and VL chains of mouse monoclonal antibody 4F3/H1 and a nucleotide sequence encoding the amino acid sequence. CDRs are bold and underlined.

[0015] Figures 9 shows the characterization of mouse 1C9 anti-tau_{2-18} monoclonal antibody. Figure 9A shows that 1C9 recognized full-length tau but not tau that lacks 2-18 domain in Western Blot. Lane 1- tauΔ2-18, lane 2-full-length tau. Figure 9B shows that 1C9 bound monomeric (spot 1), oligomeric (spot 2: cross-linked; spot 3: non-cross-linked) and fibrillar (spot 4) forms of recombinant tau protein in dot blot. Figure 9C shows anti-tau_{2-18} mAb 1C9 bound to neuropil threads and neurofibrillary tangles in AD brains (Braak stage VI-C). No binding was observed with non-AD brain (Braak stage 0). Original magnification 40X, scale bar = 20 um. Figure 9D shows that 1C9 bound different species of tau protein in brain homogenates from both AD cases and control subjects in denaturing conditions (lane 1: control 1; lane 2-control 2; lane 3-AD1; lane 4-AD2; lane 5-AD3) in Western Blot. Figure 9E shows that in non-denaturing conditions in Dot Blots 1C9 as well as

commercial TNT-1 Ab specific to N-terminus of Tau selectively bound to soluble tau in AD brains but not in controls, while HT7 and rabbit anti-tau-polyclonal Ab recognizing total tau, had bound tau in both control and AD brains.

[0016] Figure 10 shows the affinity of binding of 1C9 mouse monoclonal antibody (Figure 10A) and its chimeric humanized version (Figure 10B) with recombinant tau protein determined by Biacore analysis.

[0017] Figure 11 presents affinity ranking of selected humanized antibodies by Octet analysis.

[0018] Figure 12 presents binding validation of selected top ten humanized antibodies by ELISA. ELISA plates were coated with recombinant tau protein and incubated with different dilutions of each antibody followed by incubation with anti-human IgG secondary antibody conjugated with HRP.

[0019] Figure 13 presents affinity of binding of different clones of fully humanized antibodies and the parental mouse antibody with recombinant tau protein determined by Biacore.

[0020] Figure 14A and Figure 14B depict amino acid sequence of humanized VH and VL chains of Armanezumab and a nucleotide sequence encoding the amino acid sequence. CDRs are bold and underlined.

[0021] Figure 15A and Figure 15B depict amino acid sequence of humanized VH and VL chains of A17589 and a nucleotide sequence encoding the amino acid sequence. CDRs are bold and underlined.

[0022] Figure 16A and Figure 16B depicts amino acid sequence of humanized VH and VL chains of A17595 and a nucleotide sequence encoding the amino acid sequence. CDRs are bold and underlined.

[0023] Figure 17A and Figure 17B depicts amino acid sequence of humanized VH and VL chains of A17606 and a nucleotide sequence encoding the amino acid sequence. CDRs are bold and underlined.

[0024] Figure 18A and Figure 18B depicts amino acid sequence of humanized VH and VL chains of VH3+VH2 and a nucleotide sequence encoding the amino acid sequence. CDRs are bold and underlined.

[0025] Figure 19 shows binding specificity of Armanezumab. Figure 19A shows that Armanezumab binds to brain sections form AD, FTD, Pick's disease cases. No binding had been seen with brain section with no tauopathy. Binding of commercial anti-tau antibodies of different specificities (PHF1, AT8, AT100, TNT1, HT7) to the adjacent sections have been also shown for comparison. Figure 19B shows that Armanezumab recognized aggregated forms of tau in brain

homogenates from AD cases (Braak stage VI). HT7 recognizing total tau and TNT-1 specific to N-terminus of tau were used as positive controls.

[0026] Figure 20 shows that Armanezumab inhibited neurotoxic effect of tau oligomers on human neuroblastoma SH-SY5Y cells (Figure 20A) and mouse primary neurons (Figure 20B).

[0027] Figure 21A shows that Armanezumab (10μg/ml) inhibited the ability of pathological tau in brain lysate of tau (P301S)/Tg mice to induce the aggregation of RD-CFP/RD-YFP in transiently transfected HEK293 cells. Figure 21 B shows that Armanezumab/protein A/G complex bound and removed pathological tau from brain lysate of tau(P301S)/Tg mice significantly decreasing the ability of lysate to induce the aggregation of RD-CFP/RD-YFP in stably transfected HEK293 cells. Representative FACS images for each group are presented. Numbers indicate the percentage of gated FRET-positive cells.

[0028] Figure 22 shows that intracranial injection of Armanezumab into hippocampus of Tau/Tg mice (n=7) reduced tau recognized by antibodies specific to tau phosphorylated at position S214 and to tau phosphorylated at positions S396/S404 (PHF1) compared with control IgG analyzed by confocal microscopy.

[0029] Figure 23 shows that intracranial injection of Armanezumab into hippocampus of Tau/Tg mice (n=7) reduced tau recognized by antibodies specific to total tau (HT7), tau phosphorylated at positions Thr212 (T212), Ser214 (S214), T212/S214 (AT100), S396/S404 (PHF1), S202/T205 (AT8) and Thr231 (AT180) compared with control IgG analyzed by IHC. Density is expressed in the percentage units calculated by formula I/(I+C)x100 and C/(I+C)x100 where I-ipsilateral (Armanezumab injected), C-contralateral (control IgG injected). Average and standard deviation for each hemisphere between all mice were calculated and compared. Representative images for each antibody-injected group are presented (magnification 10x).

[0030] Figure 24 shows that passive vaccination with 1C9 moAb, mouse counterpart of Armanezumab, administered intraperitoneally, improved recognition memory of vaccinated mice compared with mice injected with control mouse IgG. Figure 24A shows that 1C9 treated tau/Tg mice spent significantly more time with the novel object when compared to IgG treated tau/Tg mice. Figure 24B shows that 1C9 treated tau/Tg mice spent significantly more time within the novel arm when compared to IgG treated tau/Tg mice.

[0031] Figure 25 shows pharmacokinetic analyses of Armanezumab in wildtype mice, in the absence of target-mediated clearance and in Tg mouse model expressing human tau.

DETAILED DESCRIPTION

[0032] The present disclosure provides humanized antibodies, including antibody fragments, single-chain antibodies, chimeric antibodies and other forms of antibodies specific to

pathological tau aggregates, immunogenic tau peptides and tau antigens or mimotope. The disclosure also provides constructs and cells producing these humanized antibodies. The disclosure also provides uses of these antibodies and their pharmaceutical compositions in the treatment of tauopathies and provides uses of the antibodies for diagnosis and monitoring of tauopathies. The disclosure also provides a method of treating tauopathy in an individual, the method comprising administering to the individual an anti-tau antibody of the present disclosure, or a pharmaceutical composition of the present disclosure.

1. Tau protein and peptides

[0033] Tau protein or fragment of tau is used as an antigen to generate an immune response. Tau is a microtubule-associated protein found primarily in neurons and glia, but also in other areas of the CNS. Six isoforms have been identified, primarily differing by their number of binding domains. The isoforms result from alternative splicing. Three isoforms have three binding domains (3R), and three have four (4R). Exons 2 and 3 are variably present. In two isoforms, both are present (2N), in two other isoforms, just exon 3 is present (1N), and in two, neither are present (0N). The binding domains of tau are located in the C-terminus region. The six isoforms are called 0N3R (SEQ ID NO.1), 0N4R (SEQ ID NO.2), 1N3R (SEQ ID NO.3), 1N4R (SEQ ID NO.4), 2N3R (SEQ ID NO.5), and 2N4R (SEQ ID NO. 6). In addition, tau may be phosphorylated. Aggregation of tau proteins is a common feature of numerous neurodegenerative disorders.

[0034] The N-terminal region of tau is normally folded to the interior of the protein, but it is exposed during aggregation of tau (Morfini, G. A. et al. J Neurosci, 2009, 29, 12776-12786; Horowitz, P. M. et al,. J Neurosci 2004, 24, 7895-7902). It is also termed as phosphatase-activating domain (PAD) and plays a role in activation of a signalling cascade involving protein phosphatase I and glycogen synthase kinase 3, which leads to anterograde FAT inhibition. The N-terminal region may be used to generate antibodies to tau. The N-terminal region may be from residues about 1 to about 100, from about 1 to about 50, from about 1 to about 25, from about 1 to about 20, from 1 to about 15. In certain embodiments, the region starts at residue 2 and extends to about residue 100, about residue 50, about residue 25, about residue 20, or about residue 15. In certain embodiments, the region is from residue 2 through 18 and comprises the sequence AEPRQEFEVMEDHAGTY (SEQ ID NO.7), called tau_{2-18}.

[0035] In another embodiments, the region is from residue 2 through 36 and comprises the sequence AEPRQEFEVMEDHAGTYGLGDRKDQGGYTMHQDQE (SEQ ID NO. 8), called tau_{2-36}. The region used for the generation of anti-tau antibodies is referred to herein as "tau antigen". It is preferable that the tau antigen be non-phosphorylated. Other regions may also be used to generate anti-tau therapeutic antibodies.

[0036] A tau antigen should be able to induce a humoral immune response. Verification that a candidate antigen induces a humoral response can be performed by a variety of methods. In one method, the protein sequence of the antigen is synthesized and coupled to a carrier protein that is used to immunize an animal, e.g. a mouse. Sera may then be tested by ELISA or other known method for the presence of antibodies to the candidate. In addition, the epitopes may be tested by any method known in the art or described herein for stimulation of T cells. Suitable epitopes do not stimulate T cells detectably.

[0037] Depending on size and immunogenicity, among other factors, the tau antigen may be coupled to a carrier molecule, typically a protein. Carrier proteins are well-known in the art and include tetanus toxin, diphtheria toxoid, Hepatitis B surface antigen, influenza virus hemagglutinin, influenza virus matrix protein, serum albumin, and the like. In some embodiments, fragments of the carrier proteins are used.

2. Antibodies to tau

[0038] Antibodies to tau are provided herein. Antibodies are raised to the N-terminal region of tau, and in certain embodiments, to tau_{2-18}. In another embodiments antibodies are raised to the tau_{2-36}. Antibodies should bind an epitope in the N-terminal region of tau. In some embodiments, the antibodies are capable of binding to the repeat domain (RD) of tau, to aggregated RD domains, to recombinant human tau; to pathologically modified tau; to pathologically aggregated tau at the pre-tangle stage, in neurofibrillary tangles (NFT), neuropil threads and dystrophic neurites in the brain; to any of the six tau isoforms; to amino acids 2-18 of tau; to amino acids 2-36, to a conformational antigenic determinant that occurs in the pathological form of tau; or to a peptide mimicking (mimotope) the tau conformational antigenic determinant.

[0039] Antibodies that bind pathological tau, but not normal tau, are highly desirable, although in general, anti-tau antibodies are not internalized in cells where they could bind functionally normal tau molecules. Antibodies may be used for a variety of purposes, including isolation of tau and inhibiting (antagonist) activity of tau, especially pathological forms of tau. As well, assays for small molecules that interact with pathological tau will be facilitated by the development of antibodies.

[0040] Within the context of the present invention, antibodies are understood to include monoclonal antibodies, polyclonal antibodies, anti-idiotypic antibodies, single chain antibodies, chimeric antibodies, antibody fragments (e.g., Fab', Fab, and F(ab')2, Fv variable regions, single chain Fv, or complementarity determining regions). Antibodies are generally accepted as specific against tau protein if they bind with a Kd equal to or greater than 10^{-7}M, preferably equal to or greater than 10^{-8}M. The affinity of an antibody or binding partner can be readily determined by one of ordinary skill in the art (see Scatchard, G. Annals of the New York Academy of Sciences, 1949, **51**, 660-672).

[0041] Monoclonal antibodies may also be readily generated from hybridoma cell lines using conventional techniques (see U.S. Patent Nos. RE 32,011, 4,902,614, 4,543,439, and 4,411,993; see also Antibodies: A Laboratory Manual, Harlow and Lane (eds.), Cold Spring Harbor Laboratory Press, 1988). Briefly, within one embodiment, a subject animal such as a rat or mouse is injected with one of the compositions taught herein. Protein are typically administered as an emulsion in an adjuvant such as Freund's complete or incomplete adjuvant, QuilA and others in order to increase the immune response. Nucleic acid constructs are administered using devices such as gene gun or electroporation device. Between one and three weeks after the initial immunization, the animal is generally boosted and may tested for reactivity to tau utilizing well-known assays. The spleen and/or lymph nodes are harvested and cells immortalized. Various immortalization techniques, such as mediated by Epstein-Barr virus or fusion to produce a hybridoma, may be used. In one embodiment, immortalization occurs by fusion with a suitable myeloma cell line to create a hybridoma that secretes monoclonal antibody. Suitable myeloma lines include, for example, NS-1 (ATCC No. TIB 18), and P3X63 - Ag 8.653 (ATCC No. CRL 1580). Preferred fusion partners do not express endogenous antibody genes. Following fusion, the cells are cultured in medium containing a reagent that selectively allows for the growth of fused cells. After about seven days, hybridomas may be screened for the presence of antibodies that are reactive against a tau protein. A wide variety of assays may be utilized, including for example countercurrent immuno-electrophoresis, radioimmunoassays, radioimmunoprecipitations, enzyme-linked immuno-sorbent assays (ELISA), dot blot assays, western blots, immunoprecipitation, inhibition or competition assays, and sandwich assays (see U.S. Patent Nos. 4,376,110 and 4,486,530; see also Antibodies: A Laboratory Manual, Harlow and Lane (eds.), Cold Spring Harbor Laboratory Press, 1988).

[0042] Other techniques may also be utilized to construct monoclonal antibodies (see Huse, W. D. et al., Science, 1989, 246, 1275-1281; Sastry, L. et al., Proc Natl Acad Sci U S A, 1989, 86, 5728-5732; Alting-Mees, M. e. a. Strategies in Molecular Biology, 1990, 3, 1-9). Briefly, mRNA is isolated from a B cell population and utilized to create heavy and light chain immunoglobulin cDNA expression libraries in suitable vectors, such as *ImmunoZap(H) and *ImmunoZap(L). These vectors may be screened individually or co-expressed to form Fab fragments or antibodies (see Huse, W. D. et al., Science, 1989, 246, 1275-1281; Sastry, L. et al., Proc Natl Acad Sci U S A, 1989, 86, 5728-5732). Positive plaques may subsequently be converted to a non-lytic plasmid that allows high level expression of monoclonal antibody fragments from E. coli.

[0043] Similarly, portions or fragments, such as Fab and Fv fragments, of antibodies that contain the antigen-binding site may also be constructed utilizing conventional enzymatic digestion or recombinant DNA techniques to yield isolated variable regions of an antibody. In one embodiment, the genes which encode the variable region from a hybridoma producing a monoclonal antibody of

Date Reçue/Date Received 2020-05-13

interest are amplified using nucleotide primers for the variable region. These primers may be synthesized by one of ordinary skill in the art, or may be purchased from commercially available sources (e.g., Stratacyte, La Jolla, CA) Amplification products are inserted into vectors such as ImmunoZAP™ H or ImmunoZAP™ L (Stratacyte), which are then introduced into E. coli, yeast, insect cells, or mammalian-based systems for expression. Utilizing these techniques, large amounts of a single-chain protein containing a fusion of the VH and VL domains (scFv) may be produced (Bird, R. E. et al., Science, 1988, 242, 423-426). In addition, techniques may be utilized to change a "murine" antibody to a "human" antibody, without altering the binding specificity of the antibody (U.S Patents No. U.S. 5,225,539, 5,530,101, 6,331,415).

[0044] Once suitable antibodies have been obtained, they may be isolated or purified by many techniques well known to those of ordinary skill in the art (see Antibodies: A Laboratory Manual, Harlow and Lane (eds.), Cold Spring Harbor Laboratory Press, 1988). Suitable techniques include peptide or protein affinity columns, HPLC or RP-HPLC, purification on protein A or protein G columns, or any combination of these techniques.

1. **Humanization of antibodies**

[0045] A humanized antibody is an antibody originating from a non-human species whose protein sequence has been modified to increase its similarity to antibodies produced naturally in humans. The process of humanization is usually applied to monoclonal antibodies. The amount of non-human sequence can range from just the CDR sequences to the entire V region. Although typically, both the light and heavy chain will have some human sequence, a humanized antibody may have human sequence in only one of the two chains (e.g., a human Fc region). For purposes herein, "humanized antibody" refers to an antibody that contains some amount of human sequence. In certain embodiments, the humanized antibody has human constant regions and the variable regions are non-human. In certain other embodiments, only the CDR sequences are non-human. Techniques to humanize an antibody are well known and widely practiced.

[0046] In a fully humanized antibody (only the CDR sequences are non-human), the framework (FR) segments of the variable region may be from any of the families and moreover, may be from different families. The constant regions of the light chain may be either kappa or lambda, the constant regions of the heavy chain may be of the μ, γ, α, ε, or δ type, although typically will be one of the four γ subclasses. Generally, all the domains of the constant region will be from the same class (e.g., all from class $\gamma 1$).

[0047] Framework segments may be chosen on a variety of bases. For example, human framework sequences that share the highest identities to the framework sequences of the non-human antibody can be chosen. Another basis is selection of the humanized antibodies with the

Date Reçue/Date Received 2020-05-13

highest binding affinity or avidity. Binding affinity of antibodies to tau protein can be determined by a variety of methods, including surface plasmon resonance (SPR) analysis.

[0048] In an exemplary method, a mouse monoclonal antibody was initially humanized by replacement of the constant regions of heavy and light chains with human sequences. Binding affinity of the chimeric antibody to tau was verified as equivalent to the parental (murine) antibody. The chimeric antibody was further humanized by replacing the heavy and light chain variable region frameworks with human frameworks. The human FR1, FR2, FR3, which shared the highest identities with those of the mouse antibody were selected and assembled with mouse CDRs using overlapping PCR. Amplified fragments were inserted into a phage display vector to create a library of phage expressing humanized Fab fragments. Strong binders to recombinant tau protein were selected through three rounds of panning. Highest affinity antibodies were chosen.

[0049] The humanized antibodies can be readily produced in cells. DNA sequence analysis of antibodies can be used to construct expression vectors. The sequences of the CDRs is the minimum information needed to construct a humanized antibody. In certain embodiments the DNA sequence encoding a fully humanized antibody or Fab fragment is obtained. As disclosed below, the DNA sequence is inserted in an expression vector. The expression vector is transduced into cells and antibody protein is produced. The cells may be prokaryotic or eukaryotic. Vectors and host cells are well known and readily obtainable for production of protein.

[0050] Antibodies may be recovered from cells or cell supernatant. They may be used with or without concentration, and with or without further purification. If purified, a typical method is HPLC, although alternative purification methods such as ion exchange chromatography and gel filtration chromatography may be used. Acceptable purity is at least 90% or at least 95% or at least 98% as assessed by analytical HPLC.

4. Construction of DNA compositions

[0051] When antibodies are to be delivered as a DNA composition or produced in a host cell, the composition will typically be an expression vector. In some embodiments, the vector is capable of autonomous replication. In other embodiments, the vector is a viral vector, insect vector, or a bacterial vector. The vector can alternatively be a plasmid, a phage, a cosmid, a mini-chromosome, a virus like particle (VLP), or a virus. Nucleic acid molecules can also be delivered in liposomes or adhered to nanoparticles. Materials and methodology for preparing liposomes and nanoparticles are well-known in the art. The sequence encoding an antibody is operatively linked to a promoter that is active in host cells. There will typically also be a polyA signal sequence, one or more introns, and optionally other control sequences, such as an enhancer. The promoter can be a constitutive promoter, an inducible promoter, or cell-type specific promoter. Such promoters are well known in the art.

[0052] The sequence of an antibody is readily determined for a monoclonal antibody. A variety of techniques can be used to clone, identify and sequence the antibody chains. In one technique, primers for the variable regions are used in an amplification reaction. The resulting amplified fragment can be inserted into a vector and grown or sequenced directly. With the sequence of the variable regions of the heavy and light chains, expression vectors can be constructed for scFv, Fv, Fab, and the like.

[0053] Antibodies, including monoclonal antibodies, scFv, an F(ab') fragment, an F(ab) fragment and an $F(ab')_2$ fragment, may be coupled to a protein transduction domain (PTD) or other molecule, e.g., polysialic acid, that can facilitate the crossing of the blood brain barrier. Two general classes of PTDs have been described, including positively charged transduction domains (cationic) and protein leader sequence derived domains (hydrophobic). Both are able to transduce wide variety of cell types. The cationic type domain is generally 10 to 30 amino acid residues in length and enriched in basic amino acids, e.g., arginine and lysine. Many PTDs are well-know and have been identified in proteins, such as synB peptide derived from protegrin, Drosophila homeodomain transcription factors antennapedia, HIV-1 transactivating protein TAT, engineered chimeric PTDs. In addition, PTDs may be identified by phage display methods. Sequences of exemplary PTDs include YGRKKRRQRRR (SEQ ID No.9) from HIV tat, MIIYRDLISH (SEQ ID No.10) from human translationally controlled tumor protein (TCTP), RQIKIWFQNRRMKW (SEQ ID No.11) from antennapedia, KLALKLALKALKAALKLA (SEQ ID NO.12), GWTLNSAGYLLGKINLKALAALAKKIL from galanin (SEQ ID NO. 13), GALFLGWLGAAGSTMGAWSQPKKKRKV (SEQ ID NO. 14) from SV40, RGGRLSYSRRRFSTSTGR (SEQ ID NO. 15) and RRLSYSRRRF (SEQ ID NO. 16) from protegrin. Antibodies may be coupled to a PTD as a fusion protein or chemically, using one of well-known methods.

5. Uses of antibodies

[0054] The antibodies may be used for treatment or diagnosis of tauopathies and related diseases. Treatment includes prevention of disease, amelioration of disease or symptoms, and the like. The antibodies may be delivered as protein, as a nucleic acid molecule that encodes the antibodies or via cells (e.g. stem cells) expressing the antibodies. As disclosed herein, the antibodies may be a single chain, double chain, tetramer, or other multimer.

[0055] The antibodies may also be used to identify mimotopes of tau, including mimotopes of aggregated tau, which could then be used in a vaccine. A mimotope is a macromolecule, often a peptide, which mimics the structure of an epitope. Because of this property it causes an antibody response similar to the one elicited by the epitope. Briefly, the anti-tau antibodies are used to query a phage display library or other type of peptide library for a peptide that mimics the conformational antigenic determinant in aggregated repeat domain of tau. Such a peptide is a

mimotope. The mimotope can be formulated and used as a therapeutic for tauopathies. For a vaccine, a mimotope that is antigenic, but not immunogenic, can be coupled to a carrier. Mimotopes can also be used for generation of antibodies, including polyclonal and monoclonal antibodies and scFv, Fab, recombinant antibodies, chimeric antibodies and the like, that will inhibit aggregation of tau and have a therapeutic effect.

[0056] Anti-tau antibodies, including scFv, Fab, Fab' or F(ab)', can be used to raise anti-idiotype antibodies, which are then used as a vaccine. Anti-idiotypic antibody may mimic the original antigen. In one embodiment, anti-idiotype antibodies are monoclonal or scFv, Fab, Fab', F(ab)' and may be generated by recombinant DNA techniques that are well-known in the art.

6. Formulations and delivery of antibodies

[0057] Humanized, anti-tau antibodies, including scFv, Fab fragment, and Fab'2, may be formulated as a pharmaceutical composition for delivery to a subject (e.g., for use as a passive antibody therapy) or for the generation of an active vaccine to raise anti-idiotypic antibodies mimicking the antigenic determinant of tau. The compositions may include adjuvants and pharmaceutically acceptable excipients.

[0058] Anti-tau antibody compositions may comprise a pharmaceutically acceptable excipient, carrier, buffer, stabilizer or other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material can depend on the route of administration, e.g. intravenous, cutaneous or subcutaneous, nasal, intramuscular, intraperitoneal routes.

[0059] For intravenous, intraperitoneal, intrathecal, cutaneous or subcutaneous injection, or injection at the site of affliction, the active ingredient will be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles such as Sodium Chloride Injection, Ringer's Injection, Lactated Ringer's Injection. Preservatives, stabilizers, buffers, antioxidants and other additives can be included, as required.

[0060] Immunogenic compositions comprise one or more anti-tau antibodies and hybrid antibodies (one antigenic determinant of antibody will bind one epitope and another one will bind another epitope).

[0061] The compositions may be packaged as a solution, but can also be packaged in dry form (e.g., desiccated), in which case, a user adds any necessary liquid. Typically, additives such as buffers, stabilizers, preservatives, excipients, carriers, and other non-active ingredients will also be present in the package. Additives are typically pharmaceutically acceptable and bio-compatible.

[0062] When antibodies are to be delivered as a DNA composition, the composition will typically be an expression vector. In some embodiments, the vector is capable of autonomous replication. In other embodiments, the vector is a viral vector, insect vector, or a bacterial vector. The vector can alternatively be a plasmid, a phage, a cosmid, a mini-chromosome, a virus like particle (VLP), or a virus. The delivery vehicle can also be a liposome or nanoparticle. The sequence encoding an antibody or will be operatively linked to a promoter that is active in host cells. There will typically also be a polyA signal sequence, one or more introns, and optionally other control sequences, such as an enhancer. The promoter can be a constitutive promoter, an inducible promoter, or cell-type specific promoter. Such promoters are well known in the art.

[0063] In addition, the protein or nucleic acid may be presented in separate containers or combined in a single container. A container can be a vial, ampoule, tube, or well of a multi-well device, reservoir, syringe or any other kind of container. The container or containers may be provided as a kit. If one or more of the containers comprises desiccated ingredients the liquids for reconstitution may be provided in the kit as well or provided by the user. The amount of solution in each container or that is added to each container is commensurate with the route of administration and how many doses are in each container. The compositions are generally provided sterile. Typical sterilization methods include filtration, irradiation and gas treatment.

[0064] Methods for treatment of tauopathies and related diseases are provided. Methods include administering a therapeutically effective amount of a composition disclosed herein. Administration is preferably in a "therapeutically effective amount" or "prophylactically effective amount" (as the case can be, although prophylaxis can be considered therapy), this being sufficient to show benefit to the individual. Effectiveness may be measured by any number of parameters or endpoints, such as an improvement in cognitive ability, a slowing down of cognitive decline, an improvement of physical abilities or slowing of physical decline, and the like. After the demise of a patient, the amount of neurofibrillary tangles and other symptoms of AD can be directly assessed and used to help guide determination of effective doses. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of protein aggregation disease being treated. Prescription of treatment, e.g. decisions on dosage etc, is within the responsibility of general practitioners and other medical doctors, and typically takes account of the disorder to be treated, the condition of the individual patient, the site of delivery, the method of administration and other factors known to practitioners. Examples of the techniques and protocols mentioned above can be found in Remington's Pharmaceutical Sciences, 16th edition, Osol, A. (ed), 1980. Furthermore, a composition can be administered alone or in combination with other treatments, either simultaneously or sequentially dependent upon the condition to be treated.

[0065] The following examples are offered by way of illustration, and not by way of limitation.

EXAMPLES

EXAMPLE 1

GENERATION OF ANTI-TAU ANTIBODIES TO N-TERMINAL REGION OF TAU

[0066] In this example, an exemplary epitope of tau is used to generate antibodies specific to the N-terminal region.

[0067] The peptide from residue 2 through 18 of tau was fused with MultiTEP protein, a string of foreign promiscuous Th (helper T cell) epitopes from tetanus toxin TT, HBV and Flu and used to immunize mice. This region of tau is hidden when tau is folded normally, but becomes exposed during aggregation of tau. The MultiTEP activates CD4+ T cells in $H2^{bxs}$ and $H2^{b}$ (data for production of IFN-γ and IL-4 not shown) mice as well as activates human helper T cells expressing various MHC class II/DR molecules.

[0068] B6SJL mice ($H2^{bxs}$ immune haplotype) were immunized with a tau_{2-18}-MultiTEP immunogen formulated in the adjuvant Quil A™ (also known as QS21). Both humoral (ELISA) and cellular (ELISPOT) immune responses were measured. Immunization induced high titers of tau_{2-18}-specific antibodies that also recognized 0N/4R full-length tau (Figure 1A and Figure 1B). The epitope vaccine also induced a strong T cell response that was specific to MultiTEP, but not tau_{2-18} (Figure 1C). In conclusion, tau_{2-18}-MultiTEP vaccine in QuilA adjuvant produced antibodies specific to various tau proteins and these antibodies can be used for passive vaccination of subjects at various stages of tauopathy (e.g. Alzheimer's disease).

EXAMPLE 2

THERAPEUTIC POTENCY OF ANTI-TAU ANTIBODY

[0069] This example shows the functional potency of administering anti-tau antibodies on inhibiting aggregation of tau.

[0070] Sera from experimental mice immunized with the epitope vaccine and control animals immunized with an irrelevant antigen were tested on brain sections from AD and non-AD cases. Figure 2 shows that immune sera at a dilution 1:500 from experimental, but not control mice recognized neurofibrillary tangles (NFT) in brain from an AD patient (Tangle stage V, Plaque stage C). Importantly, the same immune sera did not bind tau in the brain sections from non-AD case. Thus, antibodies generated after immunization of tau antigen vaccines are specific to the only pathological form of tau.

Moreover, anti-tau_{2-18} antibodies purified from the sera of vaccinated mice were tested for their ability

Date Reçue/Date Received 2020-05-13

to inhibit cell-to-cell propagation of tau aggregates, using the method of Kfoury et al. (Kfoury, N. et al.,
J Biol Chem 2012, 287, 19440-19451).

[0071] The antibodies were able to inhibit cell-cell progagation of both full-length tau and repeat domain (RD) aggregates, evidencing the therapeutic benefit of these antibodies.

[0072] More specifically, trans-cellular movement of aggregated tau was assessed in HEK293 cells transfected with tau repeat domain (RD) containing a deletion of lysine at position 280 (ΔK280) and fused to cyan or yellow fluorescent protein (RD-CFP) or (RD-YFP) (ΔK-C):(ΔK-Y). A second population of HEK293 cells was transfected with hemagglutinin-tagged tau (RD) containing two disease-associated mutations that increase aggregation: P301L and V337M (LM) (LM-HA). When the two cell populations were co-cultured, trans-cellular propagation of LM-HA aggregates from donor cells (HEK293 cells transfected with LM-HA) induces aggregation of ΔK-C:ΔK-Y in recipient cells (HEK293 transfected with RD-CFP/RD-YFP) as detected by fluorescence resonance energy transfer (FRET) between CFP and YFP, which yields a signal detected by a fluorescence plate reader. To measure baseline endogenous aggregation, a (ΔK-C):(ΔK-Y) cell population was co-cultured with mock-transfected cells (NT). To test the ability of antibodies purified from sera to block cell-to-cell transfer of RD aggregates, anti-tau_{2-18} antibody (0.4 mg/ml) was added to the culture at different dilutions (10^{-2}, 10^{-3} and 10^{-4}) and incubated for 48 h. A dose-dependent reduction of FRET signal, indicating reduced transcellular propagation of LM-HA aggregates, is observed with anti-tau_{2-18} (Figure 3A), whereas nonspecific IgG had no effect (data not shown). Because the tau_{2-18} peptide is localized outside of the RD region, these data indicate that anti-tau_{2-18} antibody recognizes a conformational antigenic determinant (mimotope(s) in aggregated RD and blocks its cell-to-cell propagation. To show possible binding of anti-tau_{2-18} to RD aggregates, HEK293 cells were transfected with RD(ΔK)-YFP or were mock-transfected (NT). Anti-tau_{2-18} antibody was added to the culture medium for 48 h. Cells were fixed, permeabilized, and stained with an anti-mouse secondary antibody labeled with Alexa 546 and analyzed by confocal microscopy. Anti-tau_{2-18}/RDΔ(K)-YFP complexes were identified when RDΔ(K)-YFP is expressed but not in its absence (NT) (Figure 3B).

[0073] In another set of experiments, the ability of anti-tau_{2-18} antibody was tested for ability to block full-length tau aggregates from entering a cell and inducing aggregation of intracellular RD. The experiment was designed as described above except that the aggregation ΔK-C:ΔK-Y in recipient cells was induced by adding aggregated tau from brain lysates of P301S Tg (transgenic) mice that were either untreated or pre-incubated with anti-tau_{2-18} antibody. As expected, addition of untreated brain lysate increased a FRET signal, whereas pre-treating of brain lysate with anti-tau_{2-18} antibody completely blocked the ability of brain lysate to induce the aggregation of RD in

recipient cells (Figure 4A). Importantly, using confocal microscopy brain lysate/anti-tau_{2-18} antibody complexes are shown to internalize into the RD-YFP transfected cells (Figure 4D), but not in control mock-transfected cells (NT) with added brain lysates from Tg mice (Figure 4C). Of course, antibodies were not detected in NT cells (Figure 4B) or in RD-YFP cells when tau aggregates from Tg mice were not added to the test tubes (Figure 4E).

[0074] This shows that therapeutic anti-tau antibodies can be generated with a non-phosphorylated tau molecules or their derivatives (e.g. B cell epitopes). Indeed, non-phosphorylated tau may be used for generation of therapeutic antibodies that will be safe to administrate to subjects with tauopathy, because such antibodies will not get inside normal cells and inhibit function of normal tau molecules.

EXAMPLE 3

GENERATION AND CHARACTERIZATION OF MONOCLONAL ANTI-TAU ANTIBODIES

[0075] In this example, monoclonal mouse anti-tau antibodies are generated and characterized.

[0076] Anti-tau_{2-18} monoclonal antibodies were generated using well-known hybridoma technology. ELISA assays identified several clones producing antibody that bound recombinant human tau protein.

[0077] Antibodies were purified from five selected clones. Their therapeutic efficacy was assessed by binding to pathological tau in brain sections from AD cases. Figure 5 shows that all five selected antibodies, but not the irrelevant control IgG, recognized neurofibrillary tangles (NFT) in brain sections from an AD patient. The intensity of staining differs among the antibodies, suggesting that the monoclonal antibodies have varying affinity of binding. 2F6/G8 binding to pathological Tau brain sections was too weak, therefore it was excluded from further testing.

The fine specificity of monoclonal antibodies was mapped by alanine scanning. More specifically, a series of peptides was constructed in which successive amino acids in the tau_{2-18} peptide was substituted by alanine, resulting in 18 peptides, each of which differs from tau_{2-18} by one amino acid. Alanines in the original peptide were replaced by serine. The data showed that antibodies recognized either $_4PXQEF_8$ or $_4PXQEXE_9$ in the tau_{2-18} peptide. The X indicates that the amino acid at that position did not affect binding of the antibody. Amino acid sequences for variable regions of selected clones, 1C9, 4F3/H1, and 2F6/H11, are presented in Figures 6-8 and as SEQ ID Nos: 17-22. The amino acid sequences of the CDR regions are presented in Figure 6 and as SEQ ID Nos:23-28 for 1C9, Figure 7 and SEQ ID Nos: 29-34 for 4F3/H1 and Figure 8 and SEQ ID Nos:35-37 for 2F6/H11. The amino acid sequences of CDRs from clone 2F6/E8 were the same as 2F6/H11 as well as CDRs of 2F6/H11 VL region were the same as CDRs of 1C9 VL region.

EXAMPLE 4

MURINE MONOCLONAL ANTIBODY SELECTIVELY RECOGNIZED PATHOLOGICAL TAU IN BRAIN EXTRACTS FROM AD CASES

[0078] One of the murine monoclonal antibodies, 1C9 was chosen for further analyses. Specificity testing showed that this novel antibody recognized full-length recombinant tau, but not tau that lacks $tau_{2\text{-}18}$ domain in western blot (Figure 9A). 1C9 mAb bound different forms of recombinant tau: monomeric, oligomeric and fibrillar tau in dot blot assay (Figure 9B). 1C9 mAb recognized pathological tau (both neuropil threads and NFT) in the fixed brain sections from AD cases (Figure 9C), but did not bind to the brain sections from a non-AD subject.

[0079] In denaturing conditions of western blot 1C9 bound different forms of tau in homogenates of postmortem AD and control brains (Figure 9D) showing typical pattern as in case of HT7 antibodies recognizing total tau. In contrast, in non-denaturing dot blot assay 1C9 selectively bound to soluble fraction of postmortem AD brain extracts (Figure 9E) indicating that PAD is more exposed and accessible in the AD brains as opposed to controls.

EXAMPLE 5

HUMANIZATION OF MONOCLONAL ANTI-TAU ANTIBODIES

[0080] One of the murine monoclonal antibodies, 1C9, was chosen and humanized.

[0081] The 1C9 antibody was first partially humanized by replacement of the mouse Fc fragment with a human IgG1 Fc fragment. The binding affinity of this chimeric humanized antibody to recombinant tau protein was determined by surface plasmon resonance (SPR) analysis. Data obtained showed that conversion of mouse mAb to chimeric mAb did not affect the affinity of binding (Figure 10).

[0082] The 1C9 chimeric antibody was further humanized by replacing the heavy and light chain frameworks with human frameworks. The human FR1, FR2, FR3, which share the highest identities with those of the mouse antibody were selected and assembled with mouse CDRs (SEQ ID Nos: 23-25 for the heavy chain CDRs and SEQ ID Nos: 26-28 for the light chain CDRs) using overlapping PCR. Amplified fragments were inserted into a phage display vector to create a library of phage expressing humanized Fab fragments. Strong binders to recombinant tau protein were selected after three rounds of panning. Affinity ranking of selected antibody was assessed with an Octet™ System (FortéBIO, Pall Corporation) (Figure 11).

Date Reçue/Date Received 2020-05-13

[0083] Several phage clones were selected for DNA sequence analysis and further testing. The VH and VL protein sequences of eight clones are presented as SEQ ID Nos: 38-53. DNA encoding the Fab VH and VL from the five clones having the highest affinity was used in construction of IgG antibodies. Purified antibodies were analyzed by ELISA (Fig. 12). For ELISA, 96-well plates were coated with recombinant tau protein and incubated with different dilutions of each antibody followed by incubation with an anti-human IgG secondary antibody conjugated with HRP (horse radish peroxidase). The reaction was developed by adding 3,3′,5,5′tetramethylbenzidine (TMB) substrate solution and stopped with 2 M H_2SO_4. The optical density (OD) was read at 450 nm. Four humanized antibodies demonstrated binding to tau protein and had similar titers to a chimeric version of the parent antibody 1C9 (Figure 12).

[0084] Affinity of the humanized antibodies was determined by SPR (Biacore) analysis (Figure 13). All humanized antibodies retain K_D of parental mouse MoAb. One of them was selected (based on binding to brain sections from AD case) and designated as Armanezumab. Protein and DNA sequences of four selected antibodies including Armanezumab are presented in Figures 14-18. Armanezumab VH/CH and VL/CL sequence is presented in SEQ ID Nos:54-55 for amino acid sequences and SEQ ID Nos: 56-57 for nucleotide sequences.

EXAMPLE 6

THERAPEUTIC EFFICACY OF ARMANEZUMAB

[0085] In this example, therapeutic efficacy of Armanezumab is assessed by several assays, including binding to pathological tau, inhibition of tau cytotoxicity, inhibition of seeding activity, and reduction of tau aggregates in brain.

[0086] Binding of Armanezumab to pathological tau. The activity of Armanezumab was tested initially by binding to pathological tau in brain sections from AD cases. Figure 19 A shows that Armanezumab recognized pathological tau in brain sections from not only AD cases, but also with pathological tau in brains from frontotemporal lobar dementia (FTLD) and Pick disease cases (tissue obtained from the Brain Bank and Tissue Repository, MIND, UC Irvine). No binding was observed with brain section from control subject without tauopathy. Binding of Armanezumab is very similar to that seen with antibodies of various specificity recognizing pathological tau in tauopathy brains.

[0087] Data in Figure 19B shows that like 1C9, Armanezumab bound to full-length recombinant tau, monomeric and aggregated forms of tau in AD brain extracts and did not recognize recombinant tauΔ2-18 (Figure 19B). It labels the similar pattern of tau protein as HT7 recognizing total tau. In contrast to commercial PAD-specific antibody TNT-1, Armanezumab bound also the aggregated forms of tau in brain extracts from AD patients.

[0088] Inhibition of cytotoxicity of tau in vitro. The ability of Armanezumab to inhibit cytotoxicity of tau oligomers was tested in vitro using SH-SY5Y human neuroblastoma cells and mouse primary neurons. Cells were incubated with toxic concentrations (0.5 μM) of tau oligomers and either 1 μM Armanezumab or 1 μM irrelevant IgG antibody. Controls included cells alone and cells incubated with tau oligomers. Cell viability was assayed in all cultures using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. Data were collected (four replicates) and expressed as percentages of control ± s.d. As shown in Figure 20, both, SH-SY5Y human neuroblastoma cells (Figure 20A) and mouse primary neurons (Figure 20B) treated with tau oligomers had significantly higher viability when incubated with Armanezumab than when incubated with an irrelevant antibody, which didn't improve viability at all. Furthermore, the viability with Armanezumab was comparable to viability of cells alone.

[0089] Armanezumab Inhibits seeding activity of aggregated tau: To assess a relevant therapeutic activity of Armanezumab we used high throughput screening test developed in the laboratory of Dr. Diamond as described in Example 2 with small modifications. Aggregation rate in HEK293 cells transiently cotransfected with RD(ΔK)-CFP and RD(ΔK)-YFP (Kfoury, N. et al., J Biol Chem 2012, 287, 19440-19451; Furman J.L. et al., J Vis Exp, 2015, 106, e53205) was tracked by FRET. More vigorous aggregation resulted higher FRET signal could be induced by adding brain lysate from P301S Tg mice containing full-length tau aggregates to the culture of co-transfected cells. We tested the ability of Armanezumab to inhibit this brain lysate induction of intracellular RD(ΔK) aggregation. As shown in Figure 21A, addition of untreated brain lysate of P301S Tg mice to the transiently transfected cells expressing RD(ΔK)-CFP/YFP increased the number of FRET-positive cells, whereas pre-treating brain lysate with Armanezumab inhibited such induction. Interestingly, the number of FRET-positive cells is even lower than background level in samples without induction (Figure 21A).

[0090] Armanezumab immunoprecipitates aggregated Tau and inhibits seeding activity of brain lysate from Tau P301S Tg mice. Immunoprecipitation of aggregated Tau from brain lysate of P301S Tau/Tg mice with Armanezumab/protein complex abrogated the ability of brain lysate to induce aggregation of RD(ΔK)-CFP/YFP in HEK293 cells stably transfected with plasmids expressing RD(ΔK)-CFP and RD(ΔK)-YFP. As shown in Figure 21B, in the absence of a seeding aggregated tau, RD(ΔK)-CFP and RD(ΔK)-YFP did not form aggregates, and only the background signal is observed in FRET. Adding the brain lysates from tau/Tg mice induced aggregation and increased the FRET signal. Immunoprecipitation (IP) of tau aggregates from brain lysate with Armanezumab (50μg)/Protein A/G complex inhibited the induction of RD(ΔK)-YFP and RD(ΔK)-CFP aggregation and significantly decreased the percentage of FRET-positive cells analyzed by flow

cytometry. In contrast, IP of brain lysates with control IgG did not affect the aggregation level. Representative FACS images for each group are presented. Numbers indicate the percentage of gated FRET-positive cells (Figure 21 B).

[0091] Reduction of tau-aggregates in the brains of tau/tg mice. The therapeutic efficacy of Armanezumab was also tested in vivo after unilateral injections of 2 µg Armanezumab or control IgG into the hippocampus of 6-8 mo old tau/Tg mice. By this age these mice exhibit substantial tau accumulation and hyperphosphorylation. Five days after a single unilateral stereotactic injection of antibodies, mice (n=7) were perfused and sections examined with different anti-human Tau antibodies.

[0092] As shown in Figure 22, analyses by confocal microscopy revealed a significant reduction in tau phosphorylated at S214 and tau phosphorylated at S396/S404 (PHF1 tau) on the side injected with Armanezumab as compared to the side injected with control IgG (n=7).

[0093] In addition, IHC analyses showed reduction of total tau (HT7), phosphorylated tau positive to AT8, pT212, pS214, PHF1, AT100 (S212/S214), AT180. Representative images of brain sections from each antibody staining group is presented in Figure 23.

[0094] Improvement of recognition memory of tau/Tg mice. To evaluate the in vivo efficacy of Armanezumab, 1C9 moAb, the mouse counterpart of Armanezumab was used for passive vaccination. 3 mo old PS19 tau/Tg mice have been i.p. injected with 250µg 1C9 weekly until the age of 8-9 mo old. Cognitive improvement was evaluated by Novel Object Recognition (NOR) and Novel Place Recognition (NPR) tests. As shown in Figure 24, mice treated with 1C9 (n=9) spent significantly more time with novel object (Figure 24A) as well as within the novel arm (Figure 24B) when compared to IgG treated tau/Tg mice (n=10).

EXAMPLE 7

PHARMACOKINETICS OF ARMANEZUMAB IN MICE AND DEVELOPMENT OF INDUSTRIAL CELL LINE

[0095] PK analyses of Armanezumab in wildtype mice, in the absence of target-mediated clearance and in Tg mouse model expressing human tau, was conducted after the administration of a single dose of Armanezumab via IV bolus injection to C57BL6 and PS19 tau/Tg mice. The concentration of Armanezumab was monitored from day 1 through day 54 following the injection. No differences were observed in the serum concentration x time profiles of Armanezumab in WT and PS19 mice (Figure 25). In both strains the peak plasma concentration (C_{max}) of Armanezumab was observed at day 1, and the average elimination half-life is 9 days (Table in Figure 25). The rate at which Armanezumab is removed from the system [Elimination rate (K_{el}) constant and the estimated clearance (CL)] also did not differ between strains indicating that the elimination was

not target-mediated. The apparent volume of distribution is 2.93 and 2.70 in Tg and wild-type mice, respectively, indicating that Armanezumab was mostly distributed in blood in both strains of mice.

[0096] Based on data generated in the studies described above, a stable CHO-DG44 cell line expressing quantities of Armanezumab amenable to scale-up production (1.5 g/L), was developed using MTX amplification.

[0097] From the foregoing, it will be appreciated that, although specific embodiments have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the invention is not limited except as by the appended claims.

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. A pharmaceutical composition for treating tauopathy, the pharmaceutical composition comprising:

a. a pharmaceutically acceptable excipient; and

b. a humanized anti-tau antibody comprising:

(i) a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 23, a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 24, a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 25, a VL CDR1 comprising the amino acid sequence of SEQ ID NO: 26, a VL CDR2 comprising the amino acid sequence of SEQ ID NO: 27 and a VL CDR3 comprising the amino acid sequence of SEQ ID NO: 28;

(ii) a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 29, a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 30, a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 31, a VL CDR1 comprising the amino acid sequence of SEQ ID NO: 32, a VL CDR2 comprising the amino acid sequence of SEQ ID NO: 33, and a VL CDR3 comprising the amino acid sequence of SEQ ID NO: 34; or

(iii) a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 35, a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 36, a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 37, a VL CDR1 comprising the amino acid sequence of SEQ ID NO: 26, a VL CDR2 comprising the amino acid sequence of SEQ ID NO: 27, and a VL CDR3 comprising the amino acid sequence of SEQ ID NO: 28.

2. The composition of claim 1, wherein the humanized antibody specifically binds to an epitope within the N-terminal region of human tau.

3. The composition of claim 1, wherein the humanized antibody binds an epitope comprising the amino acids PRQEF or PRQEFE located within the following sequences: SEQ ID NO:1, SEQ ID NO:2 SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5 and SEQ ID NO:6.

Date Reçue/Date Received 2021-10-05

4. The composition of claim 1, wherein the humanized antibody comprises:

(i) a heavy chain variable region comprising SEQ ID NO:38 and a light chain variable region comprising SEQ ID NO:39;

(ii) a heavy chain variable region comprising SEQ ID NO:40 and a light chain variable region comprising SEQ ID NO:41;

(iii) a heavy chain variable region comprising SEQ ID NO:42 and a light chain variable region comprising SEQ ID NO:43;

(iv) a heavy chain variable region comprising SEQ ID NO:44 and a light chain variable region comprising SEQ ID NO:45;

(v) a heavy chain variable region comprising SEQ ID NO:46 and a light chain variable region comprising SEQ ID NO:47;

(vi) a heavy chain variable region comprising SEQ ID NO:48 and a light chain variable region comprising SEQ ID NO:49; or

(vii) a heavy chain variable region comprising SEQ ID NO:50 and a light chain variable region comprising SEQ ID NO:51.

5. The composition of claim 1, wherein the humanized antibody comprises a human light chain framework region and a human heavy chain framework region.

6. The composition of claim 1, wherein the humanized antibody comprises a human light chain constant region and a human heavy chain constant region.

7. The composition of claim 6, wherein the heavy chain constant region of the humanized antibody comprises the heavy chain constant regions of the human immunoglobulins IgG1, IgG2, IgG3, or IgG4.

8. The composition of claim 1, wherein the humanized antibody comprises antibody Armanezumab comprising the amino acid sequences of SEQ ID NO: 54 for variable and constant regions of heavy chains and SEQ ID NO: 55 for variable and constant regions of light chains.

9. The composition of claim 1, wherein the humanized antibody is an intact antibody, a single-chain Fv fragment, an F(ab') fragment, an F(ab) fragment or an F(ab')2 fragment.

Date Reçue/Date Received 2021-10-05

10. The composition of any one of claims 1 to 9, wherein the humanized antibody:

(i) binds to pathologically aggregated tau,

(ii) reduces tau aggregates in a brain, or

(iii) inhibits transcellular propagation of pathological, aggregated tau.

11. The composition of any one of claims 1 to 10, wherein the pharmaceutical composition comprises cells or viruses expressing the humanized antibody.

12. The composition of claim 11, wherein the viruses are an adenovirus, an adeno-associated virus, or a lentivirus.

13. A pharmaceutical composition for diagnosing or screening a subject for the presence of tauopathy, the pharmaceutical composition comprising:

a. a pharmaceutically acceptable excipient; and

b. a humanized anti-tau antibody comprising:

(i) a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 23, a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 24, a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 25, a VL CDR1 comprising the amino acid sequence of SEQ ID NO: 26, a VL CDR2 comprising the amino acid sequence of SEQ ID NO: 27 and a VL CDR3 comprising the amino acid sequence of SEQ ID NO: 28;

(ii) a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 29, a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 30, a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 31, a VL CDR1 comprising the amino acid sequence of SEQ ID NO: 32, a VL CDR2 comprising the amino acid sequence of SEQ ID NO: 33, and a VL CDR3 comprising the amino acid sequence of SEQ ID NO: 34; or

(iii) a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 35, a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 36, a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 37, a VL CDR1 comprising the amino acid sequence of SEQ ID NO: 26, a VL CDR2 comprising the amino acid

Date Reçue/Date Received 2021-10-05

sequence of SEQ ID NO: 27, and a VL CDR3 comprising the amino acid sequence of SEQ ID NO: 28.

14. Use of a pharmaceutical composition for treating tauopathy, the pharmaceutical composition comprising:

a. a pharmaceutically acceptable excipient; and

b. a humanized anti-tau antibody comprising:

(i) a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 23, a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 24, a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 25, a VL CDR1 comprising the amino acid sequence of SEQ ID NO: 26, a VL CDR2 comprising the amino acid sequence of SEQ ID NO: 27 and a VL CDR3 comprising the amino acid sequence of SEQ ID NO: 28;

(ii) a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 29, a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 30, a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 31, a VL CDR1 comprising the amino acid sequence of SEQ ID NO: 32, a VL CDR2 comprising the amino acid sequence of SEQ ID NO: 33, and a VL CDR3 comprising the amino acid sequence of SEQ ID NO: 34; or

(iii) a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 35, a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 36, a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 37, a VL CDR1 comprising the amino acid sequence of SEQ ID NO: 26, a VL CDR2 comprising the amino acid sequence of SEQ ID NO: 27, and a VL CDR3 comprising the amino acid sequence of SEQ ID NO: 28.

15. The use of claim 14, wherein the humanized antibody specifically binds to an epitope within the N-terminal region of human tau.

16. The use of claim 15, wherein the humanized antibody binds an epitope comprising the amino acids PRQEF or PRQEFE located within the following sequences: SEQ ID NO:1, SEQ ID NO:2 SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5 and SEQ ID NO:6.

17. The use of claim 14, wherein the humanized antibody comprises:

(i) a heavy chain variable region comprising SEQ ID NO:38 and a light chain variable region comprising SEQ ID NO:39;

(ii) a heavy chain variable region comprising SEQ ID NO:40 and a light chain variable region comprising SEQ ID NO:41;

(iii) a heavy chain variable region comprising SEQ ID NO:42 and a light chain variable region comprising SEQ ID NO:43;

(iv) a heavy chain variable region comprising SEQ ID NO:44 and a light chain variable region comprising SEQ ID NO:45;

(v) a heavy chain variable region comprising SEQ ID NO:46 and a light chain variable region comprising SEQ ID NO:47;

(vi) a heavy chain variable region comprising SEQ ID NO:48 and a light chain variable region comprising SEQ ID NO:49; or

(vii) a heavy chain variable region comprising SEQ ID NO:50 and a light chain variable region comprising SEQ ID NO:51.

18. The use of claim 14, wherein the humanized antibody comprises a human light chain framework region and a human heavy chain framework region.

19. The use of claim 14, wherein the humanized antibody comprises a human light chain constant region and a human heavy chain constant region.

20. The use of claim 19, wherein the heavy chain constant region of the humanized antibody comprises the heavy chain constant regions of the human immunoglobulins IgG1, IgG2, IgG3, or IgG4.

21. The use of claim 14, wherein the humanized antibody comprises antibody Armanezumab comprising the amino acid sequences of SEQ ID NO: 54 for variable and constant regions of heavy chains and SEQ ID NO: 55 for variable and constant regions of light chains.

22. The use of claim 14, wherein the humanized antibody is an intact antibody, a single-chain Fv fragment, an F(ab') fragment, an F(ab) fragment or an F(ab')2 fragment.

23. The use of any one of claims 14 to 22, wherein the humanized antibody:

(i) binds to pathologically aggregated tau,

(ii) reduces tau aggregates in a brain, or

(iii) inhibits transcellular propagation of pathological, aggregated tau.

24. The use of any one of claims 14 to 23, wherein the pharmaceutical composition comprises cells or viruses expressing the humanized antibody.

25. The use of claim 24, wherein the viruses are an adenovirus, an adeno-associated virus, or a lentivirus.

26. Use of a pharmaceutical composition for diagnosing or screening a subject for the presence of tauopathy, the pharmaceutical composition comprising:

a. a pharmaceutically acceptable excipient; and

b. a humanized anti-tau antibody comprising:

(i) a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 23, a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 24, a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 25, a VL CDR1 comprising the amino acid sequence of SEQ ID NO: 26, a VL CDR2 comprising the amino acid sequence of SEQ ID NO: 27 and a VL CDR3 comprising the amino acid sequence of SEQ ID NO: 28;

(ii) a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 29, a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 30, a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 31, a VL CDR1 comprising the amino acid sequence of SEQ ID NO: 32, a VL CDR2 comprising the amino acid sequence of SEQ ID NO: 33, and a VL CDR3 comprising the amino acid sequence of SEQ ID NO: 34; or

(iii) a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 35, a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 36, a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 37, a VL CDR1 comprising the amino acid sequence of SEQ ID NO: 26, a VL CDR2 comprising the amino acid sequence of SEQ ID NO: 27, and a VL CDR3 comprising the amino acid sequence of SEQ ID NO: 28.

Date Reçue/Date Received 2021-10-05

FIG. 1A

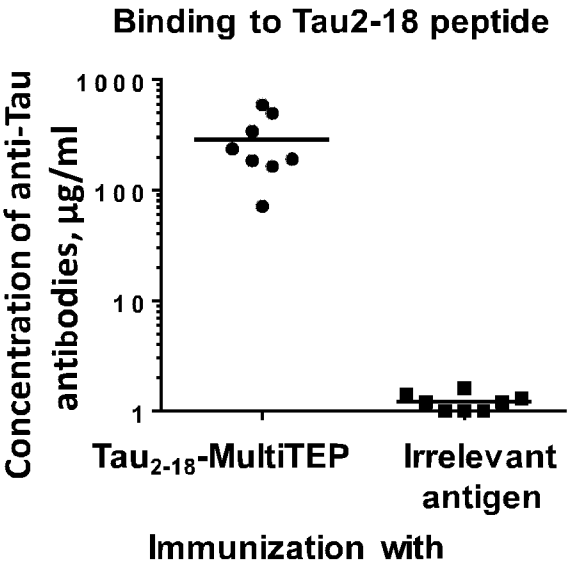


FIG. 1B

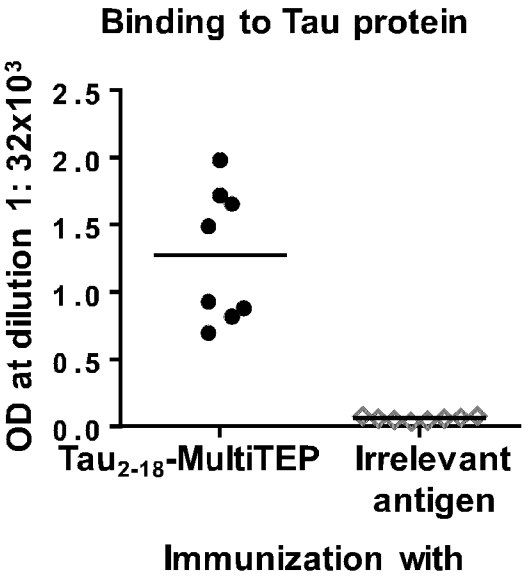
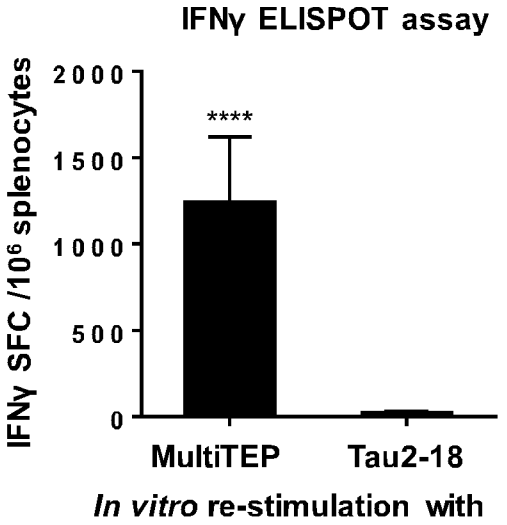
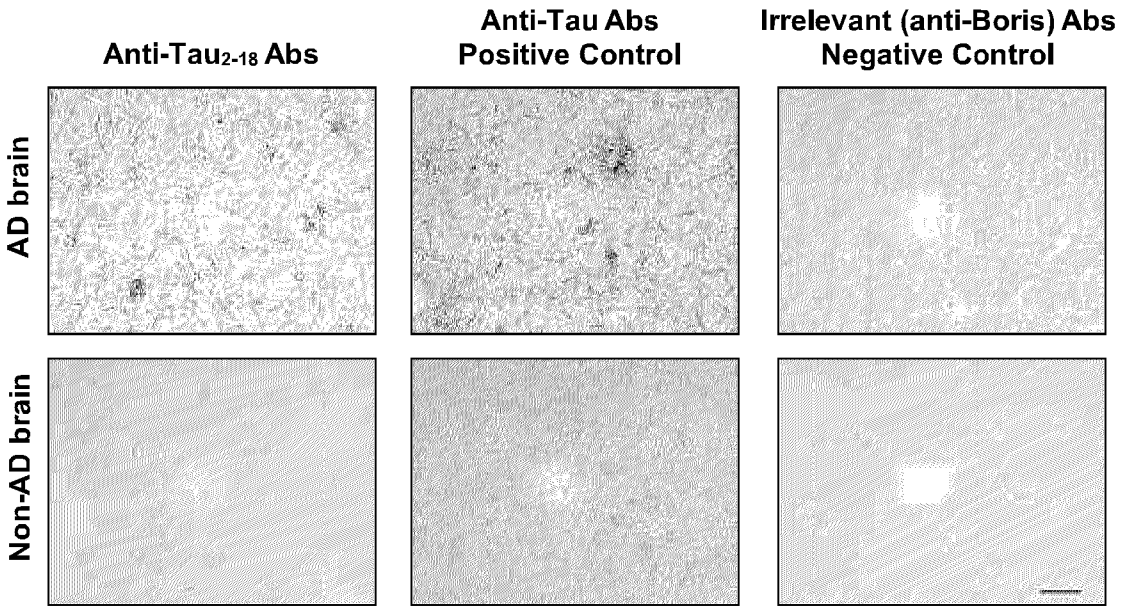


FIG. 1C



Date Reçue/Date Received 2021-03-24

FIG. 2



Date Reçue/Date Received 2021-03-24

FIG. 3A

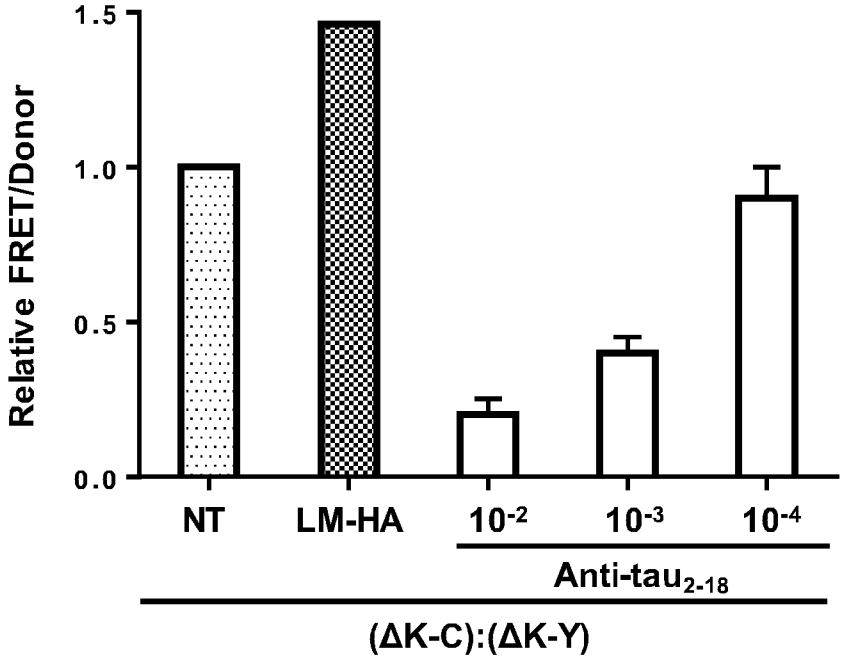

Relative FRET/Donor
1.5
1.0
0.5
0.0
NT
LM-HA
10-2
10-3
10-4
Anti-tau2-18
(ΔK-C):(ΔK-Y)

FIG. 3B

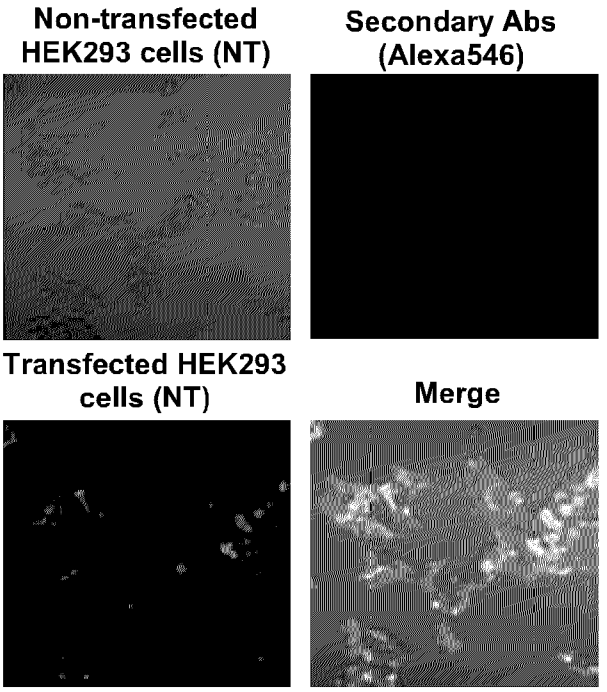

Non-transfected HEK293 cells (NT)
Secondary Abs (Alexa546)
Transfected HEK293 cells (NT)
Merge

FIG. 4A

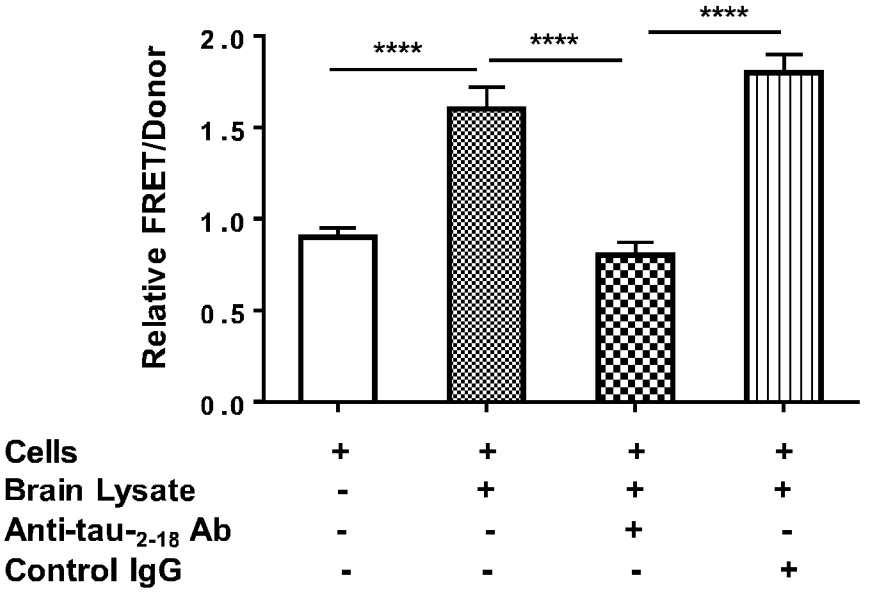


FIG. 4B

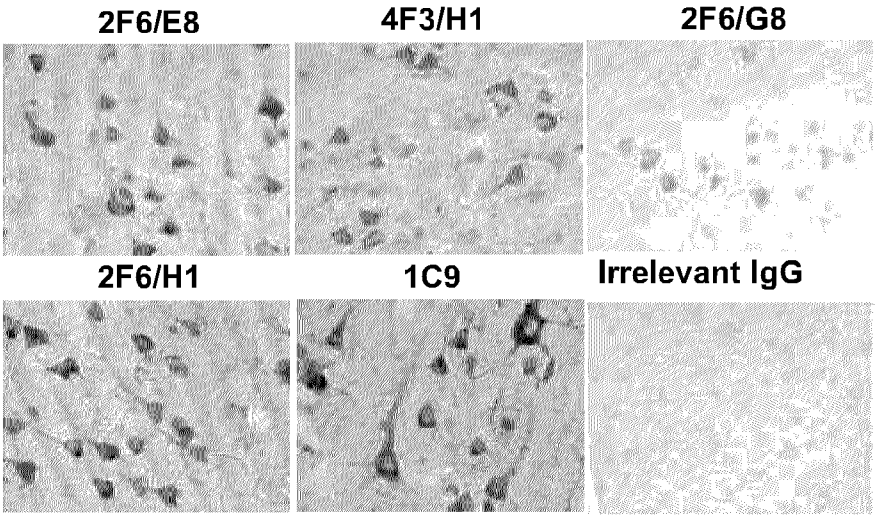
FIG. 4C

FIG. 4D

FIG. 4E

Date Reçue/Date Received 2021-03-24

FIG. 5



Date Reçue/Date Received 2021-03-24

FIG. 6A

1C9 VH

```
    M  E  C  N  W  I  L  P  F  I  L  S  V  T  S  G  V  Y  S  E  V  Q  L  Q  Q  S  G  T  V  L
  1 atggaatgta actggatact tccttttatt ctgtcggtaa cttcaggggt ctactcagag gttcagctcc agcaatctgg gactgtgctg

    A  R  P  G  A  S  V  K  M  S  C  K  T  S  G  Y  T  F  T  N  H  W  M  H  W  V  K  Q  R  P
 91 gcaaggcctg gggcttcagt gaagatgtcc tgcaagactt ctggctacac atttaccaac cactggatgc actgggtaaa acagaggcct

    G  Q  G  L  E  W  I  G  A  I  D  P  G  N  S  D  T  S  Y  N  Q  K  F  K  G  Q  A  K  L  T
181 ggacagggtc tggaatggat agggGCTATT GATCCTGGAA ATAGTGATAC TAGCTACAAC CAGAAGTTCA AGGGCcaggc caaactgact

    A  V  T  S  A  S  T  A  F  M  E  L  S  S  L  T  N  E  D  S  A  V  Y  Y  C  T  R  R  D  F
271 gcagtcacat ccgccagcac tgccttcatg gagctcagca gcctgacaaa tgaggactct gcggtctatt actgtacaag aAGGGATTTC

    F  G  G  E  Y  A  V  D  Y  W  G  Q  G  T  S  V  T  V  S  S        SEQ ID NO.17
361 TTCGGTGGTG AGTATGCTGT GGACTACtgg ggtcaaggaa cctcagtcac cgtctcctca    SEQ ID NO.58
```

FIG. 6B

1C9 VL

```
    M  K  L  P  V  R  L  L  V  L  M  F  W  I  P  A  S  S  S  D  V  V  M  T  Q  T  P  L  S  L
  1 atgaagttgc ctgttaggct gttggtgctg atgttctgga ttcctgcttc cagcagtgat gttgtgatga cccaaactcc actctccctg

    P  V  S  L  G  D  Q  A  S  I  S  C  R  S  S  Q  S  L  V  H  S  N  G  N  T  Y  L  H  W  Y
 91 cctgtcagtc ttggagatca agcctccatc tcttgcAGAT CTAGTCAGAG CCTTGTGCAC AGTAATGGAA ACACCTATTT ACATTggtac

    L  Q  K  P  G  Q  S  P  K  L  L  I  Y  K  V  S  N  R  F  S  G  V  P  D  R  F  S  G  S  G
181 ctgcagaagc caggccagtc tccaaagctc ctgatctacA AAGTTTCCAA CCGATTTTCT GGGGTCCCAG acaggttcag tggcagtgga

    S  G  T  D  F  T  L  K  I  S  R  V  E  A  E  D  L  G  V  Y  F  C  S  Q  S  T  H  V  P  W
271 tcagggacag atttcacact caagatcagc agagtggagg ctgaggatct gggagtttat ttctgctCTC AAAGTACACA TGTTCCGTGG

    T  F  G  G  G  T  K  L  E  I  K        SEQ ID NO.18
361 ACGttcggtg gaggcaccaa gctggaaatc aaa    SEQ ID NO.59
```

FIG. 7A

2F6/H11

```
    M  E  C  N  W  I  L  P  F  I  L  S  V  T  S  G  V  Y  S  E  V  Q  L  Q  Q  S  G  T  V  L
  1 atggaatgta actggatact tccttttatt ctgtcggtaa cttcaggggt ctactcagag gttcagctcc agcaatctgg gactgtgctg

    A  R  P  G  A  S  V  K  M  S  C  K  T  S  G  Y  T  F  T  T  Y  W  I  H  W  V  K  Q  R  P
 91 gcaaggcctg gggcttcagt gaagatgtcc tgtaagactt ctggctacac atttaccacc tactggatac actgggtaaa acagaggcct

    G  Q  G  L  E  W  I  G  A  I  C  P  G  D  S  D  T  S  Y  N  Q  K  F  K  G  Q  A  K  L  T
181 ggacagggtc tggaatggat aggggctatt tgtcctggag atagtgatac tagctacaac cagaagttca agggccaggc caaactgact

    A  V  P  S  A  S  T  A  Y  M  E  L  S  S  L  T  I  E  D  S  A  V  Y  Y  C  T  R  R  D  F
271 gcagtcccat ccgccagcac tgcctacatg gaactcagca gcctgacaat tgaggactct gcggtctatt actgtacaag aagggatttc

    Y  G  S  D  Y  A  V  D  Y  W  G  Q  G  T  S  V  T  V  S  S          SEQ ID NO.21
361 tacggtagtg actatgctgt ggactactgg ggtcaaggaa cctcagtcac cgtctcctca      SEQ ID NO.60
```

FIG. 7B

2F6/H11 VL

```
    M  K  L  P  V  R  L  L  V  L  M  F  W  I  P  A  S  S  S  D  V  V  M  T  Q  T  P  L  S  L
  1 atgaagttgc ctgttaggct gttggtgctg atgttctgga ttcctgcttc cagtagtgat gttgtgatga cccaaactcc actctccctg

    P  V  S  L  G  D  Q  A  S  I  S  C  R  S  S  Q  S  L  V  H  S  N  G  N  T  Y  L  H  W  Y
 91 cctgtcagtc ttggagatca agcctccatc tcttgcagat ctagtcagag ccttgtacac agtaatggaa acacctattt acattggtac

    L  Q  K  P  G  Q  S  P  K  L  L  I  Y  K  V  S  N  R  F  S  G  V  P  D  R  F  S  G  S  G
181 ctgcagaagc caggccagtc tccaaaactc ctgatctaca aagtctccaa ccgattttct ggggtcccag acaggttcag tggcagtgga

    S  G  T  D  F  T  L  K  I  S  R  V  E  G  E  D  L  G  V  Y  F  C  S  Q  S  T  H  V  P  W
271 tcagggacag atttcacact caagatcagc agagtggagg gtgaggatct gggagtatat ttctgctctc aaagtacaca tgttccgtgg

    T  F  G  G  G  T  K  L  E  I  K          SEQ ID NO.22
361 acgttcggtg gaggcaccaa gctggaaatc aaa      SEQ ID NO.61
```

FIG. 8A

4F3/H1

M K V L S L L Y L L T A I P G I L S D V Q L Q E S G P G L V
1 atgaaagtgt tgagtctgtt gtacctgttg acagccattc ctggtatcct gtctgatgta cagcttcagg agtcaggacc tggcctcgtg

K P S Q S L S L T C S V T G Y S I T **S G Y Y Y N** W I R Q F P
91 aaaccttctc agtctctgtc tctcacctgc tctgtcactg gctactccat cacc**agtggt tattactata ac**tggatccg gcagtttcca

G N K L E W M G **Y I R N D G S N N Y N P S L K N** R I S I T R
181 ggaaacaaac tggaatggat gggc**tacata agaaacgacg gtagtaataa ctacaaccca tctctcaaaa at**cgaatctc catcactcgt

D T S K N Q F F L K L N S V T T E D S A T Y Y C A R **G L T P**
271 gacacatcta agaaccagtt tttcctgaag ttgaattctg tgactacaga ggactcagcc acatattact gtgcaagag**g gcttactccg**

D Y W G Q G I T L T V S S SEQ ID NO.19
361 **gactac**tggg gccaaggcat tactctcaca gtctcctca SEQ ID NO.62

FIG. 8B

4F3/H1

M K L P V R L L V L M F W I P A S S S D V V M T Q T P L S L
1 atgaagttgc ctgttaggct gttggtgctg atgttctgga ttcctgcttc cagcagtgat gttgtgatga cccaaactcc actctccctg

P V S L G D Q A S I S C **R S S Q S L L H S N G N T Y L H** W Y
91 cctgtcagtc ttggagatca agcctccatc tcttgc**agat ctagtcagag ccttctccac agtaatggaa acacctattt acat**tggtac

L Q K P G Q S P K L L I Y **K V S D R F S** G V P D R F S G S G
181 ctgcagaagc caggccagtc tccaaagctc ctgatctac**a aagtttccga ccgcttttct** ggggtcccag acaggttcag tggcagtggc

S G T D F T L K I S R L E A E D L G V F F **C S Q T T H V P L**
271 tcagggacag atttcacact caagatcagc agactggagg ctgaggatct gggagttttt ttc**tgctctc aaactacaca tgttccgctc**

T F G A G T K L E L K SEQ ID NO.20
361 **acg**ttcggtg ctgggaccaa gctggagctg aaa SEQ ID NO.63

FIG. 9A

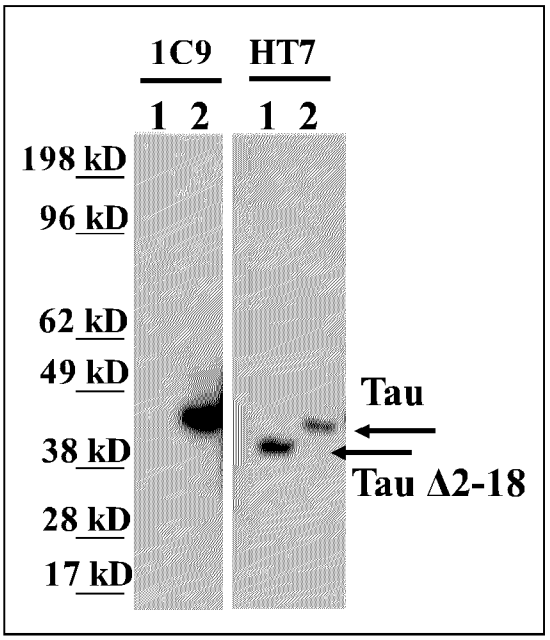


FIG. 9B

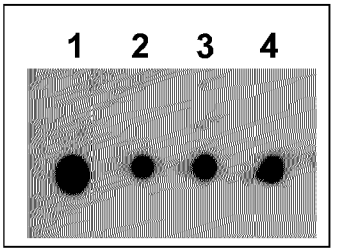


FIG. 9C

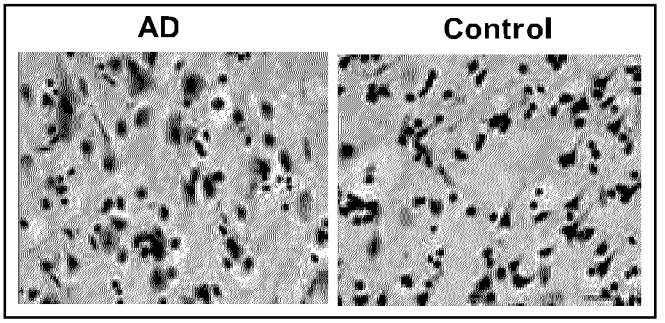


FIG. 9D

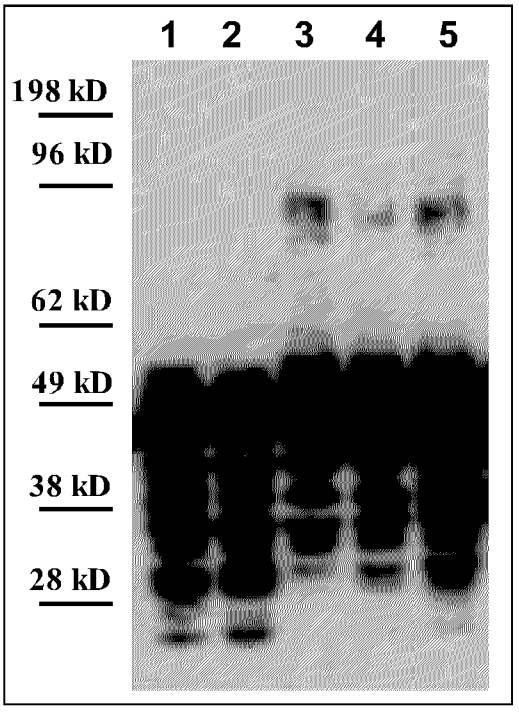
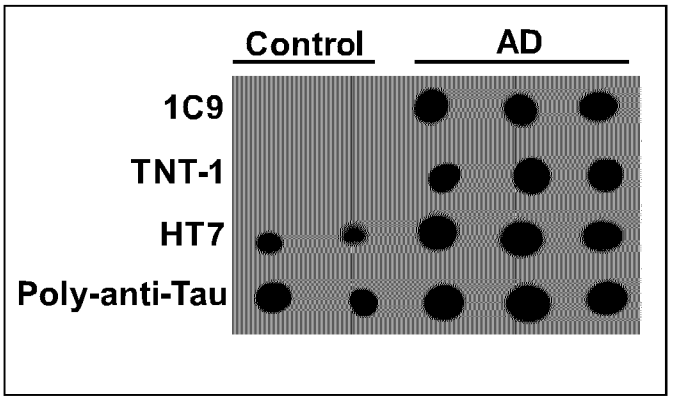


FIG. 9E



Date Reçue/Date Received 2021-03-24

FIG. 10A

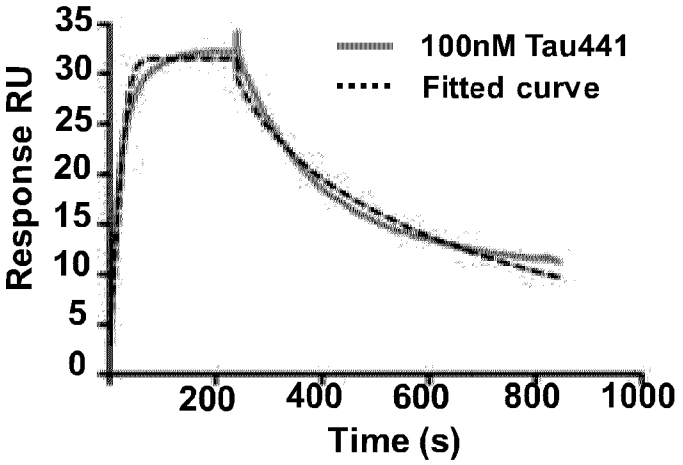
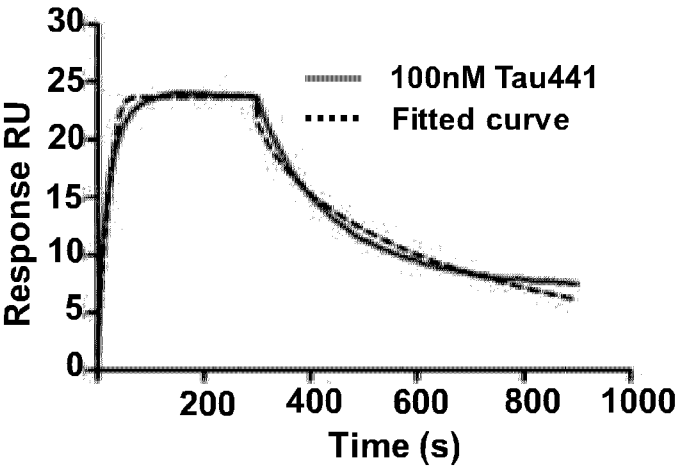


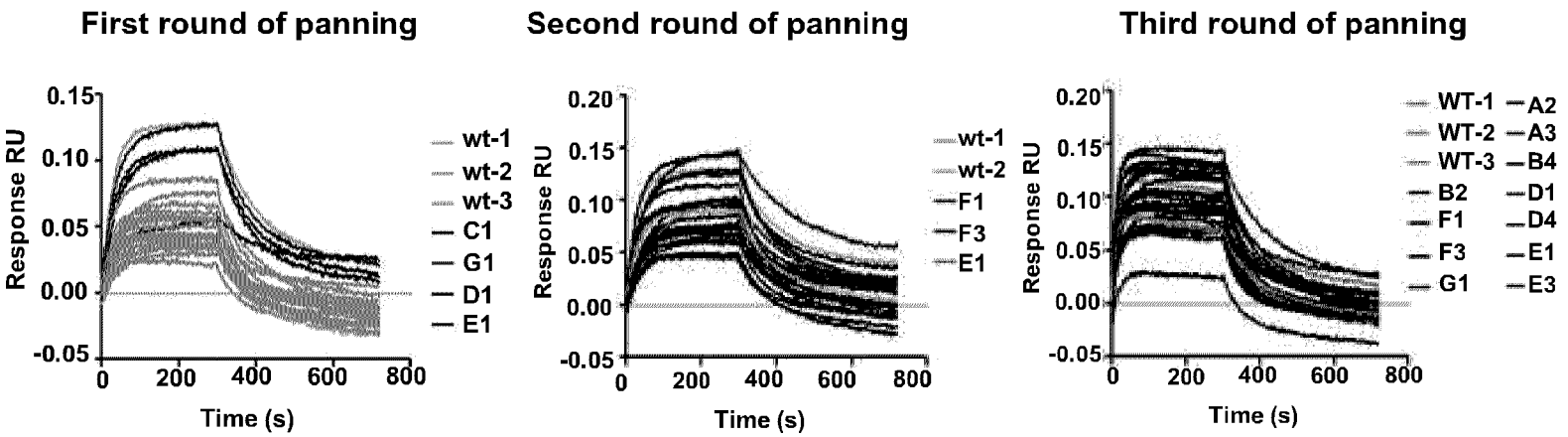
FIG. 10B



mAb	kon (1/Ms)	koff (1/s)	KD (M)	Rmax (RU)	U-value
Mouse 1C9	1.27E+06	4.30E-03	3.39E-09	30.57	3
Chimeric 1C9	1.53E+06	5.99E-03	3.91E-09	22.32	3

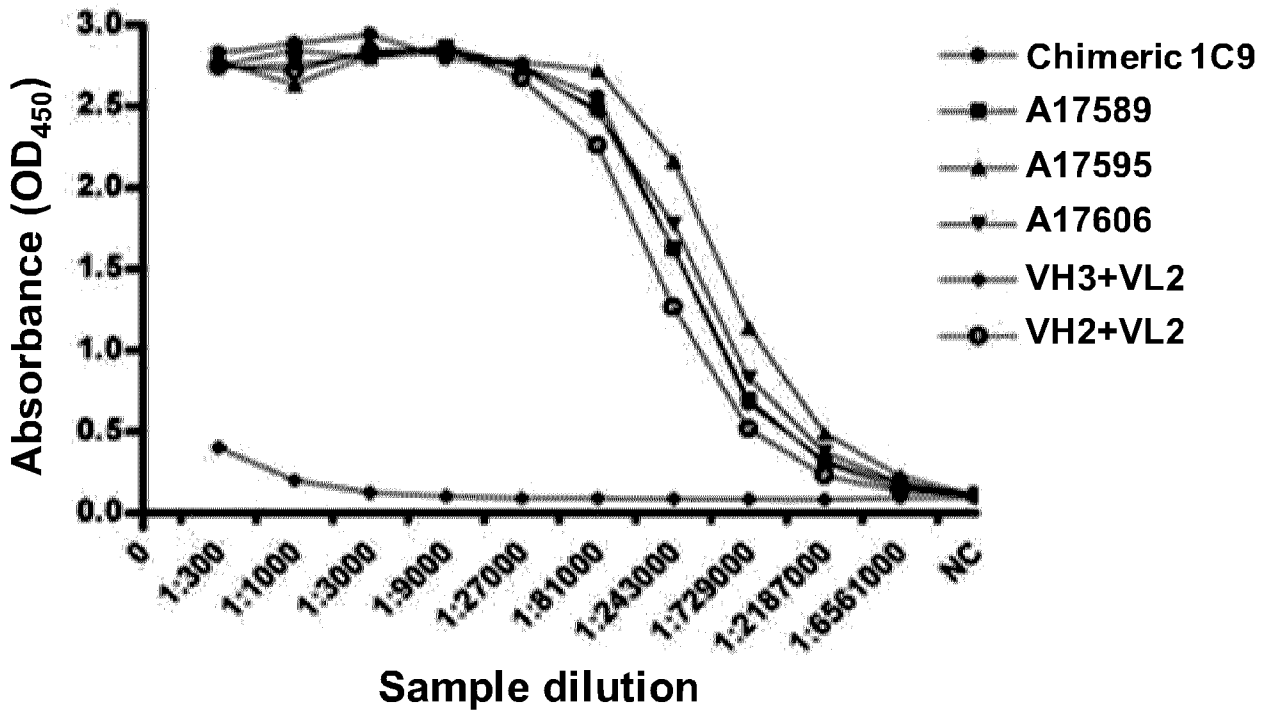
Date Reçue/Date Received 2021-03-24

FIG. 11



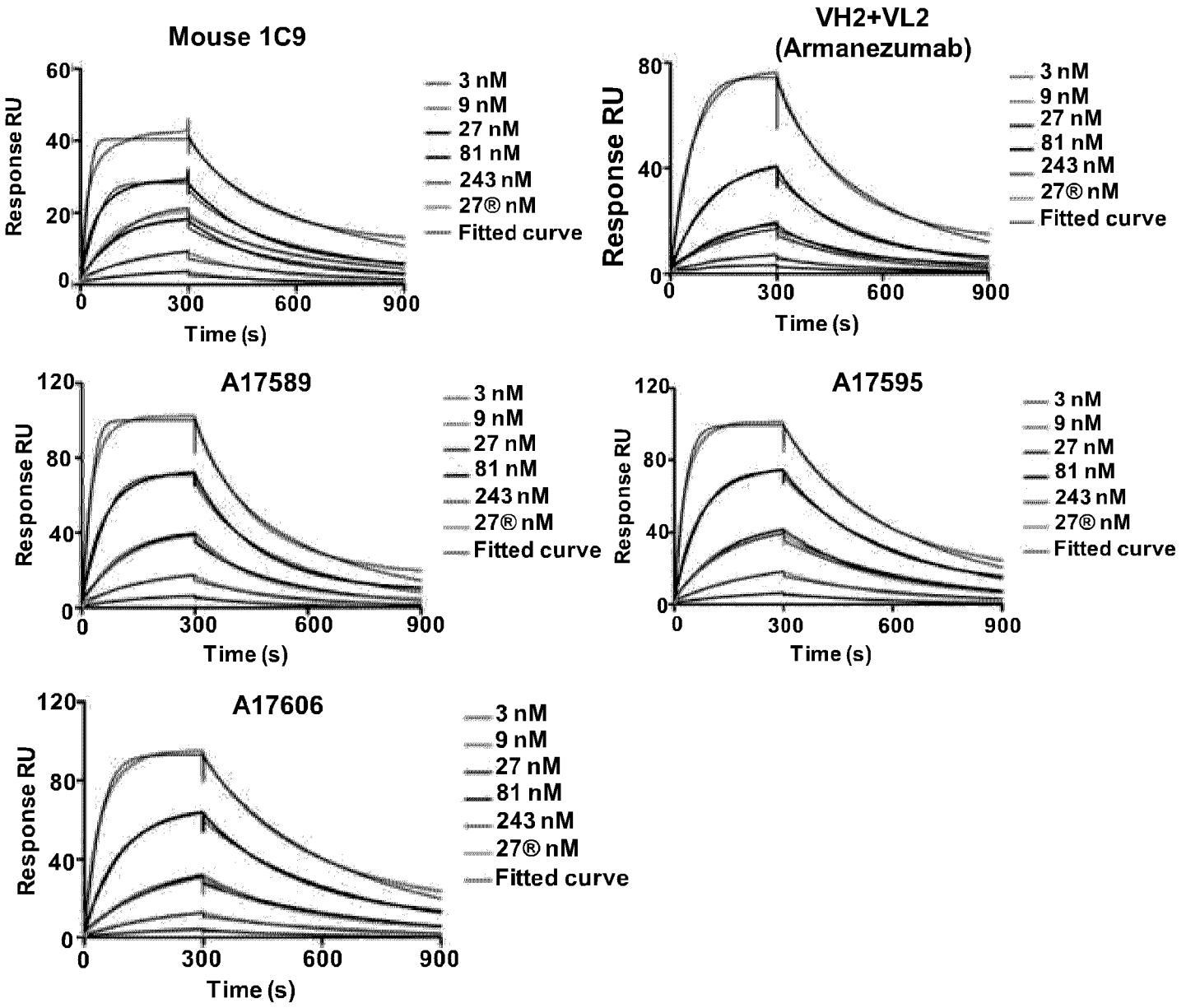
Date Reçue/Date Received 2021-03-24

FIG. 12



Date Reçue/Date Received 2021-03-24

FIG.13



mAb	k_a (1/Ms)	k_d (1/s)	K_D (M)
Mouse 1C9	5.73E+05	5.45E-03	9.51E-09
Humanized VH2+VL2 (Armanezumab)	1.38E+05	6.64E-03	4.80E-08
Humanized A17589	4.03E+05	8.37E-03	2.08E-08
Humanized A17606	1.96E+05	3.60E-03	1.83E-08
Humanized A17595	1.35E+05	3.40E-03	2.52E-08

Date Reçue/Date Received 2021-03-24

Armanezumab VH

FIG. 14A

```
    M  G  W  S  W  I  L  L  F  L  L  S  V  T  A  G  V  H  S  Q
  1 atgggctgga gctggatcct gctgttcctc ctgagcgtga cagcaggagt gcacagccag

    V  Q  L  V  Q  S  G  A  E  V  K  K  P  G  A  S  V  K  V  S
 61 gtgcagctgg tgcagtctgg ggctgaggtg aagaagcctg gggcctcagt gaaggtttcc

    C  K  A  S  G  Y  T  F  T  N  H  W  M  H  W  V  R  Q  A  P
121 tgcaaggcat ctggatacac cttcaccaac cactggatgc actgggtgcg acaggcccct

    G  Q  G  L  E  W  M  G  A  I  D  P  G  N  S  D  T  S  Y  N
181 ggacaagggc ttgagtggat gggagctatt gatcctggaa atagtgatac tagctacaac

    Q  K  F  K  G  R  V  T  M  T  R  D  T  S  T  S  T  V  Y  M
241 cagaagttca agggcagagt caccatgacc agggacacgt ccacgagcac agtctacatg

    E  L  S  S  L  R  S  E  D  T  A  V  Y  Y  C  A  R  R  D  F
301 gagctgagca gcctgagatc tgaggacacg gccgtgtatt actgtgcgag agagggattt c

    F  G  G  E  Y  A  V  D  Y  W  G  Q  G  T  L  V  T  V  S  S   SEQ ID NO.80
361 ttcggtggtg agtatgctgt ggactactgg ggccagggaa ccctggtcac cgtctcctca  SEQ ID NO.70
```

Armanezumab VL

FIG. 14B

```
    M  G  W  S  W  I  L  L  F  L  L  S  V  T  A  G  V  H  S  D
  1 atgggctgga gctggatcct gctgttcctc ctgagcgtga cagcaggagt gcacagcgat

    I  V  M  T  Q  S  P  L  S  L  P  V  T  P  G  E  P  A  S  I
 61 attgtgatga ctcagtctcc actctccctg cccgtcaccc ctggagagcc ggcctccatc

    S  C  R  S  S  Q  S  L  V  H  S  N  G  N  T  Y  L  H  W  Y
121 tcctgcagat ctagtcagag ccttgtgcac agtaatggaa acacctattt acattggtac

    L  Q  K  P  G  Q  S  P  Q  L  L  I  Y  K  V  S  N  R  F  S
181 ctgcagaagc cagggcagtc tccacagctc ctgatctata aagtttccaa ccgattttct

    G  V  P  D  R  F  S  G  S  G  S  G  T  D  F  T  L  K  I  S
241 ggggtccctg acaggttcag tggcagtgga tcaggcacag attttacact gaaaatcagc

    R  V  E  A  E  D  V  G  V  Y  Y  C  S  Q  S  T  H  V  P  W
301 agagtggagg ctgaggatgt tggggtttat tactgctctc aaagtacaca tgttccgtgg

    T  F  G  Q  G  T  K  V  E  I  K        SEQ ID NO.81
361 acgttcggcc aagggaccaa ggtggaaatc aaa     SEQ ID NO.71
```

Date Reçue/Date Received 2021-03-24

A17589 VH

FIG. 15A

```
    M  G  W  S  W  I  L  L  F  L  L  S  V  T  A  G  V  H  S  E
  1 atgggctgga gctggatcct gctgttcctc ctgagcgtga cagcaggagt gcacagcgag

    V  Q  L  V  Q  S  G  A  E  V  K  K  P  G  A  T  V  K  I  S
 61 gtccagctgg tacagtctgg ggctgaggtg aagaagcctg gggctacagt gaaaatctcc

    C  K  V  S  G  Y  T  F  T  N  H  W  M  H  W  V  Q  Q  A  P
121 tgcaaggttt ctggatacac cttcaccaac cactggatgc actgggtgca acaggcccct

    G  K  G  L  E  W  M  G  A  I  D  P  G  N  S  D  T  S  Y  N
181 ggaaaagggc ttgagtggat gggagctatt gatcctggaa atagtgatac tagctacaac

    Q  K  F  K  G  R  V  T  I  T  R  D  T  S  A  S  T  A  Y  M
241 cagaagttca agggcagagt caccattacc agggacacat ccgcgagcac agcctacatg

    E  L  S  S  L  R  S  E  D  T  A  V  Y  Y  C  A  R  R  D  F
301 gagctgagca gcctgagatc tgaagacacg gctgtgtatt actgtgcgag aagggatttc

    F  G  G  E  Y  A  V  D  Y  W  G  Q  G  T  T  V  T  V  S
361 ttcggtggtg agtatgctgt ggactactgg ggccaaggga ccacggtcac cgtctcctc
```

SEQ ID NO.82
SEQ ID NO.72

A17589 VL

FIG. 15B

```
    M  G  W  S  W  I  L  L  F  L  L  S  V  T  A  G  V  H  S  D
  1 atgggctgga gctggatcct gctgttcctc ctgagcgtga cagcaggagt gcacagcgat

    I  V  M  T  Q  T  P  L  S  L  S  V  T  P  G  Q  P  A  S  I
 61 attgtgatga cccagactcc actctctctg tccgtcaccc ctggacagcc ggcctccatc

    S  C  R  S  S  Q  S  L  V  H  S  N  G  N  T  Y  L  H  W  Y
121 tcctgcagat ctagtcagag ccttgtgcac agtaatggaa acacctattt acattggtac

    Q  Q  K  P  G  Q  A  P  R  L  L  I  Y  K  V  S  N  R  F  S
181 cagcagaaac ctggccaggc tcccaggctc ctcatctata aagtttccaa ccgattttct

    G  V  P  D  R  F  S  G  S  G  A  G  T  D  F  T  L  K  I  S
241 ggggtcccag acagattcag tggcagtggg gcagggacag atttcacact gaaaatcagc

    R  V  E  A  E  D  V  G  V  Y  Y  C  S  Q  S  T  H  V  P  W
301 agggtggaag ctgaggatgt cggggtttat tactgctctc aaagtacaca tgttccgtgg

    T  F  G  G  G  T  K  V  E  I  K
361 acgttcggcg gagggaccaa ggtggagatc aaa
```

SEQ ID NO.83
SEQ ID NO.73

FIG. 16A

A17595 VL

```
    M  G  W  S  W  I  L     L  F  L     L  S  V  T     A  G  V     H  S  Q
  1 atgggctgga gctggatcct gctgttcctc ctgagcgtga cagcaggagt gcacagccag

    V  Q  L  V  Q  S  G     A  E  V     K  K  P  G     A  S  V     K  V  S
 61 gtccagcttg tgcagtctgg ggctgaggtg aagaagcctg gggcctcagt gaaggtttcc

    C  K  A  S  G  Y  T     F  T  N     H  W  M  H     W  V  Q     Q  S  P
121 tgcaaggctt ctggatacac cttcactaac cactggatgc actgggtgca acagtcccct

    G  Q  G  L  E  W  M     G  A  I     D  P  G  N     S  D  T     S  Y  N
181 ggacaagggc ttgagtggat gggagctatt gatcctggaa atagtgatac tagctacaac

    Q  K  F  K  G  R  V     T  M  T     R  D  T  S     T  S  T     V  Y  M
241 cagaagttca agggcagagt caccatgacc agggacacgt ccacgagcac agtctacatg

    E  L  S  S  L  R  S     E  D  T     A  V  Y  Y     C  A  R     R  D  F
301 gagctgagca gcctgagatc tgaggacacg gccgtgtatt actgtgcgag aagggatttc

    F  G  G  E  Y  A  V     D  Y  W     G  Q  G  T     T  V  T     V  S  S   SEQ ID NO.84
361 ttcggtggtg agtatgctgt ggactactgg ggccaaggga ccacggtcac cgtctcctca   SEQ ID NO.74
```

FIG. 16B

A17595 VL

```
    M  G  W  S  W  I  L     L  F  L     L  S  V  T     A  G  V     H  S  D
  1 atgggctgga gctggatcct gctgttcctc ctgagcgtga cagcaggagt gcacagcgac

    I  Q  M  T  Q  S  P     S  S  L     S  A  S  V     G  D  R     V  T  I
 61 atccagatga cccagtctcc atcctccctg tctgcatctg taggagacag agtcaccatc

    T  C  R  S  S  Q  S     L  V  H     S  N  G  N     T  Y  L     H  W  Y
121 acttgcagat ctagtcagag ccttgtgcac agtaatggaa acacctattt acattggtat

    Q  Q  K  P  G  K  A     P  K  L     L  I  Y  K     V  S  N     R  F  S
181 cagcagaaac cagggaaagc ccctaagctc ctgatctata aagtttccaa ccgattttct

    G  V  P  D  R  F  S     G  S  G     S  G  T  D     F  T  L     K  I  S
241 ggggtcccag acagattcag cggcagtggg tcaggcactg atttcacact gaaaatcagc

    R  V  E  A  E  D  V     G  V  Y     Y  C  S  Q     S  T  H     V  P  W
301 agggtggagg ctgaggatgt tggggtttat tactgctctc aaagtacaca tgttccgtgg

    T  F  G  G  G  T  K     V  E  I     K      SEQ ID NO.85
361 acgttcggcg gagggaccaa ggtggagatc aaa      SEQ ID NO.75
```

A17606 VH

FIG. 17A

```
    M  G  W  S  W  I  L  L  F  L  L  S  V  T  A  G  V  H  S  Q
  1 atgggctgga gctggatcct gctgttcctc ctgagcgtga cagcaggagt gcacagccag

    V  Q  L  V  Q  S  G  S  E  L  K  K  P  G  A  S  V  K  V  S
 61 gtgcagctgg tgcaatctgg gtctgagttg aagaagcctg gggcctcagt gaaggtttcc

    C  K  A  S  G  Y  T  F  T  N  H  W  M  H  W  V  R  Q  A  P
121 tgcaaggctt ctggatacac cttcactaac cactggatgc actgggtgcg acaggcccct

    G  Q  G  L  E  W  M  G  A  I  D  P  G  N  S  D  T  S  Y  N
181 ggacaagggc ttgagtggat gggagctatt gatcctggaa atagtgatac tagctacaac

    Q  K  F  K  G  Q  V  T  I  S  A  D  K  S  I  S  T  A  Y  L
241 cagaagttca agggccaggt caccatctca gccgacaagt ccatcagcac cgcctacctg

    Q  W  S  S  L  K  A  S  D  T  A  M  Y  Y  C  A  R  R  D  F
301 cagtggagca gcctgaaggc ctcggacacc gccatgtatt actgtgcgag aagggatttc

    F  G  G  E  Y  A  V  D  Y  W  G  Q  G  T  T  V  T  V  S  S   SEQ ID NO.86
361 ttcggtggtg agtatgctgt ggactactgg ggccaaggga ccacggtcac cgtctcctca  SEQ ID NO.76
```

A17606 VL

FIG. 17B

```
    M  G  W  S  W  I  L  L  F  L  L  S  V  T  A  G  V  H  S  D
  1 atgggctgga gctggatcct gctgttcctc ctgagcgtga cagcaggagt gcacagcgat

    I  V  M  T  Q  T  P  L  S  L  S  V  T  P  G  Q  P  A  S  I
 61 attgtgatga cccagactcc actctctctg tccgtcaccc ctggacagcc ggcctccatc

    S  C  R  S  S  Q  S  L  V  H  S  N  G  N  T  Y  L  H  W  Y
121 tcctgcagat ctagtcagag ccttgtgcac agtaatggaa acacctattt acattggtac

    Q  Q  K  P  G  Q  A  P  R  L  L  I  Y  K  V  S  N  R  F  S
181 cagcagaaac ctggccaggc tcccaggctc ctcatctata aagtttccaa ccgattttct

    G  V  P  D  R  F  S  G  S  G  S  G  T  D  F  T  L  K  I  S
241 ggagtgccag ataggttcag tggcagcggg tcagggacag atttcacact gaaaatcagc

    R  V  E  A  E  D  F  G  V  Y  Y  C  S  Q  S  T  H  V  P  W
301 cgggtggagg ctgaggattt tggagtttat tactgctctc aaagtacaca tgttccgtgg

    T  F  G  G  G  T  K  V  E  I  K      SEQ ID NO.87
361 acgttcggcg gagggaccaa ggtggagatc aaa   SEQ ID NO.77
```

Date Reçue/Date Received 2021-03-24

FIG. 18A

VH3+VL2 VH

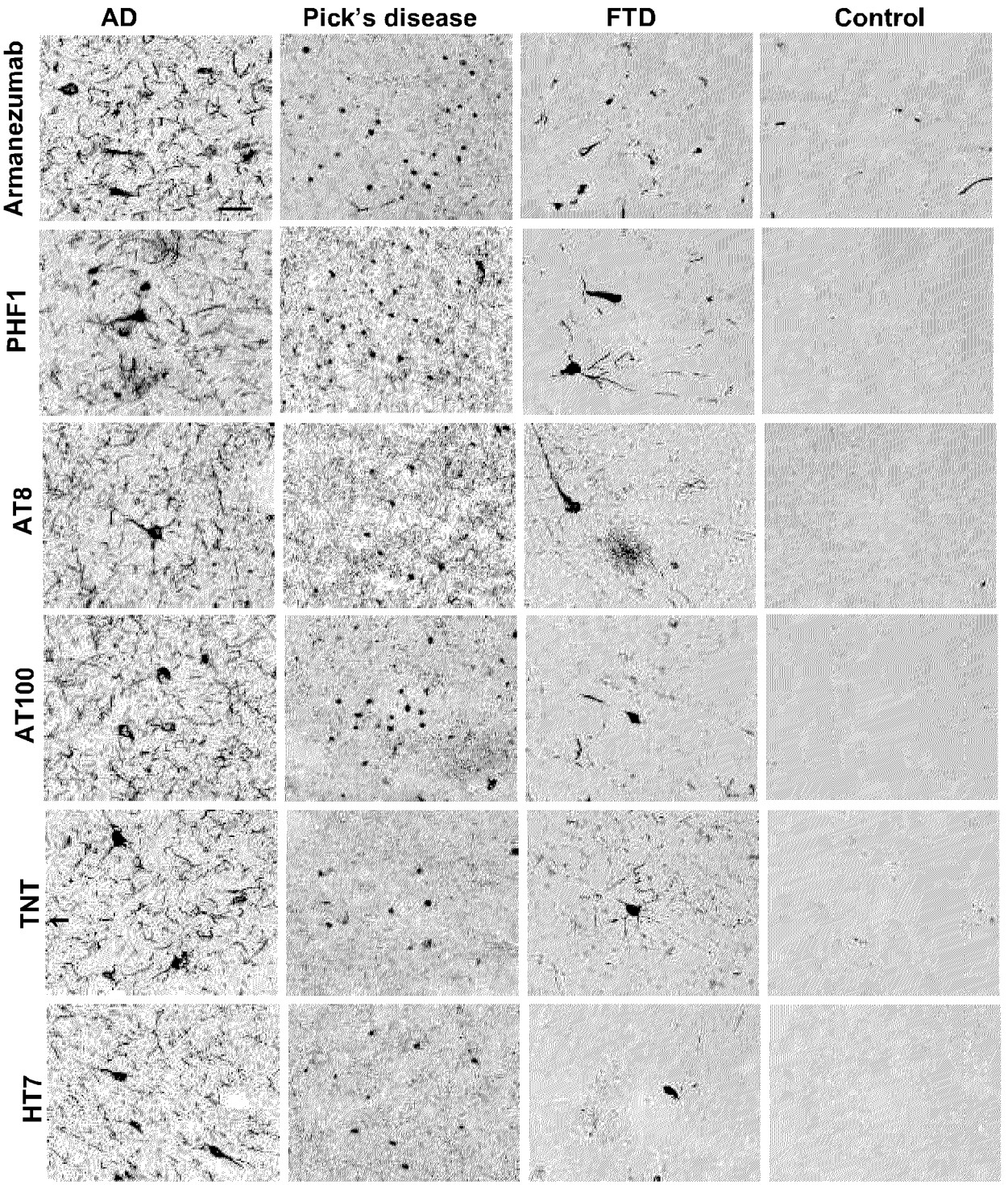
```
    M  G  W  S  W  I  L  L  F  L  L  S  V  T  A  G  V  H  S  E
  1 atgggctgga gctggatcct gctgttcctc ctgagcgtga cagcaggagt gcacagcgag

    V  Q  L  V  E  S  G  G  G  L  V  Q  P  G  G  S  L  K  L  S
 61 gtgcagctgg tggagtccgg gggaggcttg gtccagcctg gggggtccct gaaactctcc

    C  A  A  S  G  F  T  F  S  N  H  W  M  H  W  V  R  Q  A  S
121 tgtgcagcct ctgggttcac cttcagtaac cactggatgc actgggtccg ccaggcttcc

    G  K  G  L  E  W  V  G  A  I  D  P  G  N  S  D  T  S  Y  N
181 gggaaagggc tggagtgggt tggcgctatt gatcctggaa atagtgatac tagctacaac

    Q  K  F  K  G  R  F  T  I  S  R  D  D  S  K  N  T  A  Y  L
241 cagaagttca agggcaggtt caccatctcc agagatgatt caaagaacac ggcgtatctg

    Q  M  N  S  L  K  T  E  D  T  A  V  Y  Y  C  T  R  R  D  F
301 caaatgaaca gcctgaaaac cgaggacacg gccgtgtatt actgtactag aagggatttc

    F  G  G  E  Y  A  V  D  Y  W  G  Q  G  T  T  V  T  V  S  S   SEQ ID NO.88
361 ttcggtggtg agtatgctgt ggactactgg ggccaaggga ccacggtcac cgtctcctca SEQ ID NO.78
```

FIG. 18B

VH3+VL2 VL

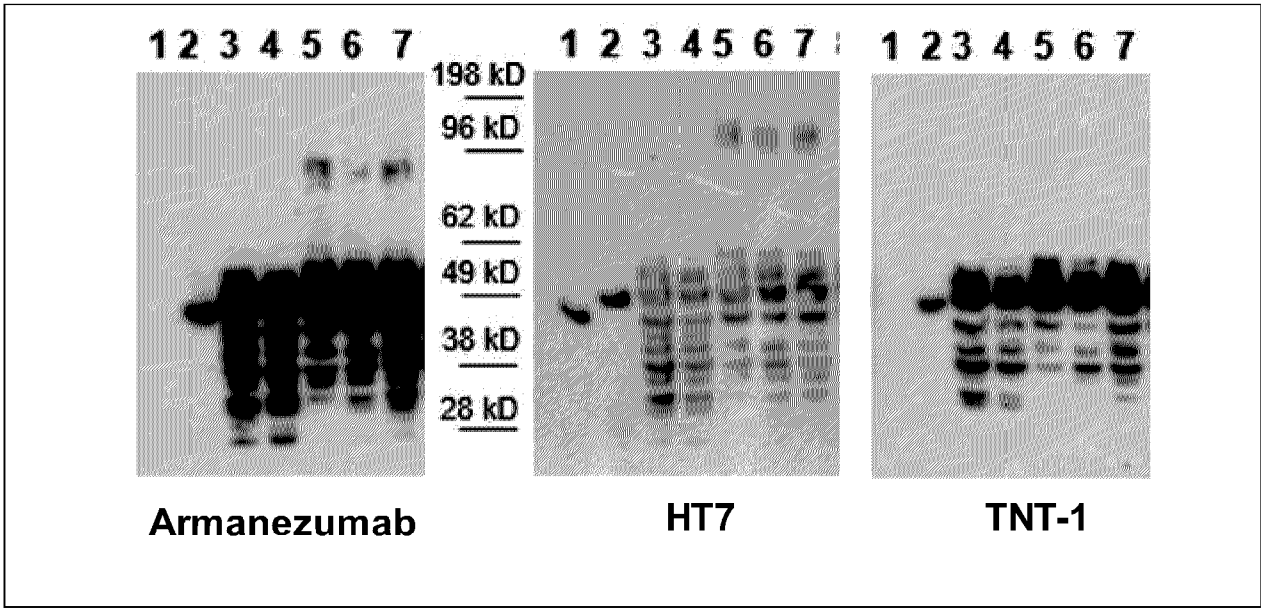
```
    M  G  W  S  W  I  L  L  F  L  L  S  V  T  A  G  V  H  S  D
  1 atgggctgga gctggatcct gctgttcctc ctgagcgtga cagcaggagt gcacagcgat

    I  V  M  T  Q  T  P  L  S  L  S  V  T  P  G  Q  P  A  S  I
 61 attgtgatga cccagactcc actctctctg tccgtcaccc ctggacagcc ggcctccatc

    S  C  R  S  S  Q  S  L  V  H  S  N  G  N  T  Y  L  H  W  Y
121 tcctgcagat ctagtcagag ccttgtgcac agtaatggaa acacctattt acattggtac

    Q  Q  K  P  G  Q  A  P  R  L  L  I  Y  K  V  S  N  R  F  S
181 cagcagaaac ctggccaggc tcccaggctc ctcatctata aagtttccaa ccgattttct

    G  V  P  D  R  F  S  G  S  G  A  G  T  D  F  T  L  K  I  S
241 ggggtcccag acagattcag tggcagtggg gcagggacag atttcacact gaaaatcagc

    R  V  E  A  E  D  V  G  V  Y  Y  C  S  Q  S  T  H  V  P  W
301 agggtggaag ctgaggatgt cggggtttat tactgctctc aaagtacaca tgttccgtgg

    T  F  G  G  G  T  K  V  E  I  K      SEQ ID NO.89
361 acgttcggcg gagggaccaa ggtggagatc aaa  SEQ ID NO.79
```

FIG. 19A



Date Reçue/Date Received 2021-03-24

FIG. 19B



Date Reçue/Date Received 2021-03-24

FIG. 20A

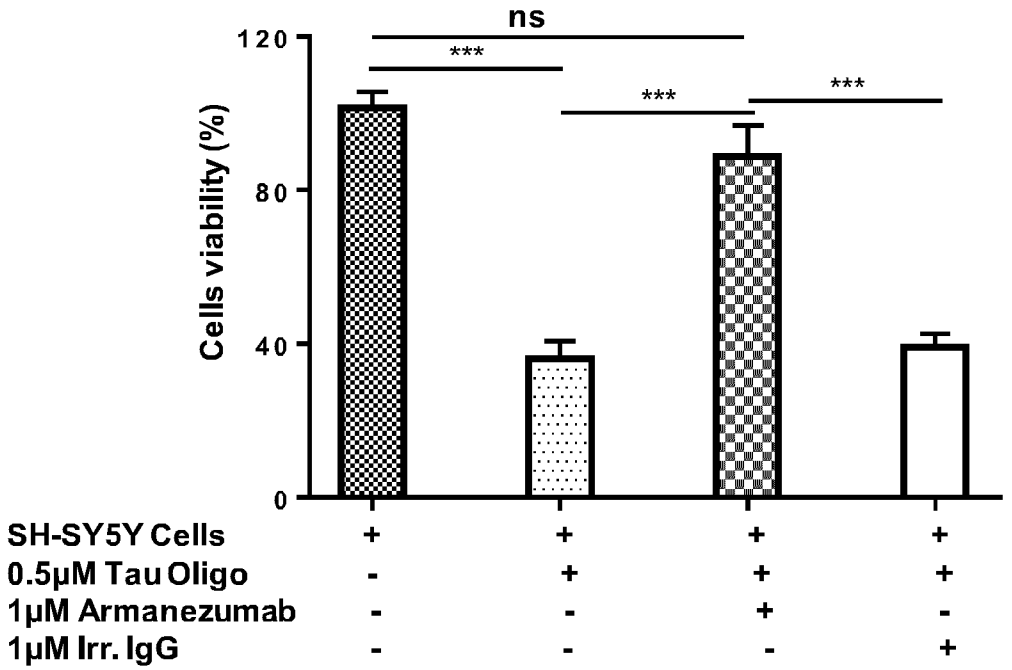
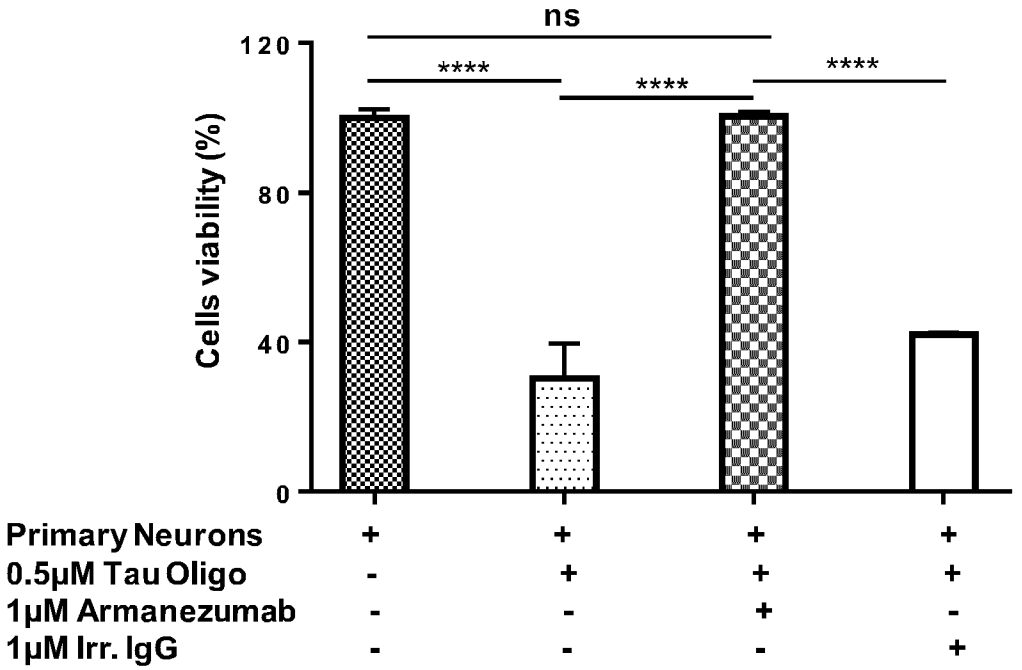


FIG. 20B



Date Reçue/Date Received 2021-03-24

FIG. 21A

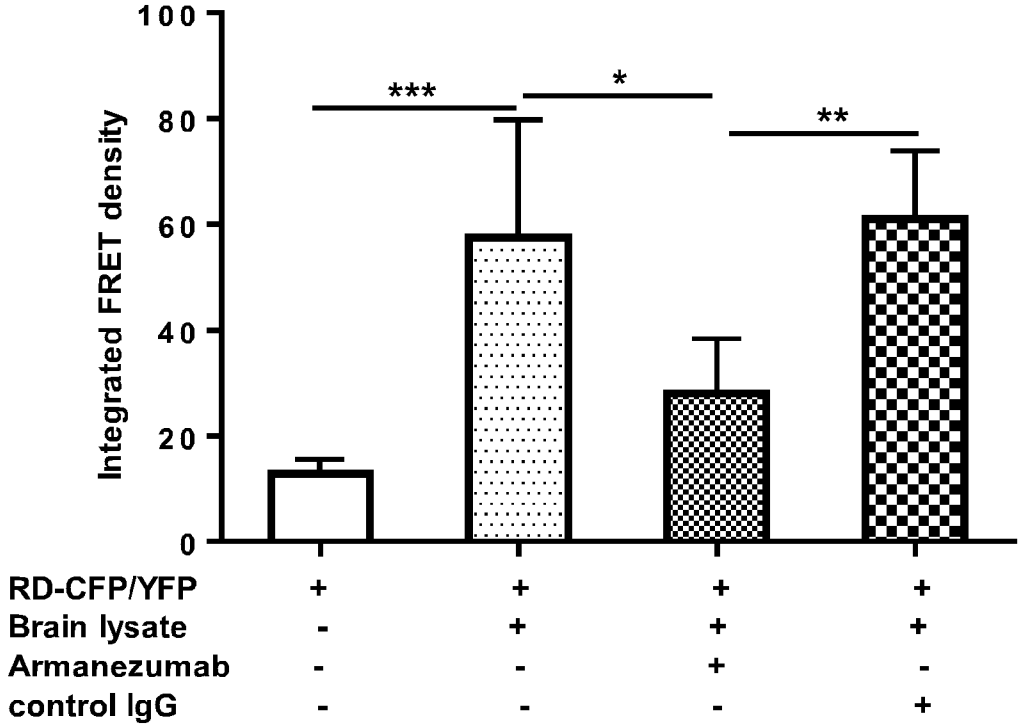
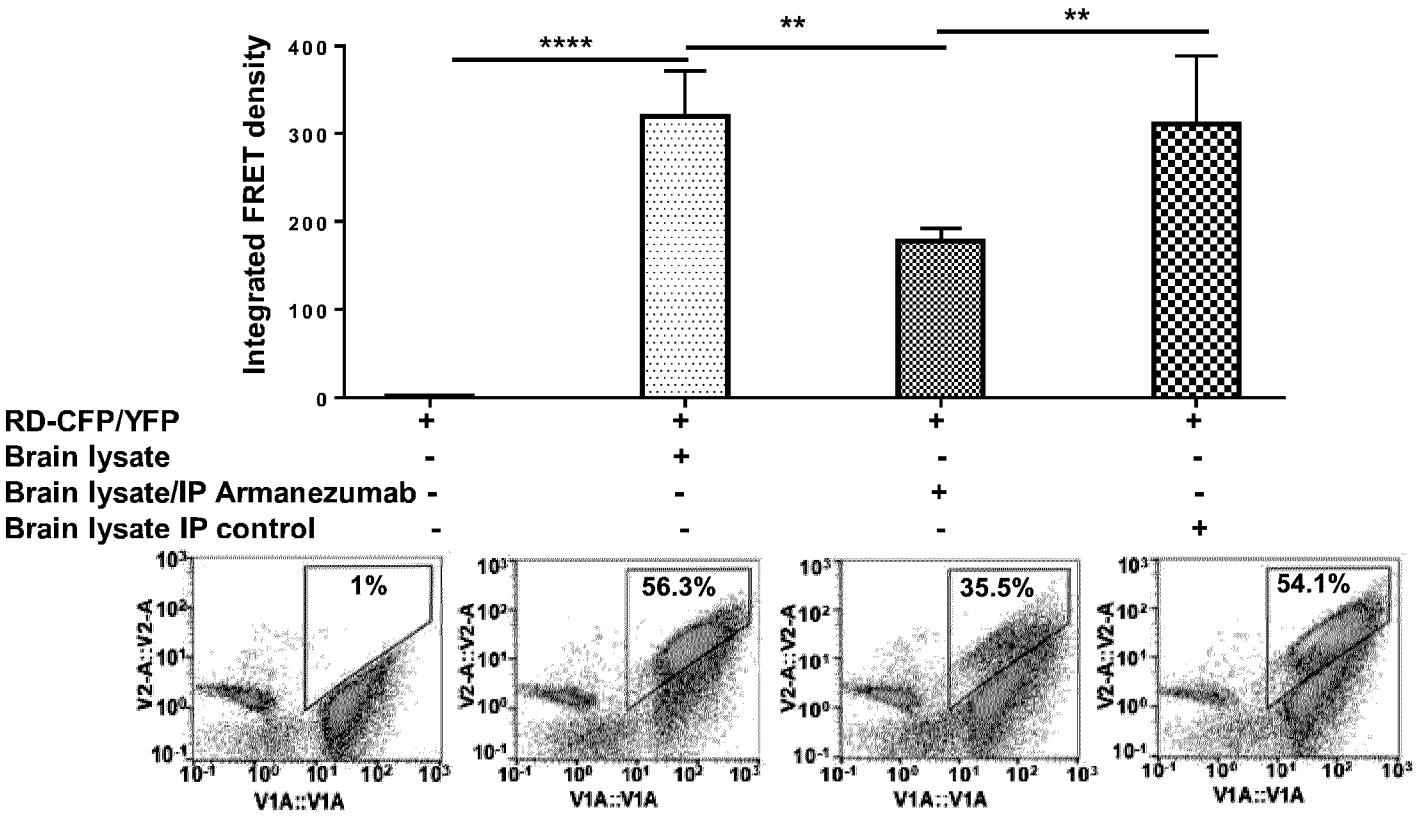


FIG. 21B



Date Reçue/Date Received 2021-03-24

FIG. 22

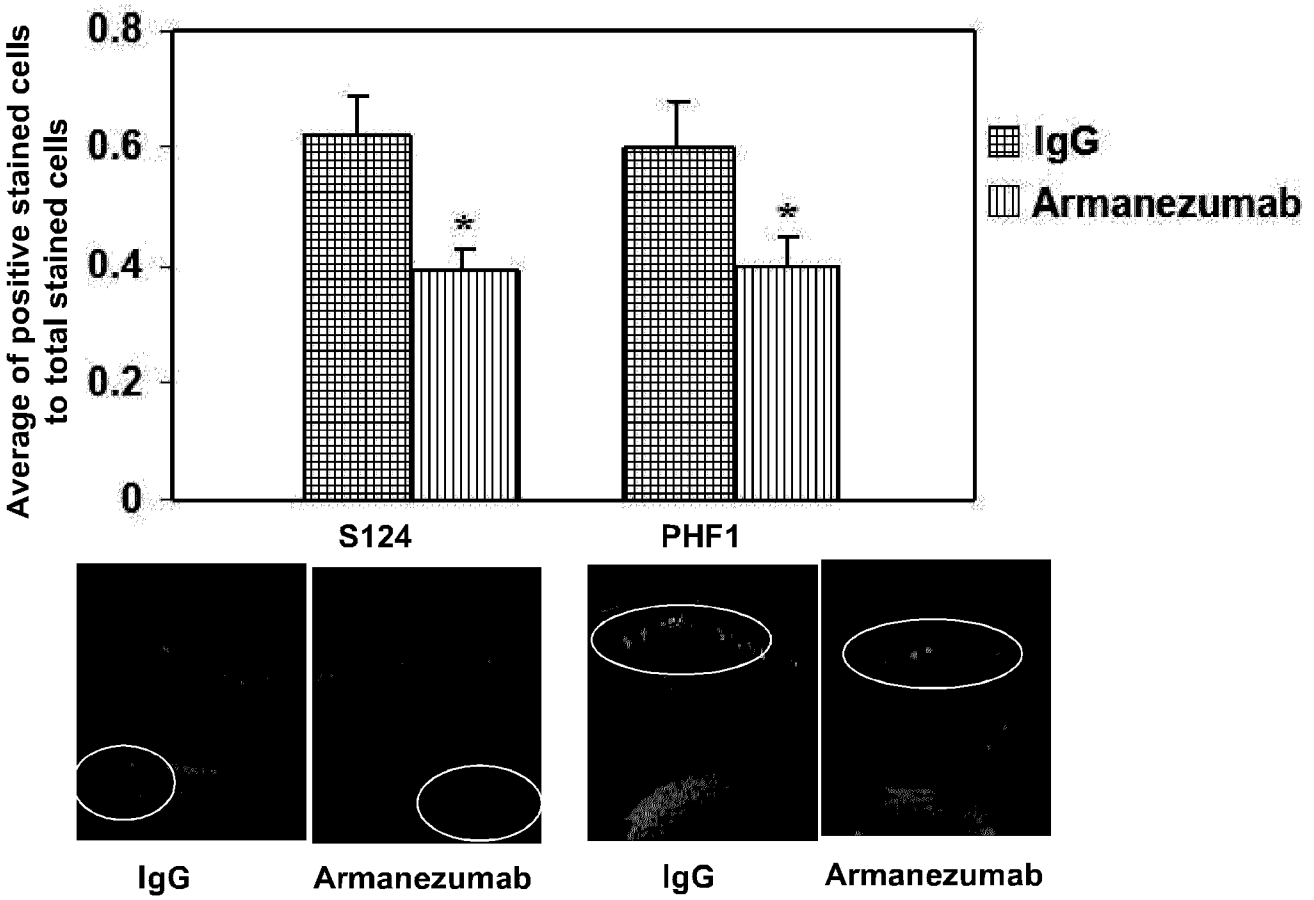
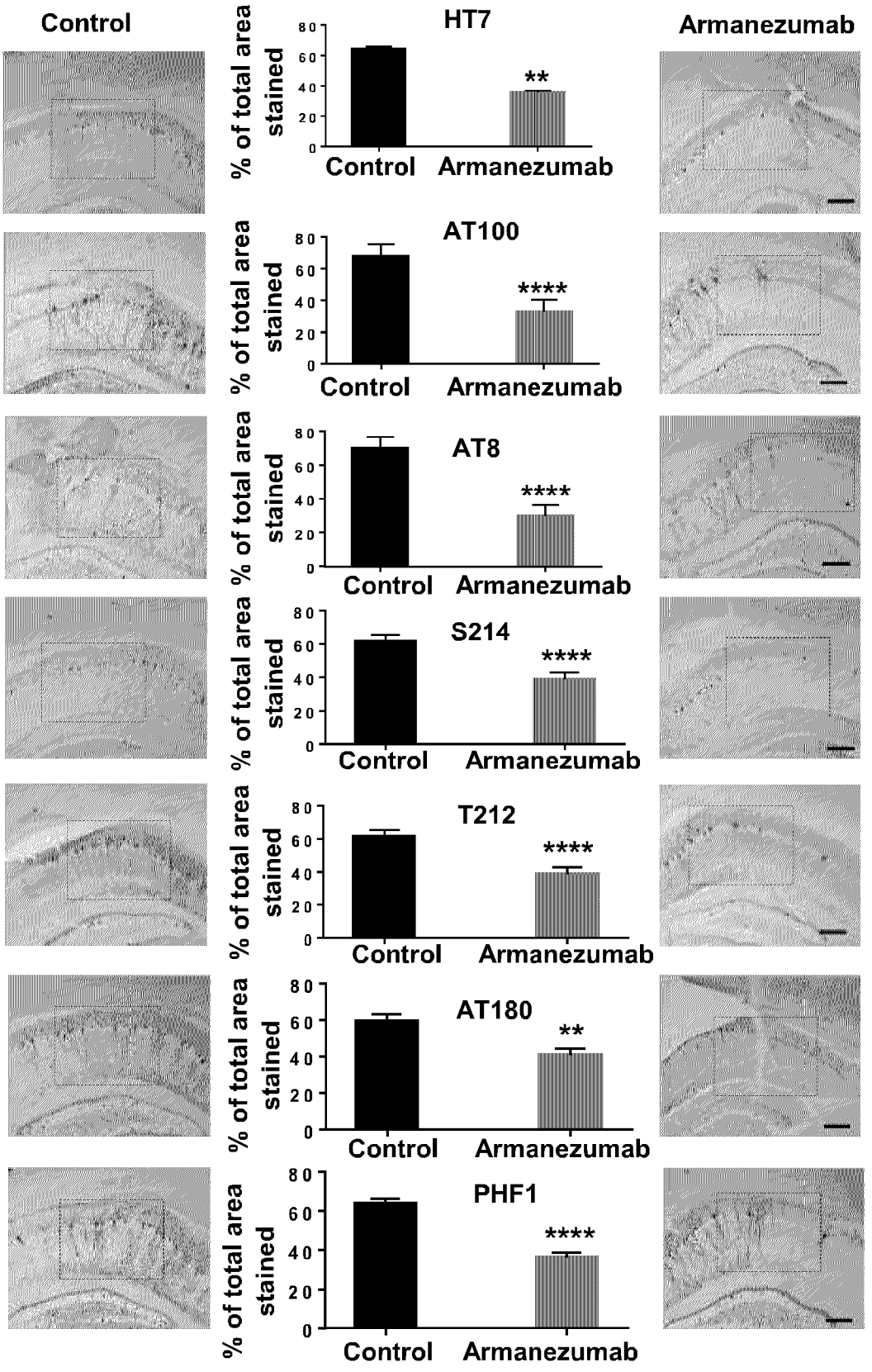

Average of positive stained cells to total stained cells
0.8
0.6
0.4
0.2
0
*
*
S124
PHF1
IgG
Armanezumab
IgG
Armanezumab
IgG
Armanezumab

FIG. 23



Date Reçue/Date Received 2021-03-24

FIG. 24A

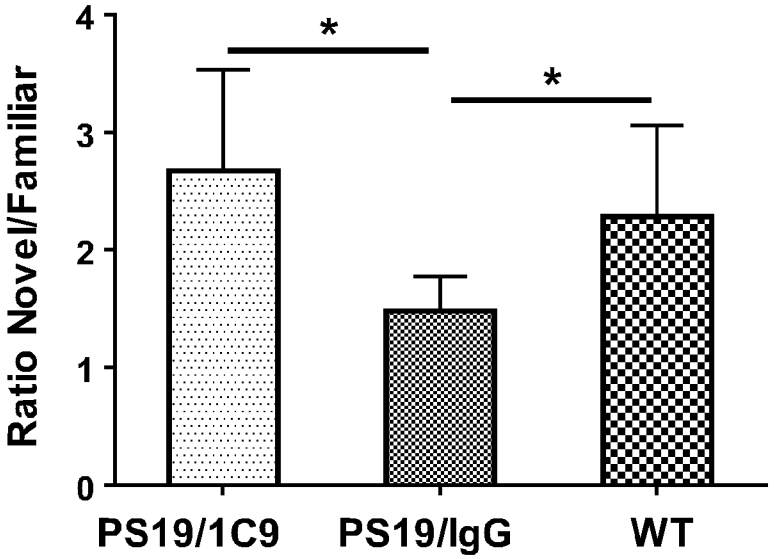
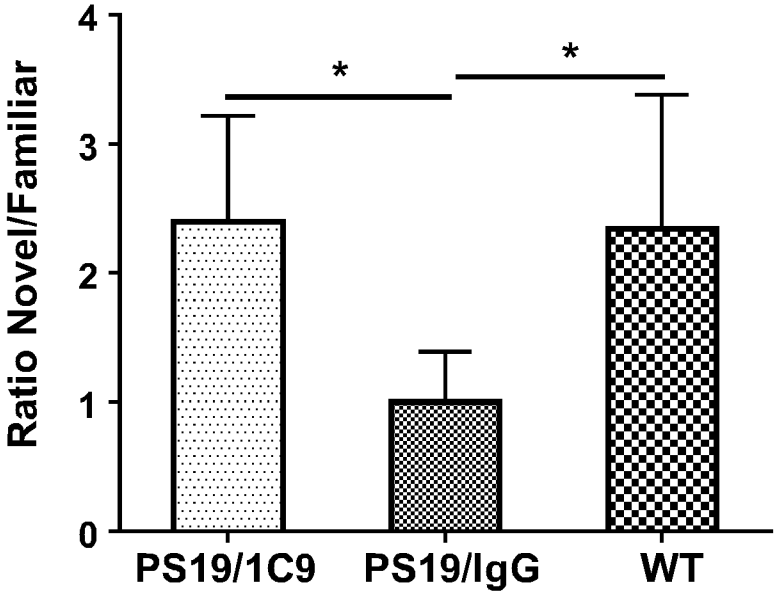
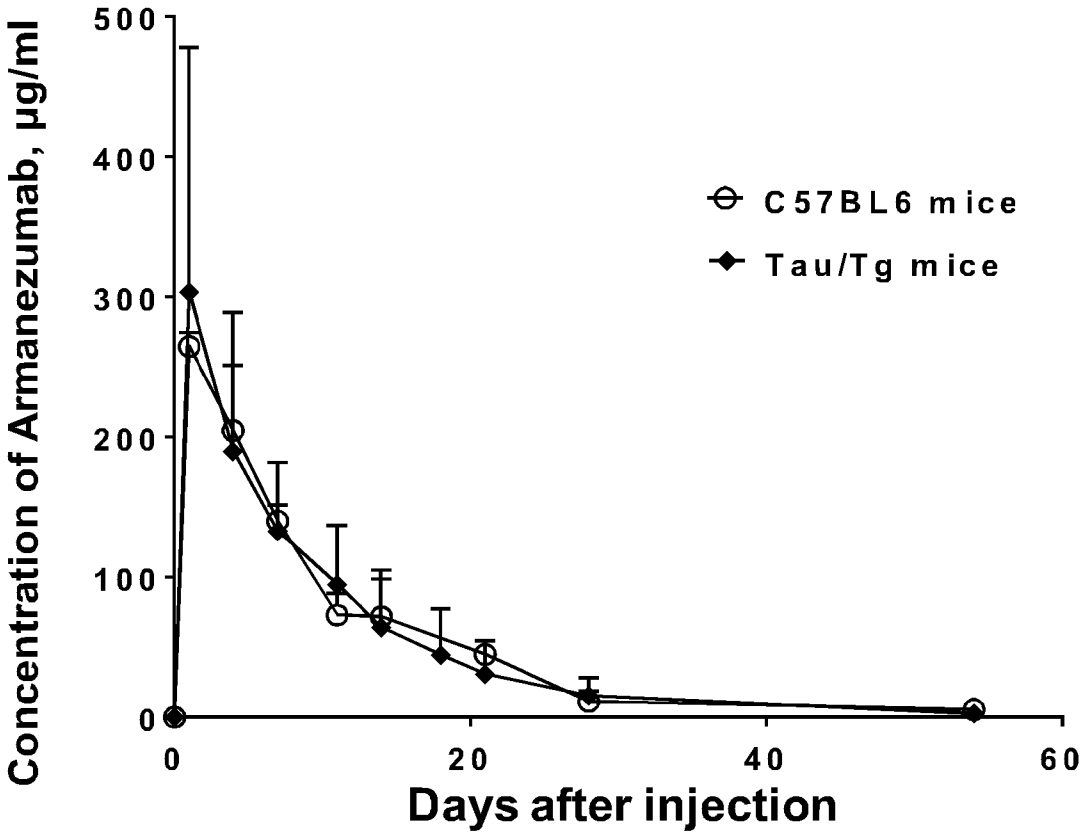


FIG. 24B



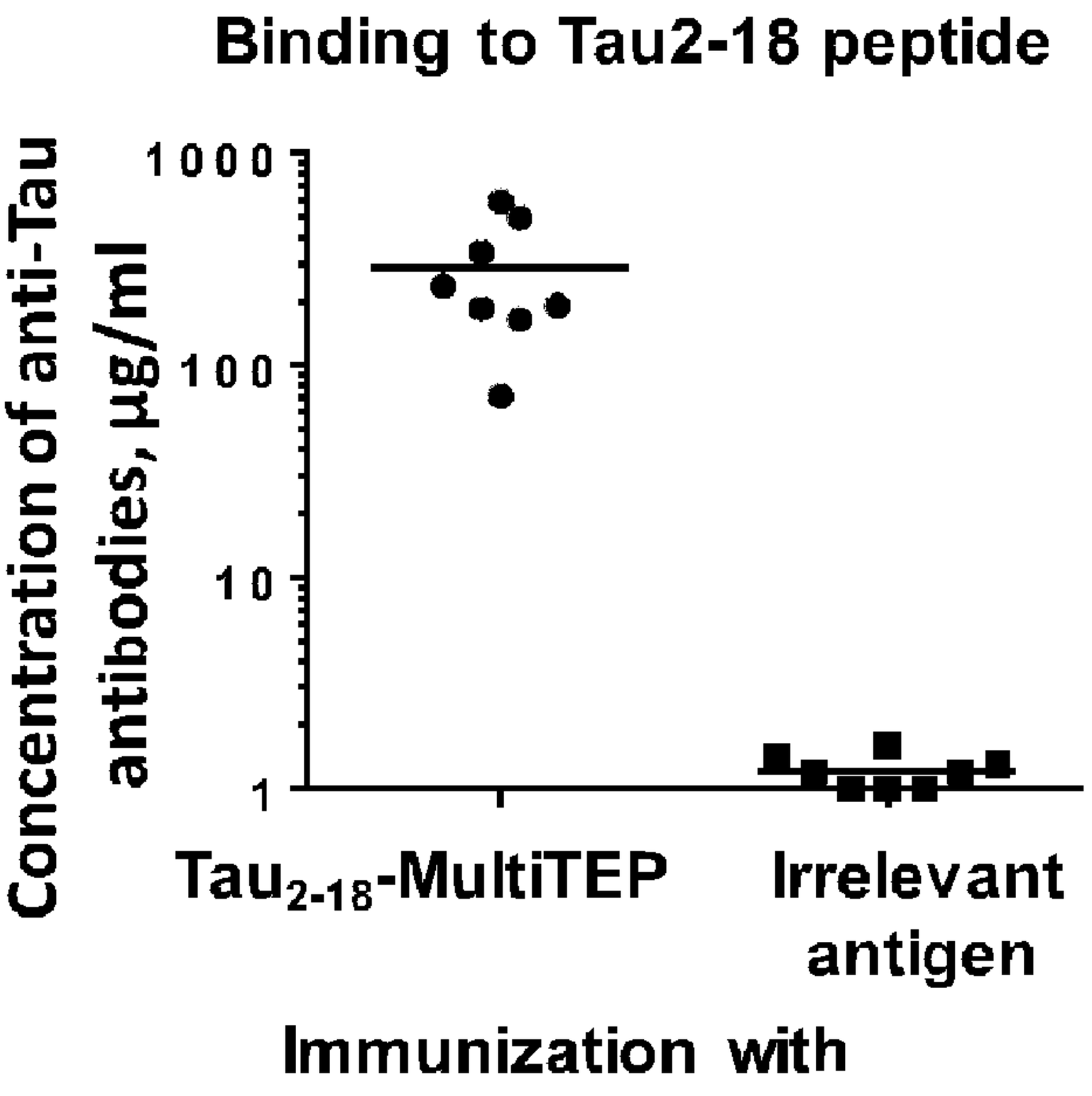
Date Reçue/Date Received 2021-03-24

FIG. 25



Mouse Type	Kel (1/day)	T-1/2 (days)	Tmax (days)	Cmax (µg/mL)	AUC 0-t (day-µg/mL)	AUC 0-inf	AUC0-inf %extrap	Vd
Tau/Tg	0.0721	9.610	1.00	303.6	2802.7	2840.1	1.32	2.9291
C57BL6	0.0753	9.203	1.00	264.8	2876.0	2951.7	2.56	2.6990

Date Reçue/Date Received 2021-03-24


Binding to Tau2-18 peptide
Concentration of anti-Tau antibodies, µg/ml
1000
100
10
1
Tau2-18-MultiTEP
Irrelevant antigen
Immunization with