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(54) **Title:** IMPROVED PROTOFIBRIL SELECTIVE ANTIBODIES AND THE USE THEREOF

(57) **Abstract:** The present invention pertains to the prevention, treatment and diagnosis of neurodegenerative diseases, in particular Alzheimer's disease, and other similar disease. More specifically to high affinity antibodies selective for amyloid beta protein (Aβ) in its protofibril conformation and of IgG class and IgGl or IgG4 subclass or combinations thereof or mutations thereof, retaining high Fc receptor binding and low Cl(CIq) binding, effective in clearance of Aβ protofibrils and with reduce risk of inflammation.

IMPROVED PROTOFIBRIL SELECTIVE ANTIBODIES AND THE USE THEREOF

5 **FIELD OF INVENTION**

This invention pertains to the prevention, treatment and diagnosis of neurodegenerative diseases, in particular Alzheimer's disease, and other similar disease. More precisely, to high affinity 10^{-7} M, preferably 10^{-8} M, even less than10^{-9}M or less than 10^{-10} M or 10^{-11} M antibodies, selective for amyloid beta protein (Aβ) in its protofibril conformation and of IgG
10 class and IgG1 or IgG4 subclass or combinations thereof or mutations thereof, retaining high Fc receptor binding and low C1(C1q) binding, effective in clearance of Aβ protofibrils and with reduce risk of inflammation.

BACKGROUND

15 Alzheimer's disease (AD) is a progressive and irreversible neurodegenerative disorder causing cognitive, memory and behavioural impairments. It is the most common cause of dementia in the elderly population affecting roughly 5% of the population above 65 years and 20% above 80 years of age. AD is characterized by an insidious onset and progressive deterioration in multiple cognitive functions. The neuropathology involves both extracellular
20 and intracellular argyrophillic proteineous deposits. The extracellular deposits, referred to as neuritic plaques, mainly consist of amyloid beta protein (Aβ) surrounded by dystrophic neurites (swollen, distorted neuronal processes). Aβ within these extracellular deposits are fibrillar in its character with a β-pleated sheet structure. Aβ in these deposits can be stained with certain dyes, e.g. Congo Red, and display a fibrillar ultra structure. These characteristics,
25 adopted by Aβ in its fibrillar structure in neuritic plaques, are the definition of the generic term amyloid. The classic intracellular AD pathologic lesion is the neurofibrillary tangle (NFT) which consists of filamentous structures called paired helical filaments (PHFs), composed of twisted strands of hyperphosphorylated microtubule-associated protein tau. Frequent neuritic plaques and neurofibrillary tangle deposits in the brain are diagnostic
30 criteria for AD, as carried out post mortem. AD brains also display macroscopic brain atrophy, nerve cell loss, local inflammation (microgliosis and astrocytosis) and often cerebral amyloid angiopathy (CAA) in cerebral vessel walls.

Two forms of Aβ peptides, Aβ40 and Aβ42, are the dominant species in AD neuritic plaques while Aβ40 is the prominent species in cerebrovascular amyloid associated with AD. Enzymatic activities allow Aβ to be continuously formed from a larger protein called the amyloid precursor protein (APP) in both healthy and AD afflicted subjects in all cells of the
5 body. Two major APP processing events through β- and γ-secretase activities enables Aβ production, while a third enzyme called α-secretase, prevents Aβ generation by cleavage inside the Aβ sequence (*Selkoe, 1994*; *Ester 2001;US5604102*). The Aβ42 is a fortytwo amino acid long peptide, i.e. two amino acids longer at the C-terminus, as compared to Aβ40. Aβ42 is more hydrophobic, and does more easily aggregate into larger structures of Aβ
10 peptides (*Jarret 1993)* such as Aβ dimers, Aβ trimers, Aβ tetramers, Aβ oligomers, Aβ protofibrils or Aβ fibrils. Aβ fibrils are hydrophobic and insoluble, while the other structures are all less hydrophobic and soluble. All these higher molecular structures of Aβ peptides are individually defined based on their biophysical and structural appearance e.g. in electron microscopy, and their biochemical characteristics e.g. by analysis with size-exclusion
15 chromatography/western blot. These Aβ peptides, particularly Aβ42, will gradually assemble into a various higher molecular structures of Aβ during the life span. AD, which is a strongly age-dependent disorder, will occur earlier in life if this assembly process occurs more rapidly. This is the core of the "amyloid cascade hypothesis" of AD which claims that APP processing, the Aβ42 levels and their assembly into higher molecular structures is a central
20 cause of AD. All other neuropathology of AD brain and the symptoms of AD such as dementia are somehow caused by Aβ or assembled forms thereof.

Aβ can exist in different lengths i.e. 1-39,1-40,1-42 and 1-43 and fragments sizes i.e. 1-28 and 25-35. Truncations might occur at the N-terminus of the peptide. All these peptides can
25 aggregate and form soluble intermediates and insoluble fibrils, each molecular form having a unique structural conformation and biophysical property. Monomeric Aβ1-42 for example, is a 42 amino acid long soluble and non toxic peptide, that is suggested to be involved in normal synapse functions. Under certain conditions, the Aβ1-42 can aggregate into dimers, trimers, tetramers, pentamers up to 12-mer and higher oligomeric forms, all with its distinct
30 physicochemical property such as molecular size, EM structure and AFM (atomic force microscopy) molecular shape. An example of a higher molecular weight soluble oligomeric Aβ form is the protofibril *(Walsh 1997*), which has an apparent molecular weight >100 kDa and a curvelinear structure of 4-11 nm in diameter and < 200 nm in length. It has recently been demonstrated that soluble oligomeric Aβ peptides such as Aβ protofibrils impair long-

term potentiation (LTP) a measure of synaptic plasticity that is thought to reflect memory formation in the hippocampus (*Walsh 2002*). Furthermore, oligomeric Arctic Aβ peptides display much more profound inhibitory effect than wtAβ on LTP in the brain, likely due to their strong propensity to form Aβ protofibrils (*Klyubin 2003).*

5

There are also other soluble oligomeric forms described in the literature that are distinctly different from protofibrils. One such oligomeric form is ADDL (Amyloid Derived Diffusible Ligand) *(Lambert 1998).* AFM analysis of ADDL revealed predominantly small globular species of 4.7-6.2 nm along the z-axis with molecular weights of 17-42 kDa *(Stine 1996).*

10 Another form is called ASPD(Amyloidspheroids) (*Hoshi 2003*). ASPD are spherical oligomers of Aβ1-40. Toxicity studies showed that spherical ASPD >10 nm were more toxic than lower molecular forms (*Hoshi* 2003). This idea has gained support from recent discovery of the Arctic (E693) APP mutation, which causes early-onset AD (*US 2002/0162129 A1; Nilsberth et al., 2001*). The mutation is located inside the Aβ peptide sequence. Mutation

15 carriers will thereby generate variants of Aβ peptides e.g. Arctic Aβ40 and Arctic Aβ42. Both Arctic Aβ40 and Arctic Aβ42 will much more easily assemble into higher molecular structures i.e. protofibrils. Thus, the pathogenic mechanism of the Arctic mutation suggests that the soluble higher molecular protofibrils are causing AD and contains a specific unique epitope i.e. “the AD disease epitope”.

20

In the Alzheimer’s disease (AD) brain, extracellular amyloid plaques are typically found in parenchyma and vessel walls. The plaques are composed of amyloid (Aβ38-43 amino acid long hydrophobic and self-aggregating peptides, which gradually polymerize prior to plaque deposition. The soluble Aβ oligomeric species have been proposed to be better

25 disease correlates than the amyloid plaques themselves (*McLean et al., 1999; Näslund et al., 2000*). Among these pre-fibrillar intermediate Aß species, oligomeric forms have been shown to elicit adverse biological effects both *in vitro* and *in vivo* (*Walsh et al., 2002*) and may thus play a central role in disease pathogenesis. Several oligomeric Aβ species of various molecular sizes are known. Importantly, the conformation of monomeric,

30 oligomeric and fibrillar forms of Aβ are different and can be targeted by conformational selective antibodies. The identity of the main Aβ pathogen is unclear, although some evidence suggests high-molecular weight Aβ oligomers to be especially neurotoxic (*Hoshi et al., 2003*).

Pathogenic mutations in the *amyloid precursor protein (APP)* gene, causing early onset AD have been described. One of them, the *Swedish APP* mutation (*Mullan et al., 1992*), causes increased levels of Aβ. The other the *Arctic APP* mutation (*E693G*) located within the Aβ domain, was found to enhance the formation of protofibrils, large Aβ oligomers, suggesting
5 these Aβ intermediates to be particularly pathogenic ((*US 2002/0162129 A1; Nilsberth et al., 2001*). The identification of the *Arctic APP* mutation and the elucidation of toxic effects for Aβ protofibrils have increased the focus on Aβ oligomers in AD pathogenesis.

Active immunization as a therapeutic strategy for Alzheimer's disease was first reported by
10 (*Schenk et al. 1999*). The target for the immunization strategy was the fibrillar form of Aβ found in Alzheimer plaques. A recent clinical phase I / II trial of active Aβ vaccination using fibrillized Aβ as a vaccine (AN-1792) had to be halted because of the development of meningoencephalitis in a small number of patients (*Bayer et al., 2005*). The side effects seen in this study were likely caused by anti-Aβ antibodies reacting against fibrillar
15 amyloid in vessel walls. The fibrillary amyloid in CAA is in close proximity to the blood-brain-barrier (BBB) and the antigen-antibody reaction could thus generate damage to the BBB leading to infiltration of T-lymphocytes into the CNS (*Pfeifer et al., 2002; Racke et al., 2005*). Moreover, only a minority of the participating patients displayed an immune response to the Aβ vaccine. Although the study ended prematurely, it seems to imply that
20 active Aβ immunization may be beneficial only to a subset of AD patients.

Monoclonal antibodies selective for human Aβ protofibrils have been described (*US 2002/0162129 A1*). The method to generate highly pure and stable human Aß protofibrils, involves the use synthetic Aβ42 peptides with the Arctic mutation (Glu22Gly). The
25 mutation facilities immunization and hybridoma screening for Aß protofibril selective antibodies. Importantly, these antibodies bind both wild-type Aβ protofibrils and Aβ-Arc protofibrils (PCT/SE 2005/000993).

Antibodies that are selective towards other conformations of Aβ such as Aβ fibrils
30 (O*'Nuallain 2002),* micellar Aβ (*Kayed 2003),* ADDL (*Lambert 2001)*, have been described. However, non of these are Aβ protofibril selective.

SUMMARY OF THE INVENTION

The present invention pertains to improved antibodies i.e. high affinity (less than 10^{-7} M) Aβ protofibril selective antibodies of class IgG and subclass IgG1 or IgG4 or combination thereof or mutations thereof, with reduced risk of inflammation, for improved prevention, treatment and diagnosis of Alzheimer's disease, Downs syndrome or other neurodegenerative disorders. Said antibodies have been developed by classical hybridoma techniques and antibody engineering.

The invention discloses the consensus amino acid sequence of the CDR1-3 regions on the VL and VH chains from antibodies that selectively bind oligomeric Aβ forms, i.e. Aβ protofibrils constituting the "Alzheimer disease epitope", combined with modifications of the Fc region to reduce complement factor C1q binding, reducing the risk for complement activation and inflammation.

The constant region of an antibody has many important functions notably binding Fc-receptors and complement factor C1q. The latter function has been inactivated to avoid inflammatory reactions.

In summary, this type of high affinity protofibril selective antibodies have the following distinct advantages as compared to other known immunotherapeutic treatment modalities:

1) targets disease causing Aβ protofibrils with high affinity
2) reduces the risk for inflammatory side-effects i.e. meningioencephalitis, by low or no binding to complement factor C1q
3) high affinity antibody reduces the clinical dose needed for an effective treatment
4) provides a modality of accurate dosing
5) less binding to Aβ fibrils in the blood vessel wall i.e. CAA, reducing the risk for inflammatory side-effects.
6) Less antibody is bound in the periphery, thus more will cross the blood brain barrier and be available for binding and elimination of Aβ oligomeric forms in the brain.

One aspect of the invention is the discovery of the antibody consensus amino acid sequence of the CDR regions that bind human wild type Aβ protofibrils (Example 1). This

discovery defines the binding sites (CDR regions) that confer high affinity and high selectivity for wild-type human Aβ protofibrils for use as therapeutics or diagnostics.
The basic structure of an immunoglobulin (IgG) molecule comprises two identical light chains and two identical heavy chains linked together by disulphide bridges (Figure 1). The light chain, which is either lambda or kappa, has a variable region (VL) and a constant region (CL) of approximately 110 amino acid residues each. The heavy chain has a variable region (VH) of about 110 amino acid residues, but a much larger constant region (CH) of 300-400 amino acid residues, comprising CHγ1, CHγ 2 and CHγ3 regions or domains.
The *constant* region (Fc) activates the complement system and binds to a Fc receptor on macrophages, microglia and neutrophiles, which ingest and destroys infecting microorganisms or foreign/non-self antigens. This function is particular important since it is part of the therapeutic principle of the antibody, i.e. Fc receptor mediated microglial phagocytosis and clearance of Aβ protofibrils. Other antibody mediated clearance mechanisms are also operating, i.e. anti-aggregation properties of Aβ antibodies and clearance of Aβ protofibrils in the periphery, according to the sink hypothesis.
The *variable* region of the heavy and light chains contains 3 hyper variable regions called *complementary determining regions* or CDRs. The CDR regions are short stretches of about 13-23 amino acid long, located in the VL and VH regions. The six CDRs regions on one "arm" of the antibody forms the "pocket" that binds the antigen. Figure 1 shows the basic structure of an IgG immunoglobulin and its subdomains.

Another aspect of the invention pertains to protofibril selective antibodies of high affinity. Affinities in the range of 10^{-7} M preferably 10^{-8} M, even less than 10^{-9} M, less than 10^{-10} M, or less than 10^{-11} M for protofibrils are described (Example 2). These antibodies have the advantage that they can be administered at lower doses compared to antibodies with affinities in the 10^{-6} M range. This has significant clinical advantage in that these high affinity antibodies, which are administered by injection, can be given subcutaneously since only a low amount of the antibody is needed to achieve efficacy. Administration modalities are not limited to subcutaneous injections. Furthermore, the lower doses needed for efficacy will reduce cost of goods for production of the antibody.
Another aspect of the invention is that the antibodies are of IgG class, suitable for therapeutic use since it can pass over the blood brain barrier. Clearance of Aβ protofibrils in the brain parenchyma is achieved by Fc receptor mediated phagocytosis by microglia

cells. Other anti-Aß clearance mechanisms are likely to operate as well. This clearance of soluble Aβ protofibrils is a central mechanism of the treatment. Aβ protofibrils are considered highly neurotoxic, initiating and driving the disease process. Clearance of Aβ protofibrils in the brain is of significant clinical value. In addition to clearance of Aß
5 protofibrils, other Aβ oligomeric forms including Aß fibrils, will be reduced indirectly via removal of Aβ protofibrils since different Aβ aggregated forms, i.e. dimers, trimers, tetramers and higher oligomeric forms including protofibrils and fibrils, are in equilibrium. Example of reduction of plaques, which contain Aβ fibrils, is shown in a Alzheimer transgenic mouse model (APPswe) after 72 hour treatment with a high affinity protofibril
10 selective antibody (mAb 158) (Example 3). Hence, clearance of Aβ protofibrils by said antibody will also have the advantage to indirectly reduce other Aβ aggregated or oligomeric forms.

Yet another aspect of the invention is a high affinity human Aß protofibril selective
15 antibody of subclass IgG1, which has a high affinity for human FcγRI receptors present on microglial cells in the brain. A high affinity antibody will lead to efficient clearance of Aß protofibrils which will be of significant therapeutic value. Hence, the antibodies will exhibit clearance of Aβ protofibrils, both in CNS and periphery as compared to other immunotherapeutic strategies such as active vaccination or monoclonal antibody
20 treatments with other monoclonal antibodies of IgG1 subclass targeting other Aß forms. Importantly, the treatment will be efficient early in the disease process when toxic soluble Aβ spices such as Aβ protofibrils are present at elevated levels but also later in the disease process. Elevated levels of oligomeric Aβ forms have been described in a transgenic mouse model exhibiting the Swedish and Arctic mutations APP swearc (*Lord A. et al. 2006*).
25 Yet another aspect of the invention is that the high affinity Aß protofibril selective antibodies can reduce or inhibit Aβ aggregation thereby reducing levels of soluble oligomeric Aβ forms in the brain.
Yet, another aspect of the invention is that the high affinity Aß protofibril selective antibodies can bind oligomeric forms of Aβ, i.e. Aβ protofibrils outside CNS as well,
30 thereby shifting the equilibrium of said Aβ forms over the blood brain barrier in such a way as to lower CNS levels of said Aβ forms (drainage).
As discussed above, the Elan clinical study using an Aβ vaccine (AN-1792) selective for Aβ fibrils to treat Alzheimer patients resulted in a side-effect, i.e. meningioencephalitis, in 6% of the cases. The strategy to target Aβ fibrils, that are the core of amyloid plaques

present in the brain parenchyma but importantly also in the blood vessel walls, resulted in severe side-effects. The side-effects was most likely caused by the binding of the antibodies to CAA (Cerebral Amyloid Angiopathy) in the blood vessel walls of the brain, starting an inflammatory process. This significant clinical problem is avoided by the
5 improved high affinity protofibril selective antibodies with reduced complement activation activity. These antibodies will retain high clearance efficacy of Aß protofibrils reduced risk of side-effects, i.e. meningioencephalitis.
Another aspect of the invention is that the high affinity protofibril selective antibodies have low Aβ fibril binding (See example 2), reducing the risk for side effects, by less binding to
10 Aß fibrils present in CAA.
Yet another aspect of the invention is that the high affinity Aß protofibril selective IgG antibodies are engineered to reduce complement factor C1q binding to the CH2 domain of IgG1 and reduce complement activation and risk of inflammation. This modification can be done in several different ways. One way is to make a chimeric antibody where the
15 CHγ2 domain of the IgG1 constant region has been deleted and exchanged for the corresponding domain from IgG4 or part of the domain that confers C1q binding. It is well established that IgG4 does not bind C1q and hence does not activate the complement cascade. To achieve this the constant region of the heavy chain (CH) is engineered is such a way as to combine the high affinity Fc-receptor domain (CHγ3) on IgG1 with the IgG4
20 domain (CHγ2) which has no binding for the complement factor C1q. This new antibody containing the chimeric constant heavy chain (IgG1:CHγ1, CHγ2:IgG4, CHγ3:IgG1) will have the important properties of both efficient clearance of Aß protofibrils through Fc-receptor mediated phagocytosis and reduced risk for side-effects, i.e inflammation such as meningioencephalitis.
25 Yet another way of reducing the risk of inflammation is to alter the oligosaccharides structure of the antibody which will reduce complement factor C1q binding and complement activation. 30 different structures of the complex biantennary oligosaccharides at Asn-297 in human IgG1 has been described. The absence of CH2 associated carbohydrates is believed to cause a conformational change in the "hinge" region of the
30 antibody, reducing interaction efficacies with effector molecules and loss of complement activation function and C1q binding.
The modification of a high affinity human Aß protofibril selective antibody by site-directed mutagenesis of Asn-297 to any other amino acid will generate an antibody of retained Fc-receptor binding with less C1q binding and hence reduced risk of inflammation in particular

at the blood brain barrier. An alternative to modify the glycosylation on the antibody is to expressing the antibody in a cell type where the enzyme N-acteylglucosaminyl-transferase I has been inactivated. This will yield an antibody with altered carbohydrate structure at Asn-297. A structure of $Man_5GlcNAc_2$, but not limited to this structure, is formed. This
5 carbohydrate modification will reduce complement factor C1q binding and inhibit inflammation (*Wright at al. 1998*). Alternatively, glycosylated protofibril selective antibodies can be achieved by culturing cells expressing antibodies in the presence of tunicamycin, which inhibits glycosylation. These antibodies will have altered complement activating activity as well as altered Fc-receptor function (*Leatherbarrow el al. 1985*). Screening of
10 clones expressing antibodies with low complement activation and high Fc-receptor binding will generate protofibril selective antibodies that exhibit high Fc-mediated clearance of Aß protofibrils and low C1q binding.

Yet another aspect of the invention is a high affinity human Aß protofibril selective antibody,
15 of IgG1 subclass, where the complement factor C1q binding site has been modified, i.e. Pro331>Ser331 (*Xu et al. 1994*), in such a way as to reduce or inhibit binding of complement factor C1q, for the treatment or prevention of AD. The proline residue at position 331 in human IgG1 can also be changed to a threonine or glycine or any other polar amino acid. This modification can be achieved by standard molecular biology techniques such as site-directed
20 mutagenesis or DNA deletions.

Yet another aspect of the invention is the use of high affinity human Aß protofibril selective IgG antibodies to specifically determine protofibril levels in human tissues, in particular in cerebrospinal fluid, blood, urine or saliva as a diagnostic tool or biomarker for Alzheimer's
25 disease. Levels of human Aß protofibrils in CSF or blood are likely to be different as compared to a matched elderly control group not having Alzheimer's disease. A person who is developing Alzheimer's disease is likely to have increased levels of Aß protofibril levels in CSF or blood. Hence, by determination of Aß protofibril levels in CSF or blood an early diagnosis of the disease can be made. This is possible to achieve with the new high affinity
30 Aß protofibril selective antibodies in combination with a sandwich ELISA method (Example 2A), where Aß protofibrils have been determined down to10 pM level. Interference of other Aß forms such as Aß fibrils, Aß monomers and Aß fragments (1-16; 17-40) in the assay, is 10% or less.

The invention further pertains to the use of a high affinity protofibril specific antibodies for determinations of Aβ protofibrils in human and animal tissues, for example, cerebrospinal fluid, blood, serum, urine and brain tissue but not limited to these tissues, providing for a possible diagnostic method for Alzheimer's disease. Suitable methods for assaying Aβ
5 protofibrils in these tissues as well as in cell cultures using an anti-Aβ protofibril antibody are immunoassays such as ELISA, RIA, Western blotting or dot blotting. The method would be suitable to follow treatment efficacy (protofibril reduction) in clinical trials and suitable as a diagnostic test for Alzheimer's disease or Down's syndrome.
Since Aß protofibrils levels are very low in CSF and blood, a high affinity Aß protofibril
10 selective antibody is needed in a diagnostic test based on an ELISA method, to be able to measure low levels of Aß protofibrils. Other supersensitive methods such as *proximity ligation* (Example 4) *(Gullberg 2004)* or similar amplification systems or Biacore or similar techniques, can be used to increase sensitivity. The proximity ligation technique is based on the discovery that different antibodies, raised against different epitopes on an analyte (in this
15 case a protein), may bind near each other on said analyte. If said different antibodies are conjugated to oligonucleotides, the distance between said oligonucleotides will be short enough for a connector oligonucleotide, with the aid of ligation components, to form a bridge between the oligonucleotides. Amplification components are also added, upon which RT-PCR may be performed. By this principle, an amplifiable DNA sequence, reflecting the identity
20 and amount of the target protein, is generated. This technique makes it possible to obtain an enhanced signal response and thus to detect lower concentrations of analyte.

The present inventors surprisingly discovered that a modified proximity ligation technique may also be used with their Aβ protofibril-specific antibodies, to detect low concentrations of
25 larger Aβ peptide structures, i.e. Aβ protofibrils but not Aβ monomers. They discovered that the Aβ peptides, in the protofibril conformation, exhibits a structure (repetitive units) that makes it possible for two antibodies, according to the present invention, to bind sufficienttly near each other on the protofibril. If said antibodies are conjugated to oligonucleotides, said oligonucleotides may be bridged using a connector oligonucleotide. PCR is performed using
30 amplification components. By this principle, an amplifiable DNA sequence, reflecting the identity and amount of the target protofibril, is generated (see Fig 4A).

Proximity ligation or a version of the technique called "rolling circle", is a highly sensitive technique and particularly well suited for detection of polymeric structures with repeated

sequences, such as Aβ protofibrils to be used for diagnosis of Alzheimer's disease and other neurodegenerative disorders.

The invention further pertains to the use of high affinity protofibril specific antibodies in imaging for detection, localization and quantitation of Aβ protofibrils in human and animal tissues. The antibody could be label with a radioactive ligand such as I^{131}, C^{14}, H^3 or Gallium^{68}, but not limited to these radioisotopes, for detection purposes. The method will be suitable as a diagnostic tool for Alzheimer's disease or Down's syndrome.

Yet another aspect of the invention is to make the antibody spices specific for use in veterinary medicine. The diagnostic methods outlined are also suitable for veterinary use.

Another aspect of the invention is the humanization of said antibodies to avoid side-effect, i.e. to avoid an immunoresponse against said antibodies in humans when used as a therapeutic or diagnostic agent.

Yet another aspect is a formulation of the antibody in a physiological buffer, for example PBS but not limited to PBS, suitable for administration to humans and animals. The antibody product can be freeze dried for better stability. The freeze dried formulation can contain an excipient such as manitol but not limited to manitol to stabilize the product after freeze drying.

The antibody product can contain an antibacterial agent.

The antibodies or fragments according to the inventions may exhibit amino acid deletions, substitutions and insertions within said CDR regions and/or its framework. Inserted or substituted amino acids may also be amino acid derivatives, with the proviso that the affinity and specificity of the antibody is still intact.

EXAMPLES

The following examples are provided for illustration and are not intended to limit the invention to these specific examples.

Example 1.

Human wild-type Aß protofibril selective monoclonal antibodies were cloned and sequenced. The amino acid sequence of the variable heavy chain region (VH) and the variable light chain region (VL) are shown in Table 1. The positions of the CDR regions 1-3 are underlined and shown as well in Table 2 and 3. The amino acid sequences of the CDR regions form the structural basis for binding human wild-type Aß protofibrils constituting the "Alzheimer disease epitope".

The amino acid sequence of the CDR regions 1-3 of the VL and VH chains for a high affinity protofibril specific antibody BA9 /158 is shown in Table 1, 2 and 3.

Sequencing data of other protofibril selective antibodies (BA2, BA3, BA4 and BA7) provide alternative amino acids sequences of the CDR regions but not limited to these. The combined amino acid sequences of the CDR1-3 regions of the VH and VL chains create the molecular "pocket" which binds human Aß wild-type protofibrils with high affinity and specificity. This "pocket" forms the structural basis of the "Alzheimer's disease epitope". Variations in the CDR amino acid sequence length are observed in both the VH chain and the VL is compatible binding to human Aß protofibrils (Table 2 and 3). A shorter CDR region provides a more restricted three dimensional structure of the binding pocket of the antibody, whereas a longer is more flexible.

We claim the CDR sequences as shown in Tables 1, 2 and 3 as well as amino acid sequences in the " mouse framework" regions of the VH and VL chains, i.e. outside the CDR regions as well as the human VL and VH framework regions for protofibril specific antibodies as shown in Table 4 and 5, but not limited to those.

The amino acid sequence of the framework region of VL and VH regions 1-3 of the VL and VH chains from a high affinity protofibril specific antibody BA9/158 is shown in Table 4 and 5.

Other amino acid substitution in the CDR regions than what is shown in Table 1, 2 and 3 are compatible with high affinity and high specificity binding to human wild-type Aß protofibrils. Where a polar amino acid is present in a particular position in a CDR region that particular amino acid can be substituted by another polar amino acid, with retained or improved high affinity and specificity binding to Aß protofibrils. Likewise, if a non-polar or negatively or positively charged amino acids is present at a certain position, that amino acid can be substituted for by a similar amino acid from the same group.

Also, a particular amino acid or amino acids are exchanged in any position in the CDR regions by functional equivalents that confers a similar function and structure to the antibody.

Example 2. Characterization of an high-affinity human Aß wild–type profibril selective monoclonal antibody by ELISA

Example 2 shows a high affinity protofibril selective antibody that cross-reacts a 200-1000-fold less with Aβ monomers and less than 40-fold with Aβ fibrils, as measured by a sandwich ELISA (Fig.2A). From competitive ELISA experiments, the antibody has a strong affinity for human Aß42 wild-type protofibrils, but only very weak affinity for the N-terminal part of the Aß peptide and Aß monomers. No binding was observed to the C-terminal fragment of Aß (Fig.2B). Furthermore, the antibody does not cross-react with other types of amyloids, like medin or transthyretin. Furthermore the antibody does not recognize human APP, the abundant precursor of Aβ.

In Figure 2A a sandwich ELISA is shown. Antibody 158 was coated in the wells and different Aß forms subsequently added to the well in increasing concentrations. Measurement of bound Aß forms was made by adding biotinylated mAb 158 and HRP labelled Streptavidine. Colour development was measured according to the procedure recommended by the manufacturer.

In Figure 2B a competitive ELISA is shown. An ELISA plate was coated with human Aß protofibrils. Antibody 158 was subsequently incubated with increasing amounts of different Aß forms (competition). The incubation mix was added to the microtiter plate wells and free antibody was allowed to bind to immobilized protofibrils in the wells. Bound 158 antibody was measured by a second antibody using standard procedures.

Example 3

The efficacy of high affinity Aβ protofibril selective antibody was determined in an Alzheimer transgenic mouse model (APPswe) by an acute intracranial injection. Transgenic mice used for efficacy evaluation express human APP, with the Swedish mutation (APP_{Swe}). In this paradigm, antibodies are injected directly into plaque-rich regions of the brain parenchyma and effects on neuropathology are assessed after 72 hours (*Wilcock et al., 2003*). Other studies have

shown that the direct application of anti-Aβ antibodies results in a rapid clearance of amyloid deposits *in vivo* (*Bacskai et al., 2001; Brendza et al., 2005*). The injection of high affinity Aβ protofibril selective antibody leads to a significant plaque reduction in the APP_{Swe} mouse model (Figure 3).

5

In Figure 3 the therapeutic efficacy of a high affinity protofibril selective antibody in transgenic mouse model (APPswe) was tested. A: A 14 months old APPSwe transgenic mouse was intracranially injected with PBS and B: high affinity protofibril selective antibody (158) at 1 μg/μl and examined 72 hours following injection. Marked clearance of Aβ burden is
10 noticeable in the subiculum close to the injection site (B; arrow) as compared to the control side (A; arrow).

Example 4

Proximity ligation in combination with high affinity protofibril selective antibody for
15 measurement of Aβ protofibrils. Human wild-type Aβ protofibrils were detected down to 10 pM-range whereas the Aß monomer preparation were not detected at all. The combination of the hypersensitive proximity ligation method and a high affinity antibody is particularly advantageous since it provides a system to determine only oligomeric forms of the analyte, which is particularly suitable when diagnosing Alzheimer's disease and other
20 protein "aggregation" diseases such as prion disease, Creutzfelt-Jacob, amyloidosis and Parkinson's disease.

In Figure 4 human Aß protofibrils are measured at pM levels by the proximity ligation technique. Proximity ligation assay: Method description (from Gullberg et al., 2004): Step
25 1, incubation of sample with proximity probe pair (≈1 h); step 2, addition of all components required for ligation and detection by quantitative PCR (≈5 min ligation time). A high affinity protofibril selective monoclonal antibody was used in the assay; step 3, quantitative PCR (≈2 h). Synthetic Aβ monomer and Aβ protofibril preparations were diluted and tested for their reactivity in proximity ligation assay described above.

30

Example 5

mAb 158 does not recognize a generic amyloid epitope.

Previously reported Aβ conformation dependent antibodies have been shown to bind oligomers and fibrils of other amyloidogenic proteins, suggesting a common epitope present

on all amyloid aggregates. Due to technical difficulties in generating protofibrils from other amyloidogenic proteins than Aβ, mAb158 was instead tested against different amyloid fibrils. The dot blot assay was used for these experiments since inhibition ELISA, where the antibody-antigen reactions take place in solution, is not suitable for insoluble antigens like
5 fibrils. The dot blot assay is however not suitable for evaluation of antibody specificity for various Aβ forms, i.e. for measuring differences in selectivity for profibrils and fibrils. Fibrils of medin, islet amyloid polypeptide (IAPP) and α-synuclein were immobilized on a nitrocellulose membrane to maintain their native conformations. mAb158 did not exhibit reactivity with any amyloid other the Aβ fibril (Fig 5A**).** The binding of mAb 158 to Aβ fibrils
10 suggests that part of the Aβ protofibril epitope is present also in the Aβ fibril structure. As positive controls the antibodies 6E10 (Aβ), pAb179 (medin), pAbA110 (IAPP) and mAb211 (α-synuclein) were used (Fig 5B). Representative blots from repeated experiments (n=3).

mAb158 does not bind APP
15 Levels of APP and soluble APP fragments commonly exceed the levels of Aβ in biological samples such as CSF and brain homogenate, and therefore an Aβ-antibody's cross-reactivity to APP could inhibit a treatment by binding to APP, resulting in less free antibody for binding and elimination of Aβ protofibrils and/or Aβ oligomers. Also, it could disturb measurements of Aβ protofibrils in biological samples by a sandwich ELISA assay of Aβ. To elucidate
20 whether mAb158 binds to native APP, immunoprecipitation experiments were performed. HEK-cell culture media (mock, APP_{Swe} and $APP_{Arc\text{-}Swe}$) and mouse brain homogenates (non-transgenic, APP_{Swe} and $APP_{Arc\text{-}Swe}$) were immunoprecipitated with mAb158 or 6E10, followed by a denaturing Western blot with 6E10 as detecting antibody (Fig 5C). As seen in Figure 5C, mAb158 did not immunoprecipitate αAPPs from cell culture media or full length
25 APP from mouse brain homogenates, whereas, as expected, 6E10 did. The synthetic Aβ protofibrils used as control were immunoprecipitated equally well by both antibodies (Fig 5C). Representative blots from repeated experiments (n=3).

Example 6
30 *Establishment of an Aβ protofibril specific sandwich ELISA.* To enable measurements of Aβ protofibrils in biological samples a sandwich ELISA with mAb158 as both capturing and detecting antibody was established. This assay measures Aβ protofibrils with a detection limit of 1 pM and with a linear range up to 250 pM (Fig 6A, lines indicate linear regression of the standard curves). Due to uncertainties concerning the size of the Aβ protofibrils used in the

standard curve, the concentration 1 pM is based on the molecular weight of one Aβ monomer (4514 g/mol), Though, since the molecular weight of a protofibril has been estimated to be at least 100 kDa, the limit of detection calculated as molar Aβ protofibrils could be as low as 50 fM. A standard curve of AβArc protofibrils gave a lower signal than wild type Aβ
5 protofibrils, possibly due to differences in Aβ protofibril size (Fig 6A, 6B). Titrated synthetic LMW-Aβ (Low Molecular Weight Aβ). By the term "Low Molecular Weight Aβ", it is meant monomers, dimers and trimers of Aβ having a molecular weight of approximately 4-12 kDa. Aβ protofibrils and Aβ1-16 were used to validate the conformation specificity of the ELISA (Fig 6B), where the hydrophilic Aβ1-16 peptide was used since it is not expected to
10 aggregate. An ELISA composed of two identical antibodies requires at least a dimer of a protein to produce a signal and as predicted, Aβ1-l6 was not detected with the mAb158 sandwich-ELISA even at μM–concentrations (Fig 6B). When pre-treating the LMW-Aβ and Aβ protofibrils with 70% formic acid (FA), known to dissociate aggregated Aβ into monomers, the sandwich ELISA the signal was lost (data not shown). Hence, the detection of
15 LMW-Aβ at high nM concentrations (Fig 6B) is probably due to a small aggregate content of the peptide preparation.

A large excess of monomeric Aβ, holoAPP and APP-fragments, naturally occurring in biological samples, could interfere with the Aβ protofibril analysis by occupying binding sites
20 of the capture antibody coat, thus inhibiting the protofibrils from binding. This problem was investigated by adding an increasing excess of Aβ1-16 to a fixed concentration of Aβ protofibrils (50 pM, expressed as monomer units) and analyzing it with both the mAb158 ELISA and a 6E10-6E10 sandwich ELISA (Fig 6C). A 500 000-fold molar excess of Aβ1-l6, as compared to Aβ protofibrils, did not disturb the measurements with the mAb158 sandwich
25 ELISA, as expected since Aβ1-16 binds poorly to the capture antibody. In contrast, a 500 fold excess of Aβ1-16 was enough to decrease the signal in the 6E10-6E10 ELISA, where Aβ1-16 binds with high affinity to the capture antibody (Fig 6C). Moreover, when synthetic Aβ protofibrils was added to mock HEK cell culture media or non-transgenic mouse brain homogenates, 90% of the signal was recovered (data not shown).
30

Example 7

Measurement of Aβ protofibrils in biological samples.

The presence of Aβ protofibrils in cell and mouse models carrying the Arctic mutation have been suggested, though until now there has been no method for direct assaying of Aβ

protofibrils in biological samples. The mAb158 sandwich ELISA therefore provides the first opportunity to measure Aβ protofibril levels in such cell and mouse models and to compare them to models without this intra-Aβ mutation. Samples from cells and mice carrying only the Swedish mutation were compared to the wild type Aβ protofibril standard curve, whereas samples from cells and mice expressing Aβ with the Arctic mutation were compared to AβArc protofibril standard curve (Fig 6A). To ensure that all Aβ measured in this assay was in a soluble state, and to exclude any possible interference from Aβ fibrils, all samples were centrifuged for 5 min at 17 900 x g before analysis. Groups of cell media from transiently transfected APP_{Swe} and $APP_{Arc\text{-}Swe}$ HEK-cells were analyzed and compared to mock HEK-cell culture media. Aβ protofibril levels were calculated from the standard curves (Fig 6A) as the mean value of triplicates and were then normalized to APP levels to compensate for differences in transfection levels (according to Stenh et al.). The Aβ protofibril concentration in $APP_{Arc\text{-}Swe}$ HEK -cell culture media was 28 pM (±2), significantly higher ($p<0.0001$) than the 8.2 pM (±0.3) seen in APP_{Swe} (Fig 7A). No Aβ protofibrils could be detected in mock media. Levels of Aβ protofibrils were also measured in brains from 10 months old $APP_{Arc\text{-}Swe}$ and APP_{Swe} transgenic mice with both plaques and intraneuronal Aβ pathology (according to Lord et al.). Brains were homogenized in TBS and centrifuged prior to analysis in order to recover the soluble Aβ fraction. Similar to the analysis using cell culture media, Aβ protofibril levels differed significantly ($p=0.005$) between the groups, with 397 pM (±59) in APP_{ArcSwe} and 108 pM (±14) in APP_{Swe} transgenic mouse brains (Fig 7B).

In the above-mentioned figures (Figs. 6 and 7) the number of samples were; mock cells (n=3) and transiently transfected with APP_{Swe} (n=8) and $APP_{Arc\text{-}Swe}$(n=11). Levels of Aβ protofibrils in $APP_{Arc\text{-}Swe}$ media were approximately 9 fold higher than in APP_{Swe} media, whereas mock media gave no signal (A). Measurements of Aβ protofibril levels in the TBS-soluble fraction of non-transgenic mouse brain homogenates (n=6) were compared to transgenic mice (APP_{Swe}, n=3, and $APP_{Arc\text{-}Swe}$, n=6) (B). Similar to the cell culture media, Aβ protofibril levels of $APP_{Arc\text{-}Swe}$ mice were 7 fold higher than in APP_{Swe} mice. Error bars show ± SEM.

Example 8

mAb158 significantly lowers Aβ protofibrils and total Aβ in APPswearc transgenic mice after i.p. administration

mAb158 (12 mg/kg) was injected i.p. once weekly for 18 weeks in 9-10 months old APPswearc mice. After the study, brains were isolated and homogenised in TBS and

subsequently centrifuged to sediment insoluble material. The insoluble material was solubilised in formic acid. Hence, two fractions were obtained from mouse brains i.e. a TBS fraction and a formic acid fraction. Aβ protofibril levels in the TBS fractions were determined by an ELISA. A significant reduction of Aβ protofibrils was found in the
5 mAb158 treatment group compared to the placebo group (Fig 8). Figure 8 shows the Aβ protofibril levels in APPswearc transgenic mouse brain TBS extracts after 4 months treatment with either mAb158 or placebo.

Total Aβ in the formic acid fraction was determined by an ELISA (the formic acid was
10 used to solubilise all Aβ forms, in order to make all Aβ forms detectable). A significant reduction of total Aβ was observed in the treatment group compared to the placebo group (Fig 9). Figure 9 shows the total Aβ levels in APPswearc transgenic mouse brain formic acid extracts after 4 months treatment with either mAb158 or placebo.

15 **Examples 9-11**

Abbreviations

A	Adenine
A*b* protocol	AERES *biomedical* protocol
20 BHK	baby hamster kidney
bp	base pairs
C	Centrigrade
C	Cytosine
CHO	Chinese Hamster Ovary
25 CMF	Calcium and Magnesium Free
COS 7	African green monkey kidney fibroblast cell line
dhfr	Dihydrofolate-reductase
DMEM	Dulbecco's Modified Eagles Medium
DMSO	Dimethyl sulphoxide
30 DNA	Deoxyribonucleic acid
ELISA	Enzyme linked immuno-adsorbent assay
FCS	Foetal Calf Serum
g	grams
G	Guanine

	hr	hour
	HRP	Horseradish peroxidase
	IgG	Immunoglobulin
	K	G or T (IUPAC convention)
5	LSAP	Large Soluble Amyloid Product
	mAb	monoclonal antibody
	sec	second
	min	minute
	M	A or C (IUPAC convention)
10	MTX	Methotrexate
	NIMR	National Institute for Medical Research (UK)
	nm	nanometre
	OD	optical density
	PBS	Phosphate Buffered Saline
15	PCR	Polymerase chain reaction
	R	A or G (IUPAC convention)
	RT	Room Temperature
	S	C or G (IUPAC convention)
	T	Thymine
20	UV	Ultra Violet
	V	variable
	V	A or C or G (IUPAC convention)
	VH	Immunoglobulin heavy chain variable region
	VK	Immunoglobulin kappa light chain variable region
25	W	A or T (IUPAC convention)
	Y	C or T (IUPAC convention)

Materials

Equipment

Equipment	UK Supplier	Catalog Number
DNA thermal cycler: GeneAmp 9600	Perkin Elmer	N801-0177
A designated tissue culture laboratory containing a class II microbiological safety cabinet fitted with a UV-lamp	Walker Safety Cabinets Ltd.	N/a
Innova® bench top incubator shaker	New Brunswick Scientific	4000
Bench top centrifuge	Fisher Scientific	CEK-126-010N
CO2-gassed 37° incubator	RossLab plc	HSO-501TVBB
Microbiological incubator	Kendro / Heraeus	B6060
Electroporator Model: Gene Pulser II	Bio-Rad Laboratories Ltd.	341BR-3092
ELISA reader: Microplater Reader 3550	Bio-Rad Laboratories Ltd.	3550
Microplate Manager® 2.2 data analysis software package for Macintosh computer	Bio-Rad Laboratories Ltd.	N/a
96-Well GeneAmp PCR System 9700	ABI	N8050200
ABI PRISM 310 Genetic Analyzer	Applied Biosystems	310-00-100/120
T100 surface plasmon resonance detector	Biacore	

5 *Plastic consumables*

Article	UK Supplier	Catalog Number
175 cm2 tissue culture flask	Sarstedt Ltd	83.1812.002
25 cm2 tissue culture flask	Corning Costar	3056
30 ml universal container	Sterilin	128C
75 cm2 tissue culture flask	Sarstedt Ltd	83.1813.002
Electroporation cuvettes	Bio-Rad Laboratories Ltd.	165-2088
ELISA plates:Nunc MaxiSorp	Invitrogen Life Technologies	43945A
GeneAmp™ PCR reaction tubes	Perkin Elmer	N801-0180
Glasstic® disposable cell-counting slide	Bio-stat Diagnostic	887144
Nunc inoculating needles	Life Technologies	254399
tissue culture petri 100x20mm, multi-vent	Helena Biosciences	93100
tissue culture plate: 6-well + lid	Corning	C3516
tissue culture plate: 24-well + lid	Corning	C3526

Immunology and molecular biology reagents

Article	UK Supplier	Catalog No.	Lot No.
1st strand synthesis kit	Amersham Biosciences	27-9261-01	3375313
Advantage®-HF 2 PCR Kit	Clontech	639123	6040151
Agarose (UltraPure™)	Invitrogen	15510-027	3019491
Albumin bovine (BSA)	Calbiochem	126575	B65755
Ampicillin	Sigma	A-9518	63H0992
Apa I	Promega	R636	16007003
Themoprime+ DNA Polymerase	Abgene	AB0301	014/0103/11 019/0607/13 020/1808/13
Bam HI	Promega	R602	15851606
BigDye® Terminator v3.0 Cycle Sequencing Ready Reaction Kit	ABI	4390242	0605143 0608154
Ethidium Bromide (10 mg/ml)	Sigma	E-1510	43H9414
Goat anti-human IgG (Fc fragment specific) antibody	Stratech Scientific	109-005-098	68215
Goat anti-human kappa chain horseradish peroxidase conjugate	Sigma	A7164	032K9157
Hind III	Promega	R604	16834803
Human IgG1/kappa antibody.	The Binding Site	BP078	223729
K-Blue HRP substrate	SkyBio	308176	060823
Oligonucleotides	Sigma	n.a.	
PBS Tablets	Sigma	P4417	11K8204
QIAGEN Plasmid Maxi Kit (25)	Qiagen	12162	124114870
QIAprep Spin Miniprep Kit	Qiagen	27106	124117906
QIAquick gel purification kit	Qiagen	28704	11549740
QIAquick PCR purification kit	Qiagen	28106	G10.1.12
Red Stop Solution (For K Blue)	SkyBio Ltd,	301475	060104
	Qiagen	74106	10916587
Shrimp alkaline phosphatase	USB	70092Y	107635
Subcloning Efficiency™ DH5α™ Chemically Competent E. coli	Invitrogen	44 0098	1164658
T4 DNA Ligase	Promega	M1801	167080
TMB One-Step substrate for HRP	SkyBio Ltd,	KB176	
TOPO-TA Cloning® kit	Invitrogen	45-0641	1350772
X-Gal	Sigma	B-9146	20965701

Solutions from National Institute of Medical Research

Solution name:	Components	Amount
PBS 'A' Dulbeccos (Ca & Mg Free)	NaCl KCl Na_2HPO_4 KH_2PO_4 water	8g 0.2g 1.15g 0.2g 1L
LB	Bacto Tryptone Yeast Extract NaCl water	10g 5g 10g 1L
LB agar	LB Agar (Difco)	1L 15g

Culture Reagents

Article	UK Supplier	Catalog Number	Lot Numbers	Expiry date
DMEM (1X) Dulbecco's Modified Eagle Medium (High glucose) with GlutaMAX™ I, 4500mg/L D-Glucose, Sodium Puruvate	Invitrogen	41966-047	9206	07/07
DMSO (Dimethyl sulfoxide)	Sigma	D2650	125K2409	12/07
Penicillin & Streptomycin	Invitrogen	15070-063	1298401	
Serum: Fetal Clone I	Perbio Science	SH30080	AMM17779	12/07
SOC	Invitrogen	15544-034	1306051	
Trypan Blue	Sigma	T8154	19H2388	
Trypsin-EDTA solution, cell culture tested, 0.25%	Sigma	T4049	48K2342	04/08

5

Example 9 - DNA sequence of 158 antibody

9.1 - RNA preparation

Snap-frozen cell pellets of the mouse hybridoma 158, (labelled vials
5 060824#158 5x10^6 cells) were received by TAG on October 3 2006. These cells were stored frozen until processing using the Qiagen RNeasy midi kit to isolate RNA following the manufacturers protocol.

9.2 – 1st strand synthesis

10 About 5 micrograms of 158 RNA was subjected to reverse transcription to produce 158 cDNA using the Amersham Biosciences 1st strand synthesis kit following the manufacturers protocol -This was repeated to generate 3 independent cDNA products (rounds 1, 2 and 3) in order to obviate DNA mutations due to the RT reaction.

15 **9.3 Cloning of the 158 immunoglobulin cDNA**

Hybridoma 158 cDNA was amplified by PCR in 23 separate reactions. Immunoglobulin kappa chain variable region (VK) cDNA was amplified using 11 VK primers (MKV1-11) in combination with the kappa constant region primer MKC (Table 6). Similarly, immunoglobulin heavy chain variable region (VH) cDNA was amplified by PCR using 12
20 different VH primers (MHV1-12) in combination with a mix of the four IgG constant region primers (MHCG1/2a/2b/3: Table 7).

The result of the initial set of IgH PCR reactions was the single amplification product using MHV5 primer. None of the other 11 primer pairs gave a PCR product. The product of the PCR reaction primed by the oligonucleotide primers: MHV5 + (MHCG1/2a/2b/3 mixture)
25 was ligated into the pCR2.1®-TOPO® vector using the TOPO-TA cloning® kit . The result of the initial set of IgK PCR reactions was two single amplification products using primers MKV1 and MKV2 with MKC. The other 9 primer pairs generated no product. The products of the PCR reaction primed by the oligonucleotide primers: MKV1 or MKV2 + MKC were ligated into the pCR2.1®-TOPO® vector using the TOPO-TA cloning® kit.

30 *E.coli* TOP10 bacteria transformed with the ligated vector were cloned on LB/ ampicillin /X-gal agar plates, by picking onto agar grid and into PCR screening mixture. The cloned plasmid inserts were screened by PCR amplification. The PCR products were gel electrophoresed and clones producing the correct-sized PCR amplification product (500bp

approx) were identified. Overnight cultures (5ml) of each clone were processed using the QIAprep Spin Miniprep Kit Protocol, to produce DNA plasmid minipreps.

9.4 - cDNA sequence determination

5 The complete cycle of RT-PCR, cloning, and DNA sequence analysis was repeated to obtain three completely independent sets of sequence information for each immunoglobulin chain. Plasmid clones from each independent set of RT-PCR reactions were sequenced in both directions using the 1212 and 1233 primers (Table 10). Plasmids were sequenced using the BigDye® Terminator v3.0 Cycle Sequencing Ready Reaction Kit (ABI), cycled on a
10 GeneAmp9600 PCR machine and analysed on an ABI 310 capillary sequencer.

9.5 - 158 VK DNA sequence

Sequences of VK clones generated using PCR primers MKV2 and MKC on 1st strand cDNAs rounds 1 and 2, were identical to a sterile kappa transcript originating from the myeloma
15 fusion partner such as MOPC-21, SP2 and Ag8. This is a sterile transcript
The consensus sequence (158 VK) of VK clones generated using PCR primers MKV1 and MKC on 1st strand cDNAs rounds 1-3 is shown in Table 11. This is a functional rearrangement. Table 11 shows some differences from the sequence shown in Tables 1, 4 and 5. These differences are in the FW1 region where the PCR primer was located. The mouse
20 VK leader sequence most identical to the fragment of leader in 158 VK, not encoded by our primers, was K5.1# (Table 12). The prediction for the signal peptide to cleave correctly the #K5.1 signal sequence was done by a prediction program. Most likely predicted cleavage site was correctly between amino acid residue 19 and 20. (Table 13). The chimeric 158VK protein and DNA sequence is shown in Table 14.

25

9.6 - 158 VH DNA sequence

The consensus sequence (158 VH) of VH clones generated using PCR primers MHV5 and MHCG1/2a/2b/3 mixture on 1st strand cDNAs rounds 1-3 is shown in Table 15. As with 158 VK, there are some differences from the FW1 sequence shown in Tables 1, 4 and 5. The most
30 identical mouse VH leader sequence to the fragment of leader, not encoded by our primers, was NL-1 (Table 16).

Example 10 - Construction of chimeric expression vectors

Construction of chimeric expression vectors entails adding a suitable leader sequence to VH and VK, preceded by a *Hin* dIII restriction site and a Kozak sequence. The Kozak sequence (Table 8) ensures efficient translation of the variable region sequence. It defines the correct AUG codon from which a ribosome can commence translation, and the most critical base is the adenine at position -3, upstream of the AUG start. The leader sequence is selected as the most similar mouse leader sequence in the Kabat database. These additions are encoded within the forward primers (Table 9). Furthermore, the construction of the chimeric expression vectors entails introducing a 5' fragment of the human γ_1 constant region, up to a natural *Apa* I restriction site, contiguous with the 3' end of the J region of 158. The CH is encoded in the expression vector downstream of the inserted VH sequence but lacks the V-C intron. For the light chain, the natural splice donor site (Table 8) and a *Bam* HI site is added downstream of the V region. The splice donor sequence facilitates splicing out the kappa V:C intron which is necessary for in-frame attachment of the VK to the constant region.
The mouse VH and VK genes were analysed to identify any unwanted splice donor sites, splice acceptor sites, Kozak sequences and for the presence of any extra sub-cloning restriction sites which would later interfere with the subcloning and/or expression of functional whole antibody. In this case none were found.

10.1 - Expression vectors

Plasmid DNA preparations of the expression vectors pKN100, and pG1D200 were purified using Qiagen Maxi kits following the manufacturers protocol. Plasmid DNA Purification using QIAGEN Plasmid Midi and Maxi Kits, from 500ml cultures of TOP10 bacteria transfected with either vector. The vector maps are shown in Figs 10 and 11.

10.2 - The light chain chimerisation primers

The mouse leader sequence K5.1# was incorporated into the design of the chimeric 158 VK. Primers were designed to generate a PCR product containing this complete leader, and 158 VK, with terminal restriction sites *Hind* III and *Bam* HI for cloning into the pKN100 expression vector (Table 9). The forward primer 158vl introduces a *Hind* III restriction site; a Kozak site and the K5.1# leader sequence. The back primer 158vlrev introduces: a splice donor site and a *Bam* HI restriction site.

10.3 - The heavy chain chimerisation primers

The leader sequence NL-1 was incorporated into the design of the chimeric 158 VH. Primers were designed to generate a PCR product containing this leader, and the 158 VH region, with
5 terminal restriction sites *Hin* dIII and *Apa* I for cloning into the pG1D200 expression vector. These are shown in Table 9. The forward primer, 158vh, introduces a *Hin* dIII restriction site; a Kozak translation initiation site and the NL-1 leader sequence. The back primer, 158vhrev, introduces the 5' end of the γ1 C region and a natural *Apa* I restriction site. The signal peptide cleavage site prediction for K5.1 leader sequence of VK is shown in Table 17.
10

10.4 - Generation of the chimeric 158 VH construct: pG1D200158VH

The 158 VH DNA fragment was amplified with primers: 158vh and 158vhrev (Table 9). The 450bp (approx) PCR product was T-A ligated into the vector pCR2.1 and used to transform chemically competent TOP10 bacteria. Clones were selected by appropriate insert size and
15 sequenced using the 1212 primer (Table 10). The correct expression insert was subcloned into pG1D200 expression vector and the correct subclone was selected by DNA sequencing using primer BDSH61R (Table 10). This clone was grown in 200 ml culture to produce plasmid DNA using the Qiagen Maxi Kit using the manufacturers protocol. The chimeric 158VH protein and DNA sequence is shown in Table 18.
20

10.5 - Generation of the chimeric 158 VK construct: pKN100158VK

- The 158 VK DNA fragment was amplified with primers 158vl and 158vlrev (Table 9). The 450bp (approx) PCR product was T-A ligated into vector pCR2.1 and used to transform chemically competent TOP10 bacteria. Clones were selected by insert size and sequenced
25 using the 1212 primer (Table 10). The correct clone was subcloned into pKN100 expression vector. The correct subclone was selected by screening for insert size and DNA sequencing using primer Hu-K2 (Table 10). This clone was grown in 200 ml culture to produce plasmid DNA using the Qiagen Maxi Kit using the manufacturers protocol.

30 **Example 11 - Production and binding properties of chimeric 158 antibody**

11.1 - COS 7 cell transformation and cell culture

One vial of COS 7 cells was thawed and grown in DMEM supplemented with 10% Fetal clone I serum and antibiotics. One week later, cells (0.8ml at 10^7/ml) were electroporated with

pG1D200158VH plus pKN100158VK (10μg DNA each). The cells were grown in 8ml of growth medium in petri dishes for 3 days.

11.2 - Chimeric antibody production

5 A sandwich ELISA was used to measure antibody concentrations in the COS 7 supernatants. Chimeric 158 VH x 158 VK antibody was expressed at 0.3μg/ml and subsequently at 3.7μg/ml (Table 19) in transiently co-transfected COS cell conditioned media.

11.3 - Chimeric antibody activity

10 Two ELISAs was used to analyse the antigen binding of chimeric 158. Using the 3.7μg/ml chimeric antibody conditioned medium, binding to Aβ monomer was measured by a direct ELISA protocol (Figure 12) and compared to the mouse 158 IgG. Secondly, a competition ELISA was done using either monomer or protofibril mixed in the fluid phase with antibody, which subsequently bound to Aβ monomer in the solid phase (Figure 13). These showed that 15 the chimeric 158 antibody binds to amyloid Aβ monomer and protofibril similarly to the original 158 mouse antibody.

Comment

Later sequencing has shown that the mouse antibody sequence data, as shown in Tables 1 and 20 4 contain errors in both VH and VK chains at the 5' end. We suggest that this is due to the use of primers located within the V region. In later sequencing, primers located within the leader sequences, which cannot introduce mutations within the V regions, were used. The later sequencing showed sequence differences (see Tables 15 and 11). Said differences are however not located within the CDR regions.

25

The chimeric antibody binds amyloid Aβ monomer and protofibrils as shown by the direct binding ELISA and the competition ELISA respectively. This evidence confirms that the combination of 158 VH and 158 VK chains encodes the anti-LSAP antibody 158 and indicates that these sequences are suitable for the humanisation procedure to generate a 30 humanised 158 antibody.

Example 12 - Humanised antibody design and discussion

Abbreviations and definitions

158	mouse monoclonal anti-LSAP™ antibody 158
158 VH	VH of mouse 158 antibody
158 VK	VK of mouse 158 antibody
158RKAss	Humanised version of 158 VK retaining cryptic splice sites
158RKA	Humanised version of 158 VK with cryptic splice sites removed
158RHAss	Humanised version of 158 VH retaining cryptic splice sites
158RHA	Humanised version of 158 VH with cryptic splice sites removed
A	Adenine
bp	base pairs
C	Cytosine
CDR	Complementarity determining region in the immunoglobulin variable regions, defined using the Kabat numbering system
D-gene	Diversity gene
DNA	Deoxyribonucleic acid
FW	Framework region: the immunoglobulin variable regions excluding the CDR regions
G	Guanine
IgG	Immunoglobulin G
J-gene	Joining gene
Kabat	an immunoglobulin alignment and numbering system pioneered by Elvin A Kabat
mAb	monoclonal antibody
MRCT	Medical Research Council Technology
T	Thymine
VCI	Framework residue classified as vernier or canonical or VH-VL interface
V-gene	The gene segment that is rearranged together with a J (and D for VH) gene to generate a complete VH or VK
V region	The segment of IgG chains which is variable in sequence between different antibodies. It extends to Kabat residue 109 in the light chain and 113 in the heavy chain.
VH	Immunoglobulin heavy chain variable region
VK	Immunoglobulin kappa light chain variable region

Equipment

Hardware & software	Origin
SGW02 computer	Silicon Graphics
PC computer	Hewlett Packard
SR 7.6	Steve Searle, Wellcome Trust Sanger Institute, Cambridge.
Lasergene 6.0	DNAstar Inc
Modeler 9.0	Accelrys Ltd.
SignalP	www.cbs.dtu.dk
BlastP	www.ncbi.nlm.nih.gov

12.1 - Human V gene databases

The protein sequences of human and mouse immunoglobulins from the International
5 Immunogenetics Database 2006 and the Kabat Database Release 5 of Sequences of Proteins of Immunological Interest (last update 17-Nov-1999) were used to compile a database of immunoglobulin protein sequences in Kabat alignment. Our database contains 9322 human VH and 2689 human VK sequences. The sequence analysis program, SR 7.6, was used to query the human VH and VK databases with 158 VH and 158 VK protein
10 sequences (Table 20).

12.2 - Selection of a human framework for 158RHA

12.2.1 - Comparison of 158 VH with human VH sequences

Human VH sequences with highest identity to 158 VH at Vernier (Foote,J. and G.Winter. 1992.
15 Antibody framework residues affecting the conformation of the hypervariable loops. *J Mol.Biol.* 224:487-499.), Canonical (Morea,V., A.M.Lesk, and A.Tramontano. 2000. Antibody modeling: implications for engineering and design. *Methods* 20:267-279.) and VH-VL Interface (Chothia,C., J.Novotny, R.Bruccoleri, and M.Karplus. 1985. Domain association in immunoglobulin molecules. The packing of variable domains. *J Mol.Biol.* 186:651-663.)
20 (VCI) residues, located within the V-region framework (FW), are shown in Table 21. The number of VCI residues (VCI score) and FW residues (FW score) identical to 158 are also shown. All these VH sequences share identical VCI residues, and CDR lengths, as shown in Table 22. AJ556669 has an unusual Pro74 not seen in the other human sequences in this dataset, leading us to discount it in the initial analysis. Pro74 is, however, present in the
25 158VH sequence, so AJ556669 could be considered as an alternative FW for humanisation, if

the VH construct based on AF062243 does not bind antigen. The alignment of these sequences (Table 23) highlights their differences. AF062243 uniquely within this dataset has the conservative change T(82a)S and the conservation of F79. The other features of AF062243 are the conservative changes D1E, K19R, A23S, T77S, S118T. All other FW
5 changes were common to all the frameworks in Table 23. AF062243 was selected as the framework on which to base 158RHA.

12.3 - Generation of 158RHA

The design of 158RHA is simply the grafting of CDR 1, 2 and 3 from 158 VH into the
10 acceptor FW of AF062243. The human germline V-gene most identical to AF062243 is VH M99649 (VH3-07), (Table 24) from which the leader peptide was extracted (Table 25). The SignalP algorithm (Nielsen,H., J.Engelbrecht, S.Brunak, and G.von Heijne. 1997. Identification of prokaryotic and eukaryotic signal peptides and prediction of their cleavage sites. *Protein Eng* 10:1-6.) predicted that it would cut appropriately with signal peptidase
15 (Table 26). Table 27 shows the scheme of grafting 158 VH CDR 1, 2 and 3 into the AF062243 FW, to generate 158RHA protein sequence. Table 28 shows the generation of the DNA sequence 158RHAss from the natural DNA sequences of 158 VH and AF062243. Analysis of the 158RHAss DNA sequence predicted the presence of splice donor sites, the prediction scores of which are shown in Table 29. Non-coding mutations were introduced to
20 inactivate these predicted splice sites, as shown in Table 30 to generate the final 158RHA DNA sequence (Table 31).

12.4 - Selection of a human framework for 158RKA

12.4.1 - Comparison of 158 VK with human VK sequences

25 The human VK sequences with highest identity to 158 VK at VCI residues are shown in Table 32 together with the number of VCI residues (VCI score) and FW residues (FW score) identical to 158 VK. Eleven sequences have all VCI residues identical to 158 VK. Table 33 shows that all these sequences have CDR lengths identical to 158 VK. Table 34 highlights their differences, showing that K45 is retained in AB064054 only, which also
30 retains I85. The G100P change is unremarkable because P100 is common, having an incidence of 15% in our human VK database. The two substitutions: T7S and K74R, are conservative, and all other substitutions are common to all the sequences in Table 34. For these reasons AB064054 was selected to generate 158RKA.

12.5 - Generation of 158RKA

The design of 158RKA is the simple grafting of the CDRs 1, 2 and 3 from 158 VK into the acceptor FW of human AB064054. The nearest germline V-gene to AB064054 is A19 (Table 35), from which the leader peptide was extracted (Table 36). The SignalP algorithm predicted appropriate cutting (Table 37) of this leader peptide. Table 38 shows the generation of the protein sequence of 158RKA by intercalation of the 158 VK CDRs into the FW of AB064054. Table 39 shows the generation of the DNA sequence of 158RKAss from the natural DNA sequence of 158 VK and AB064054. Analysis of the 158RKAss predicted the presence of splice donor sites, the scores of which are shown in Table 40. Non-coding mutations (41) were introduced to inactivate these sites and generate the final 158RKA DNA construct (Table 42).

12.6 Humanized antibody (BAN2401) binding activity

The 158RKA and 158RHA genes were inserted into an expression vector containing the IgG1 constant region. This construct was expressed in COS cells to generate the humanized 158 antibody. The humanized 158 antibody was tested for binding activity and specificity in a competitive ELISA. The humanised antibody exhibited identical binding properties as to mAb158 and the 158 chimeric antibody (see Figure 14.)

12.7 Additional mutations in the 158RHA and 158RKA chains.

By comparing mouse germline V genes VH AAK71612 to 158 VH a single somatic mutation A60G in the CDR2 was identified. Furthermore, the molecular model of antibody 158 which contains three VH FW residues within 5Å of CDR residues which are unconserved in 158RHA. These substitutions are D1E, P74A and T82S (Table 43). Similarly, there are two VK FW residues within 5Å of CDR residues which is unconserved in 158RKA. This substitution is L3V and G100P (Table 44). Introduction of back mutations at positions VH-1, VH-74, VH-82, VK-3 and VK-100 into 158RHA and 158RKA, in humanised versions 158RHB, 158RHC, 158RHD, 158RKB and 158RKC are shown in Table 43 and 44.

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30

CLAIMS

1. An antibody or fragment thereof being selective and having high affinity for wild type human Aβ protofibrils, wherein the antibody or fragment in its six CDR regions has the following consensus sequences:

VH-CDR1	**SFGMH**
VH-CDR2	**YISSGSSTIYYGDTVKG**
VH-CDR3	**EGGYYYGRSYYTMDY**
VL-CDR1	**RSSQSIVHSNGNTYLE**
VL-CDR2	**KVSNRFS**
VL-CDR3	**FQGSHVPPT**

wherein said antibody or fragment may exhibit amino acid deletions, substitutions and insertions within said CDR regions.

2. An antibody according to claim 1, wherein the antibody exhibits 1-10 amino acid deletions, substitutions and insertions within said CDR regions.

3. An antibody according to claim 1, wherein the antibody exhibits 1-5 amino acid deletions, substitutions and insertions within said CDR regions.

4. An antibody according to claim 1, wherein the antibody exhibits 1-3 amino acid deletions within said CDR regions.

5. An antibody according to any of the preceding claims, wherein said antibody is monoclonal.

6. An antibody according to any of the preceding claims, wherein said antibody has reduced complement activation activity.

7. An antibody according to claim 6, wherein said reduced complement activation activity has been achieved by changing the amino acid proline in position 331 to serine or an other polar amino acid.

8. An antibody according to claim 6, wherein said reduced complement activation activity has been achieved by inhibiting or lowering or changing the glycosylation.

9. An antibody according to any of the preceding claims, wherein said antibody is of IgG class.

10. An antibody according to claim 9, wherein said antibody is of IgG1 or IgG4 subclass.

11. An antibody according to claim 10, wherein said antibody is a chimera of IgG1 or IgG4 subclass, where the heavy chain constant region CH2 or part of CH2 is derived from IgG4 and the regions CH1 and CH3 are derived from IgG1, for reduced complement activation.

12. An antibody according to any of the preceding claims, wherein said antibody comprises the complete heavy chain sequence according to Table 31 and the complete light chain sequence according to Table 42.

13. An antibody according to any of claims 1-11, wherein said antibody comprises mutations in the heavy chain (VH) according to Table 43, said mutations being selected from A60G, D1E, P74A and T82S, and/or mutations in the light chain (VK) according to Table 44, said mutations being selected from L3V and G100P, or combinations of these VH and VK mutations.

14. An antibody according to any of claims 1-11, wherein said antibody comprises the complete heavy chain sequence according to Table 31 and the complete light chain sequence according to Table 42, with the exception that the eighth amino acid to the left of the heavy chain sequence of CDR1 is S.

15. An antibody according to any of the preceding claims, wherein said antibody is human or humanized or mutated to reduce antigenicity in humans.

16. An antibody according to any of claims 1-11, wherein said antibody is a mouse antibody.

17. An antibody according to any of the preceding claims, wherein the specificity ratio between Aβ monomers and protofibrils is at least 1:200.

5 18. An antibody according to claim 1, wherein said antibody in its six CDR regions has the following consensus sequences:

VH-CDR1	**AASGFTFSSFGMHWVR**
VH-CDR2	**WVAYISSGSSTIYYGDTVKGRFT**
VH-CDR3	**CAREGGYYYGRSYYTMDYWGQ**
VL-CDR1	**ISCRSSQSIVHSNGNTYLEWYL**
VL-CDR2	**LIYKVSNRFSGVP**
VL-CDR3	**YYCFQGSHVPPTFGG**

10

wherein said antibody or fragment may exhibit amino acid deletions, substitutions and insertions within said CDR regions.

15

19. An antibody according to claim 18, wherein said antibody is a mouse antibody.

20. An antibody according to claim 18 or 19, wherein said antibody comprises the complete light chain sequence 158 VK according to Table 11 and the complete heavy chain sequence 158 VH according to Table 15.

20

21. Composition comprising the antibody defined in any of the preceding claims and a pharmaceutically acceptable buffer for human and veterinary use.

25 22. Composition according to claim 21, further comprising an antibacterial agent.

23. Composition according to claim 22, wherein the composition is freeze-dried.

24. Composition according to claim 22, wherein the composition is freeze-dried together with an excipient to increase stability of the antibody during and after freeze-drying.

30

25. Composition according to claim 23, wherein the excipient is mannitol or trehalose.

26. Method of preventing or treating Alzheimer's disease, comprising the step of administering to a patient having or suspected of having Alzheimer's disease the antibody defined in any of claims 1-20 or the composition defined in claims 21-25.

5 27. Method of preventing or treating Down's syndrome, Lewybody dementia, vascular dementia and other neurodegenerative disorders, comprising the step of administering to a patient having or suspected of having Down's syndrome, Lewybody dementia, vascular dementia and other neurodegenerative disorders having the antibody defined in any of claims 1-20 or the composition defined in
10 claims 21-25.

28. A method of detecting Aβ protofibrils *in vitro,* comprising the steps of:
- adding the antibody defined in any of claims 1-20 to a biological sample comprising or suspected of comprising Aβ protofibrils.
15 - measuring the concentration of the complex formed between said Aβ protofibril and said antibody.

29. A method according to claim 28, where said detection method is an immunoassay.

20 30. A method according to claim 28, where said detection method is an proximity ligation assay.

31. A method of detecting Aβ protofibrils *in vivo,* comprising the steps of:
- adding the antibody defined in any of claims 1-20 to a mammal comprising or
25 suspected of comprising Aß protofibrils.
- measuring the concentration of the complex formed between said Aß protofibril and said antibody.

32. A method of diagnosing Alzheimer's disease, comprising the steps of:
30 - taking a biological sample from a subject,
- adding the antibody defined in any of claims 1-20 to said sample,
- measuring the concentration of the complex formed between said antibody and any Aβ protofibrils in said sample.

33. A method of diagnosing Down's syndrome, Lewybody dementia, vascular dementia and other neurodegenerative disorders, comprising the steps of:
- taking a biological sample from a subject,
- adding the antibody defined in any of claims 1-20 to said sample,
5 - measuring the concentration of the complex formed between said antibody and any Aβ protofibrils in said sample.

34. Use of the antibody defined in any of claims 1-20 or the composition defined in any of claims 21-25 for the preparation of a medicament for treatment of Alzheimer's
10 disease.

35. Use of the antibody defined in any of claims 1-20 or the composition defined in any of claims 21-25 for the preparation of a medicament for treatment of Down's syndrome, Lewybody dementia, vascular dementia and other neurodegenerative
15 disorders.

Table 1.

Amino acid sequence of variable regions of the heavy chain (VH) and light chain (VL/Vκ) from six different monoclonal antibodies specific for human wild-type Aβ protofibrils.

Chain	Clone	Sequence
VH-BA1:	X731	EVKLVESGGGLVQPGGSRKLSCAASGFTFSSFGMHWVRQAPEKGLEWVAYISSGSSTIYYADTVKGRFTISRDNPKNTLFLQMTSLRSEDTAMYYCARYGNYAM-------DYWGQGTSVTVSS
VH-BA2:	X736	EVKLVESGGGLVKPGGSLKLSCAASGFTFSSYAMSWVRQTPEKRLEWVATISSGGSYTYYPDSVKGRFTISRDNAKNTLYLQMSSLRSEDTAMYYCARNYGSRRYF-----DVWGAGTSVTVSS
VH-BA3:	X745	QVHLQQSGPELVKPGASVKMSCKASGYTFTSYVMHWVKQKPGQGLEWIGYINPYNDGTKYNEKFKGKATLTSDKSSSTAYMELSSLTSEDSAVYYCARRVSPLTSYAM---DYWGQGTSVTVSS
VH-BA7:	X746	QVQLKESGPGLVAPPQSLSITCTVSGFSLTSYGVHWVRQPPGKGLEWLGVIWAGGSTNYNSALMS-RLSISKDNSKSQVFLKMNSLQTDDTAMYYCARGRYDGKTRFA---YWGQGTLVTVSS
VH-BA4:	X748	EVKLMESGGGLVQPGGSRKLSCAASGFTFSSFGMHWVRQAPEKGLEWVAYISSGSSTIYYADTVKGRFTISRDNPKNTLFLQMTSLRSEDTAMYYCARGDSF--------DYWGQGTTLTVSS
VH-BA9:	X758	EVQRVESGGGLVQPGGSRKLSCAASGFTFSSFGMHWVRQAPEKGLEWVAYISSGSSTIYYGDTVKGRFTISRDNPKNTLFLQMTSLRSEDTAMYYCAREGGYYYGRSYYTMDYWGQGTSVTVSS
Vκ-BA1:	X731	DVVMTQTPLSLPVSLGDQASISCRSSQSIVHSNGNTYLE-WYLQKPGQSPKLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYCFQGSHVPPTFGGGTKLEIK
Vκ-BA2:	X736	DIVMTQAPKFLLVSAGDRVTITCKASQSVSNDVA------WYQQKPGQSPKLLIYYASNRYTGVPDRFTGSGYGTDFTFTISTVQAEDLAVYFCQQDYSSPFTFGSGTKLEIK
Vκ-BA3:	X745	DIVMTQAPSSLAVSAGEKVTMSCKSSQSLLNSRTRKNYLAWYQQKPGQSPKLLIYWASTRESGVPDRFTGSGSGTDFTLTISSVQAEDLAVYYCKQSYNL-WTFGGGTKLEIK
Vκ-BA7:	X746	ENVLTQSPAIMSASPGEKVTMTCRASSSVSSSYLH----WYQQKSGASPKLWIYSTSNLASGVPARFSGSGSGTSYSLTISSVEAEDAATYYCQQYSGYPLTFGAGTKLELK
Vκ-BA4:	X746	DIVMTQAPLSLPVSLGDQASISCRSSQSLVHSNGNTYLH-WYLQKPGQSLKLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYFCSQSTHVPLTFGAGTKLELK
Vκ-BA9:	X758	DIVMTQAPLSLPVSLGDQASISCRSSQSIVHSNGNTYL-EWYLQKPGQSPKLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGIYYCFQGSHVPPTFGGGTKLEIK

*Position of the various CDR regions (1-3) are underlined in VL and VH. The boundaries of the CDR regions (1-3) are shown in Table 3 and Table 4. Antibody BA9, also named 158 in the patent application., is an example of a high affinity protofibril specific antibody according to the invention.

Table 2.

Amino acid sequences of CDR1-2 regions from VH chain from a protofibril selective antibody and amino acid substitutions that are compatible with high affinity binding to human wild–type Aß protofibrils.

VH chain CDR-1 region	
AASGFTFSSFGMHWVR	Antibody 158
---------YA-S---	Substitutions*

VH chain CDR-2 region	
WVAYISSGSSTIYYGDTVKGRFT	Antibody 158
--------------A--------	Substitutions*
---T----G-YT--P-S------	Substitutions*

VH chain CDR-3 region	
CAREG-GYYYGRSYY-TMDYWGQ	Antibody 158
CARYGxxxxxNYxxxxAMDYWGQ	Substitutions and deletions*
CARNYxxxxGSRRxxxYFDVWGA	Substitutions and deletions*

---

* The amino acid substitutions (other amino acid than in antibody 158) are shown with one amino acid letter code. Deletions are shown with (x).

Table 3.

Amino acid sequences of CDR 1-3 regions from VL chain from a protofibril selective antibody and amino acid substitutions that are compatible with high affinity binding to human wild–type Aß protofibrils

VL chain CDR-1 region	
ISCRSSQSIVHSNGNTYLEWYL	Antibody 158
ITCKASQSVxxSNDxxxVAWYQ	Substitutions and deletions*
VL chain CDR-2 region	
LIYKVSNRFSGVP	Antibody 158
---YA---YT---	Substitutions*
VL chain CDR-3 region	
YYCFQGSHVPPTFGG	Antibody 158
-F-Q-DYSS-F---S	Substitutions*

* The amino acid substitutions (other amino acid than in antibody 158) are shown with one amino acid letter code. Deletions are shown with (x).

Table 4.

Amino acid sequence of mouse framework regions of the mouse and human variable light chain (VL) region from protofibril specific antibodies

```
Mouse framework* VL regions
Divmtqaplslpvslgdqasiscwylqkpgqspklliygvpdrfsgsgsgtdftlkisrveaedlgiyyc   antibody 158
......................................................................   BA9_VL_fr123
.v....t...........................................................v...   BA1_VL_fr123
........kf.l..a..rvt.t...q..................t...y.....ft..t.q....av.f.   BA2_VL_fr123

Human framework VL regions
......t......tp.ep...............q..............................v.v...   VKII-3-1-(1)-O11
......s......tp.ep...............q..............................v.v...   VKII-4-1-(1)-A19
......t....s.tp.qp...............q..............................v.v...   VKII-4-1-(1)-A18
......t....s.tp.qp.............p.q..............................v.v...   VKII-4-1-(1)-A2
.v....s......t..qp......fq.r.....rr.............................v.v...   VKII-4-1-(1)-A17
```

* Framework region is the region outside the CDR regions. The CDR regions has been deleted for clarity.

Table 5.

Amino acid sequence of mouse and human framework regions of the mouse and human variable light heavy (VH) region from protofibril specific antibodies

```
Mouse framework* VH regions
Evklmesggglvqpggsrklscaaswvrqapekglewvarftisrdnpkntlflqmtslrsedtamyycar   antibody 158
.......................................................................   BA9_VH_fr123
....v..................................................................   BA1_VH_fr123
....v.......k....l...........t...r.............a....y...s..............   BA2_VH_fr123

Human framework VH regions
..q.v............lr............g...............a..s.y...n...a....v.....   VH3-7_fr123
..q.v......i.....lr............g......s........s....y...n...a....v.....   VH3-53_fr123
..q.l............lr............g......s........s....y...n...a....v....k   VH3-23_fr123
..q.v............lr............g......s........a..s.y...n...d....v.....   VH3-48_fr123
..q.v............lr............g...v..s........a....y...n...a....v.....   VH3-74_fr123
```

* Framework region is the region outside the CDR regions. The CDR regions has been deleted for clarity.

Table 6. PCR primers for cloning mouse VK

Name	Sequence (5'→ 3')
MKV1	ATGAAGTTGVVTGTTAGGCTGTTGGTGCTG
MKV2	ATGGAGWCAGACACACTCCTGYTATGGGTG
MKV3	ATGAGTGTGCTCACTCAGGTCCTGGSGTTG
MKV4	ATGAGGRCCCCTGCTCAGWTTYTTGGMWTCTTG
MKV5	ATGGATTTWAGGTGCAGATTWTCAGCTTC
MKV6	ATGAGGTKCKKTGKTSAGSTSCTGRGG
MKV7	ATGGGCWTCAAGATGGAGTCACAKWYYCWGG
MKV8	ATGTGGGGAYCTKTTTYCMMTTTTTCAATTG
MKV9	ATGGTRTCCWCASCTCAGTTCCTTG
MKV10	ATGTATATATGTTTGTTGTCTATTTCT
MKV11	ATGGAAGCCCCAGCTCAGCTTCTCTTCC
MKC	ACTGGATGGTGGGAAGATGG

Table 7. PCR primers for cloning mouse heavy VH

Name	Sequence (5'→ 3')
MHV1	ATGAAATGCAGCTGGGGCATSTTCTTC
MHV2	ATGGGATGGAGCTRTATCATSYTCTT
MHV3	ATGAAGWTGTGGTTAAACTGGGTTTTT
MHV4	ATGRACTTTGGGYTCAGCTTGRTTT
MHV5	ATGGACTCCAGGCTCAATTTAGTTTTCCTT
MHV6	ATGGCTGTCYTRGSGCTRCTCTTCTGC
MHV7	ATGGRATGGAGCKGGRTCTTTMTCTT
MHV8	ATGAGAGTGCTGATTCTTTTGTG
MHV9	ATGGMTTGGGTGTGGAMCTTGCTATTCCTG
MHV10	ATGGGCAGACTTACATTCTCATTCCTG
MHV11	ATGGATTTTGGGCTGATTTTTTTTATTG
MHV12	ATGATGGTGTTAAGTCTTCTGTACCTG
MHCG1	CAGTGGATAGACAGATGGGGG
MHCG2a	CAGTGGATAGACCGATGGGGC
MHCG2b	CAGTGGATAGACTGATGGGGG
MHCG3	CAAGGGATAGACAGATGGGGC

Legend: Wobble bases are defined in Abbreviations (Section 2).

Table 8.
Sequences important for efficient expression of immunoglobulin in mammalian cells

Name	Consensus DNA Sequence (5'→ 3')
Kozak translation initiation site	G C C G C C **R** C C^{-1} **A^{+1}** **U** **G** **G**
Kappa light chain splice donor site	A C :: **G** **T** R A G T
Heavy chain splice donor site	M A G :: **G** **T** R A G T
Immunoglobulin splice acceptor site	Y Y Y Y Y Y Y Y Y Y Y N C A **G** :: G

Legend: Bases shown in **bold** are considered to be invariant within each consensus sequence. Splice sites are defined by the symbol " : : ". Wobble bases are defined in Abbreviations (see Examples 9-11).

Table 9. Oligonucleotide primers used to generate chimeric 158

Oligonucletotide name	Sequence (5'→ 3')
158vh	AAGCTT**GCCGCCACC**ATGGACTCCAGGCTC
158vhrev	GGGCCCTTGGTGGAGGCTGAGGAGACGGTGACTGAGG
158vl	AAGCTT**GCCGCCACC**ATGAAGTTGCCTGTTAGG
158vlrev	GGATCCACTCACGTTTGATTTCCAGCTTGG

Legend: Restriction sites are underlined. Kozak sequences are in **bold type.**

Table 10. Oligonucleotide primers used for sequencing

Oligonucletotide name	Sequence (5'→ 3')
1212 (17mer)	GTTTTCCCAGTCACGAC
1233 (24mer)	AGCGGATAACAATTTCACACAGGA
Hu-K2 (17mer)	CTCATCAGATGGCGGGA
BDSH61R	CGCTGCTGAGGGAGTAGAGTC

Table 11. DNA sequence of 158 VK, primer MKV1 and the VK sequence derived using primers located within the V region

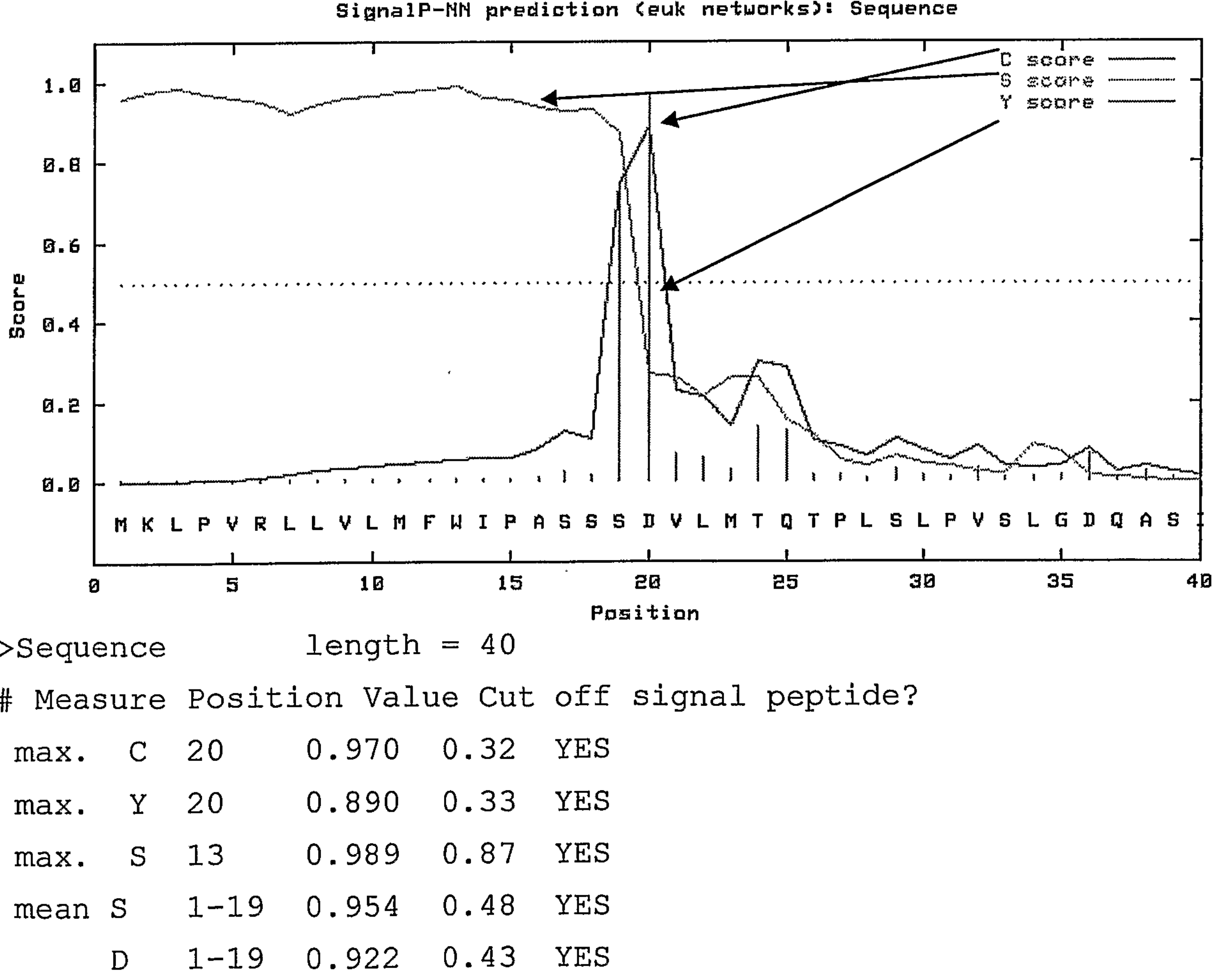
```
  1 ATGAAGTTGCCTGTTAGGCTGTTGGTGCTGATGTTCTGGATTCCTGCTTCCAGCAGTGATGTTTTGATGACCCAAACTCCACTCTCCCTG 158 VK
  1 .........VV...................                                                             MKV1
  1 ---------------------------------------------------------...A..G..........GG.............. *** VK

 91 CCTGTCAGTCTTGGAGATCAAGCCTCCATCTCTTGCAGATCTAGTCAGAGCATTGTACATAGTAATGGAAACACCTATTTAGAATGGTAC 158 VK
 34 .......................................................................................... *** VK

181 CTGCAGAAACCAGGCCAGTCTCCAAAGCTCCTGATCTACAAAGTTTCCAACCGATTTTCTGGGGTCCCAGACAGGTTCAGTGGCAGTGGA 158 VK
124 .......................................................................................... *** VK

271 TCAGGGACAGATTTCACACTCAAGATCAGCAGAGTGGAGGCTGAGGATCTGGGAATTTATTACTGCTTTCAAGGTTCACATGTTCCTCCG 158 VK
214 .......................................................................................... *** VK

361 ACGTTCGGTGGAGGCACCAAGCTGGAAATCAAACGGGCTG                                                   158 VK
304 .................................                                                          *** VK
```

Legend: Residues identical to 158 VK are indicated by a dot. ***Sequencing using primers located within the V region.

Table 12. Chimeric VK leader sequence selection - K5.1# leader selection for the chimeric VK

158 VK	MKLPVRLLVLMFWIPASSS
K5.1#Protein	MKLPVRLLVLMFWIPASSS
K5.1#DNA	ATGAAGTTGCCTGTTAGGCTGTTGGTGCTGATGTTCTGGATTCCT GCTTCCAGCAGT

Table 13. SignalP result 6 for K5.1# leader




```
>Sequence        length = 40
# Measure Position Value Cut off signal peptide?
 max.  C  20     0.970  0.32  YES
 max.  Y  20     0.890  0.33  YES
 max.  S  13     0.989  0.87  YES
 mean S   1-19   0.954  0.48  YES
      D   1-19   0.922  0.43  YES
```

Highest probability for cleavage is between amino acid residue 19 and 20 (SSS-DV)

Legend: The signal P algorithm[6] generates the combination score Y ,from the cleavage site score C, and the signal peptide score S.

Table 14. Protein and DNA sequence of chimeric 158 VK construct

```
HindIII
AAGCTTGCCGCCACCATGAAGTTGCCTGTTAGGCTGTTGGTGCTGATGTTCTGGATTCCTGCTTCCAGCAGTGATGTTTTG 81
   kozak   K5.1# leader                                                   158 VK
 K  L  A  A  T  M  K  L  P  V  R  L  L  V  L  M  F  W  I  P  A  S  S  S  D  V  L
ATGACCCAAACTCCACTCTCCCTGCCTGTCAGTCTTGGAGATCAAGCCTCCATCTCTTGCAGATCTAGTCAGAGCATTGTA 162
                                    158 VK
 M  T  Q  T  P  L  S  L  P  V  S  L  G  D  Q  A  S  I  S  C  R  S  S  Q  S  I  V
CATAGTAATGGAAACACCTATTTAGAATGGTACCTGCAGAAACCAGGCCAGTCTCCAAAGCTCCTGATCTACAAAGTTTCC 243
                                    158 VK
 H  S  N  G  N  T  Y  L  E  W  Y  L  Q  K  P  G  Q  S  P  K  L  L  I  Y  K  V  S
AACCGATTTTCTGGGGTCCCAGACAGGTTCAGTGGCAGTGGATCAGGGACAGATTTCACACTCAAGATCAGCAGAGTGGAG 324
                                    158 VK
 N  R  F  S  G  V  P  D  R  F  S  G  S  G  S  G  T  D  F  T  L  K  I  S  R  V  E
GCTGAGGATCTGGGAATTTATTACTGCTTTCAAGGTTCACATGTTCCTCCGACGTTCGGTGGAGGCACCAAGCTGGAAATC 405
                                    158 VK
 A  E  D  L  G  I  Y  Y  C  F  Q  G  S  H  V  P  P  T  F  G  G  G  T  K  L  E  I
       BamHI
AAACGTGAGTGGATCC 421
15 Human K intron
 K  R  E  W  I
```

Table 15. DNA sequence of 158 VH, primer MHV5 and the sequence derived using primers located within the V region

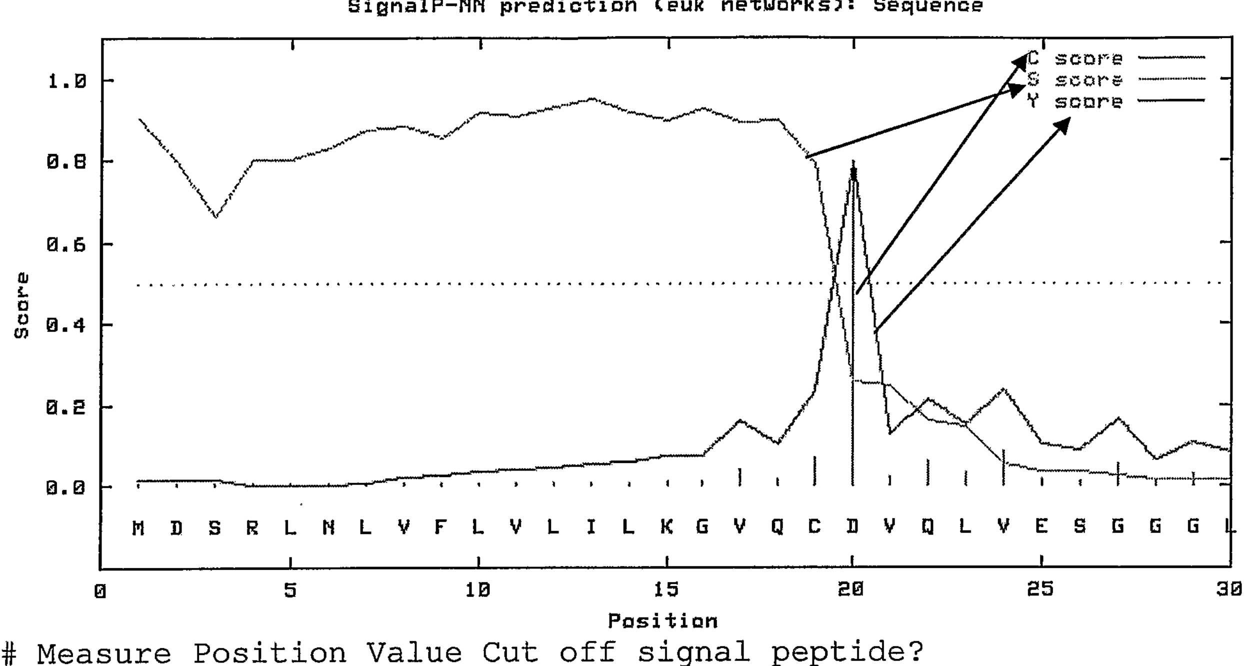
```
  1 ATGGACTCCAGGCTCAATTTAGTTTTCCTTGTCCTTATTTTAAAAGGTGTCCAGTGTGATGTGCAGCTGGTGGAGTCTGGGGGAGGCTTA 158 VH
  1 ---------------------------------------------------------..G...A.....A....A............... *** VH
  1 ..............................                                                            MHV5

 91 GTGCAGCCTGGAGGGTCCCGGAAACTCTCCTGTGCAGCCTCTGGATTCACTTTCAGTAGCTTTGGAATGCACTGGGTTCGTCAGGCTCCA 158 VH
 34 .......................................................................................... *** VH

181 GAGAAGGGGCTGGAGTGGGTCGCATACATTAGTAGTGGCAGTAGTACCATCTACTATGGAGACACAGTGAAGGGCCGATTCACCATCTCC 158 VH
124 .......................................................................................... *** VH

271 AGAGACAATCCCAAGAACACCCTGTTCCTGCAAATGACCAGTCTAAGGTCTGAGGACACGGCCATGTATTACTGTGCAAGAGAGGGGGGA 158 VH
214 .......................................................................................... *** VH

361 TATTACTACGGTAGGAGTTACTATACTATGGACTACTGGGGTCAAGGAACCTCAGTCACCGTCTCCTCAGCCAAAACAACAGCCCCA    158 VH
304 .......................................................................................    *** VH
```

Legend: Residues identical to 158 VH are indicated by a dot . ***Sequencing using primers located within the V region.

Table 16. Chimeric VH leader selection - NL-1 VH leader sequence

158 VH leader	MDSRLNLVFLVLILKGVQC
NL-1 protein	MDSRLNLVFLVLILKGVQC
NL-1 DNA	ATGGACTCCAGGCTCAATTTAGTTTTCCTTGTCCTTATTTTAAAAGGTGTCCAGTGT

Table 17 - SignalP result 6 for NL-1 VH leader sequence



# Measure	Position	Value	Cut off	signal peptide?
max. C	20	0.775	0.32	YES
max. Y	20	0.795	0.33	YES
max. S	13	0.953	0.87	YES
mean S	1-19	0.866	0.48	YES
D	1-19	0.830	0.43	YES

Highest probability for cleavage is between amino acid residue 19 and 20 (VQC-DV)
19 and 20: VQC-DV

Legend: The signal P algorithm[6] generates the combination score Y ,from the cleavage site score C, and the signal peptide score S.

Table 18 - Protein and DNA sequence of chimeric 158 VH

HindIII

AAGCTTGCCGCCACCATGGACTCCAGGCTCAATTTAGTTTTCCTTGTCCTTATTTTAAAAGGTGTCCAGTGTGATGTGCAG 81

kozak — NL-1 leader — 158 VH

K L A A T M D S R L N L V F L V L I L K G V Q C D V Q

CTGGTGGAGTCTGGGGGAGGCTTAGTGCAGCCTGGAGGGTCCCGGAAACTCTCCTGTGCAGCCTCTGGATTCACTTTCAGT 162

158 VH

L V E S G G G L V Q P G G S R K L S C A A S G F T F S

AGCTTTGGAATGCACTGGGTTCGTCAGGCTCCAGAGAAGGGGCTGGAGTGGGTCGCATACATTAGTAGTGGCAGTAGTACC 243

158 VH

S F G M H W V R Q A P E K G L E W V A Y I S S G S S T

ATCTACTATGGAGACACAGTGAAGGGCCGATTCACCATCTCCAGAGACAATCCCAAGAACACCCTGTTCCTGCAAATGACC 324

158 VH

I Y Y G D T V K G R F T I S R D N P K N T L F L Q M T

AGTCTAAGGTCTGAGGACACGGCCATGTATTACTGTGCAAGAGAGGGGGGATATTACTACGGTAGGAGTTACTATACTATG 405

158 VH

S L R S E D T A M Y Y C A R E G G Y Y Y G R S Y Y T M

ApaI

GACTACTGGGGTCAAGGAACCTCAGTCACCGTCTCCTCAGCCTCCACCAAGGGCCC 461

158 VH — Hum HC region

D Y W G Q G T S V T V S S A S T K G P

Table 19 - Expression of chimeric 158 antibody in COS cells

Number of Co-transfections	Expression Vector Constructs Co-Transfected	Antibody Concentration (ng/ml)
2 pooled	pG1D200158 and pKN100158	300
2 pooled	pG1D200158 and pKN100158	3700

Legend: Antibody concentration was measured by ELISA in 3-day cultures of transfected COS 7 cells. COS cells were co-transformed with 10μg each of the heavy and light chain chimeric expression vectors pG1D200158 and pKN100158.

Table 20. Amino acid sequence of 158 VH and 158 VK

VH	DVQLVESGGGLVQPGGSRKLSCAASGFTFSSFGMHWVRQAPEKGLEWVAYISSGSSTIYYGDTVKGRFTISRDNPKNTLFLQMTSLRSEDTAMYYCAREGGYYYGRSYYTMDYWGQGTSVTVSS
VK	DVLMTQTPLSLPVSLGDQASISCRSSQSIVHSNGNTYLEWYLQKPGQSPKLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGIYYCFQGSHVPPTFGGGTKLEIK

Table 21. Best human VH framework VCI scores compared with 158 VH

Kabat Number[6]			2 24 26 27 28 29 30 37 39 45 47 48 49 67 69 71 73 78 91 93 94 103
Canonical Residue[8]			-111-1---------2----1-
Vernier Residue[7]			*--****---*******-**-
Interface Residue[9]			-------IIII-------II-I
Sequence name	FW score	VCI score	VCI Residues
158 VH	87	22	VAGFTFSVQLWVAFIRNLYARW
38687	79	22	....
AB021520	77	22	....
AJ556669	77	22	....
38672	77	22	....
38673	77	22	....
DQ322738	77	22	....
AB067108	76	22	....
AB021531	76	22	....
AB021532	76	22	....
AB063892	76	22	....
AB067237	76	22	....
AB021507	76	22	....
AF471177	76	22	....
AF471184	76	22	....
AF062243	76	22	....
AF174030	76	22	....
AF466141	76	22	....
AF466142	76	22	....
AJ245279	76	22	....
AJ579216	76	22	....

Legend: Canonical residues are numbered in this table according to which CDR they are associated with. FW score and VCI score are the number of residues in the FW or VCI definition respectively, which are identical to their counterpart in 158. Residues identical to those in 158 VH are indicated by a dot.

Table 22. Sequences of best VCI-scoring human VH, compared with 158 VH

Kabat Number[6]	1 2 3 4 5 6 7 8 9 10 11 -12345678901234567890123456789012345AB678901234567890l2ABC345678901234567890123456789012ABC3456789012345678900ABCDEFGHIJKlmnopqrstuv1234567890123
Canonical	1 11 1 1 2 22 2 1
Vernier	* **** *** * * * * * ** *
Interface	I I I I I I I I I I
Kabat CDR	******* ******************* ******************************
158 VH	-DVQLVESGGGLVQPGGSRKLSCAASGFTFSSFGMH--WVRQAPEKGLEWVAYISS--GSSTIYYGDTVKGRFTISRDNPKNTLFLQMTSLRSEDTAMYYCAREGGYYYGRSYYT---------------MDYWGQGTSVTVSS
AB021520	-EVQLVESGGGLVQPGGSLKLSCAASGFTFSSYWMS--WVRQAPGKGLEWVANIKQ--DGSEKYYVDSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARPDDSSGYYSAEY---------------FQHWGQGTLVTVSS
AJ556669	-EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYWMS--WVRQAPGKGLEWVANIKE--DGGEKFYVDSVKGRFTISRDNPKNSLFLQMNSLRAEDTAVYYCARERGHDFWSIYYTH--------------FDYWGQGALVTVSS
DQ322738	PLVQLVESGGGLVQPGGSLRLSCAASGFTFSSYWMS--WVRQAPGKGLEWVAVIWY--DGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDGGSI----------------------FDYWGQGTLVTVSS
AB067108	-EVQLVESGGGVVQPGGSLRLSCAASGFTFSNYAMH--WVRQAPGKGLEWVAVISY--DGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARARDYYYP--------------------MDVWGQGTTVTVSS
AB021531	-QVQLVESGGGVVQPGRSLKLSCAASGFTFSSYAMH--WVRQAPGKGLEWVAVISY--DGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDQSWSRIAAAGTPPSL-----------FDPWGQGTLVTVSS
AB021532	-QVQLVESGGGVVQPGRSLKLSCAASGFTFSSYAMH--WVRQAPGKGLEWVAVISY--DGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARARNYYDSSGYS----------------FDYWGQGTLVTVSS
AB063892	-EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYWMS--WVRQAPGKGLEWVANIKQ--DGSEKYYVDSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARVRRGS----------------------GDSWGQGTLVTVSS
AB067237	-EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYWMS--WVRQAPGKGLEWVANIKQ--DGSEKYYVDSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAREQQLGPHNW------------------FDPWGQGTLVTVSS
AB021507	-QVQLVESGGGVVQPGRSLKLSCAASGFTFSSYAMH--WVRQAPGKGLEWVAVISY--DGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDQETGTTFDYYYYG-------------MDVWGQGTTVTVSS
AF471177	-EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYWMS--WVRQAPGKGLEWVANIKQ--DGSEKYYVDSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARDPMTTVVKPSLAT--------------NDYWGQGTLVTVSS
AF471184	-EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYWMS--WVRQAPGKGLEWVANIKQ--DGSEKYYVDSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARDCVGALGA-------------------FDIWGQGTMVTVSS
AF062243	CEVQLVESGGGLVQPGGSLRLSCSASGFTFSTYWMT--WVRQAPGKGLEWVANIKP--HGSEAYYVDSVKGRFTISRDNAKNSLFLQMSSLRAEDTAVYYCARANS------------------------LDVWGQGTTVTVSS
AF174030	CEVQLVESGGGLVQPGGSLRLSCAASGFTFSSYWMS--WVRQAPGKGLEWVANIKQ--DGSEKYYVDSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARDGDIGDWW-------------------FDPWGQGTLVTVSS
AF466141	-QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMH--WVRQAPGKGLEWVAVIWY--DGSNKYYADSAKGRFTISRDNSKNTLFLQMNSLRAEDTAVYYCARDKGYYDYVWGSYRSNPKNDA-------FDIWGQGTMVTVSS
AF466142	-QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMH--WVRQAPGKGLEWVAVIWY--DGSNKYYADSAKGRFTISRDNSKNTLFLQMNSLRAEDTAVYYCARDKGYYDYVWGSYRSNPKNDA-------FDIWGQGTMVTVSS
AJ245279	-QVQLVESGGGVVQPGGSLRLSCAASGFTFSSYGMH--WVRQAPGKGLEWVAVIWY--DGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDRFF-----------------------FDNWGQGTLVTVSS

Table 23. Alignment of 158 VH with the best VCI-scoring human VH

```
         1         2         3         4         5          6         7         8            9         10                 11
1234567890123456789012345678901234567890123456789012A3456789012345678901234567890123456789012ABC34567890123456789012345678901234567--------890ABCDEFv1234567890123
DVQLVESGGGLVQPGGSRKLSCAASGFTFSSFGMHWVRQAPEKGLEWVAYISSGSSTIYYGDTVKGRFTISRDNPKNTLFLQMTSLRSEDTAMYYCAREGG--------YYYGRSYYTMDYWGQGTSVTVSS   158 VH
E................L.............YW.S......G.......N.KQDG.EK..V.S...........A..S.Y...N...A....V.....PDD--------SSGYY.AEYFQH.....L.....   AB021520
L................LR............YW.S......G.......V.WYDG.NK..A.S...........S....Y...N...A....V.....D.----------S---IF.......L.....   DQ322738
E.........V......LR...........NYA........G.......V..YDG.NK..A.S...........S....Y...N...A....V.....AR--------D...YP----..V.....T.....   AB067108
Q.........V....R.L.............YA........G.......V..YDG.NK..A.S...........S....Y...N...A....V.....DQSWSR-I--AAAGTPPS-LF.P.....L.....   AB021531
Q.........V....R.L.............YA........G.......V..YDG.NK..A.S...........S....Y...N...A....V.....ARNYY-----DSSGYS----F.......L.....   AB021532
E................LR............YW.S......G.......N.KQDG.EK..V.S...........A..S.Y...N...A....V.....VRR--------GSG-------.S.....L.....   AB063892
E................LR............YW.S......G.......N.KQDG.EK..V.S...........A..S.Y...N...A....V......Q--------QLGP--HNWF.P.....L.....   AB067237
Q.........V....R.L.............YA........G.......V..YDG.NK..A.S...........S....Y...N...A....V.....DQETGTTF--D...YG----..V.....T.....   AB021507
E................LR............YW.S......G.......N.KQDG.EK..V.S...........A..S.Y...N...A....V.....DPMT-------TVVKP.LA.N.......L.....   AF471177
E................LR............YW.S......G.......N.KQDG.EK..V.S...........A..S.Y...N...A....V.....DPMT-------TVVKP.LA.N.......L.....   AF471184
E................LR...S........TYW.T......G.......N.KPHG.EA..V.S...........A..S.....S...A....V.....AN-----------------SL.V.....T.....   AF062243
```

Legend: Residues identical to 158 VH are represented by a dot. CDRs are grey-shaded

Table 24. VH signal peptide selection - 5.5.1 V-gene alignment of 158 VH, human AF062243 and human germline M99649 (VH3-07)

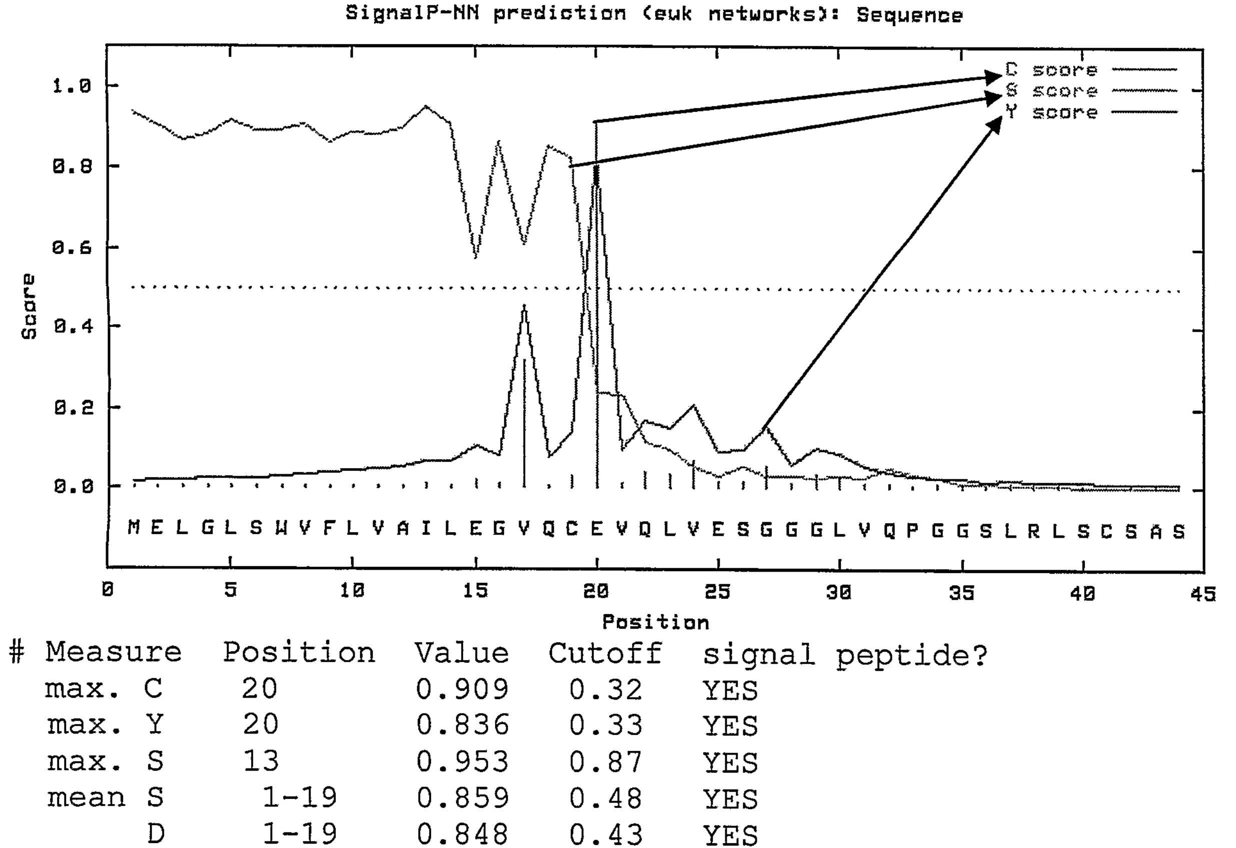
```
DVQLVESGGGLVQPGGSRKLSCAASGFTFSSFGMHWVRQAPEKGLEWVAYIS 158 VH
E................LR...S.......TYW.T......G.......N.K AF062243
E................LR............YW.S......G.......N.K M99649

SGSSTIYYGDTVKGRFTISRDNPKNTLFLQMTSLRSEDTAMYYCAR         158 VH
PHG.EA..V.S...........A..S.....S...A....V.....         AF062243
QDG.EK..V.S...........A..S.Y...N...A....V.....         M99649
```

Legend: V-gene residues identical to 158 VH are represented by a dot.

Table 25. Signal peptide of M99649 human germline VH gene

DNA	ATGGAATTGGGGCTGAGCTGGGTTTTCCTTGTTGCTATTTTAGAAGGTGTCCAGTGT
protein	MELGLSWVFLVAILEGVQC

Table 26. M99649 signal peptide cutting prediction



# Measure	Position	Value	Cutoff	signal peptide?
max. C	20	0.909	0.32	YES
max. Y	20	0.836	0.33	YES
max. S	13	0.953	0.87	YES
mean S	1-19	0.859	0.48	YES
D	1-19	0.848	0.43	YES

Highest probability for cleavage is between amino acid residue 19-20: VQC-DV.

Legend: The signal P algorithm[10] generates the combination score Y ,from the cleavage site score C, and the signal peptide score S

Table 27. Generation of 158RHA protein sequence

Kabat Number[6]	1 2 3 4 5 6 7 8 9 10 11 012345678901234567890123456789012345AB6789012345678901 2ABC345678901234567890123456789012ABC3456789012345678 90ABCDEFGHIJK1234567890123
CDR	===H1== =====H2=========== =========H3========
158 VH	-DVQLVESGGGLVQPGGSRKLSCAASGFTFSSFGMH--WVRQAPEKGLEWVAYISS--GSSTIYYGDTVKGRFTISRDNPKNTLFLQMTSLRSEDTAMYYCAREGGYYYGRSYYT----MDYWGQGTSVTVSS
158 CDR	SFGMH-- YISS--GSSTIYYGDTVKG EGGYYYGRSYYT----MDY
158RHA	-EVQLVESGGGLVQPGGSLRLSCSASGFTFSSFGMH--WVRQAPGKGLEWVAYISS--GSSTIYYGDTVKGRFTISRDNAKNSLFLQMSSLRAEDTAVYYCAREGGYYYGRSYYT----MDYWGQGTTVTVS
AF062243 FW	-EVQLVESGGGLVQPGGSLRLSCSASGFTFS WVRQAPGKGLEWVA RFTISRDNAKNSLFLQMSSLRAEDTAVYYCAR WGQGTTVTVS
AF062243	CEVQLVESGGGLVQPGGSLRLSCSASGFTFSTYWMT--WVRQAPGKGLEWVANIKP--HGSEAYYVDSVKGRFTISRDNAKNSLFLQMSSLRAEDTAVYYCARANS------------LDVWGQGTTVTVSS

Table 28. Generation of 158RHA DNA sequence - Generation of 158RHAss DNA sequence

Region	DNA	Protein
VH3-07 leader	ATGGAATTGGGGCTGAGCTGGGTTTTCCTTGTTGCTATTTTAGAAGGTGTCCAGTGT	MELGLSWVFLVAILEGVQC
AF062243 FW1	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTGGGGGGTCCCTGAGACTCTCCTGTTCAGCCTCTGGATTCACCTTTAGT	EVQLVESGGGLVQPGGSLRLS CSASGFTFS
158 CDR1	AGCTTTGGAATGCAC	SFGMH
AF062243 FW2	TGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTGGCC	WVRQAPGKGLEWVA
158 CDR2	TACATTAGTAGTGGCAGTAGTACCATCTACTATGGAGACACAGTGAAGGGC	YISSGSSTIYYGDTVKG
AF062243 FW3	CGATTCACCATCTCCAGAGACAACGCCAAGAACTCACTGTTTCTGCAAATGAGCAGCCTGAGAGCCGAGGACACGGCCGT GTATTATTGTGCGAGA	RFTISRDNAKNSLFLQMSSLR AEDTAVYYCAR
158 CDR3	GAGGGGGGATATTACTACGGTAGGAGTTACTATACTATGGACTAC	EGGYYYGRSYYTMDY
AF062243 FW4	TGGGGCCAAGGGACCACGGTCACCGTCTCC	WGQGTTVTVS

Legend: Human VH3-07 leader and AF062243VH FWs intercalated with 158 VH CDRs (grey-shaded) to generate 158RHAss. (relate to Tables 27 and 28)

Table 29. DNA and protein sequence of 158RHAss

```
ATGGAATTGGGGCTGAGCTGGGTTTTCCTTGTTGCTATTTTAGAAGGTGTCCAGTGTGAGGTGCAGCTGGTGGAGTCTGGG   81
                                        |splice 4.3|  |splice 6.9|
                                                  |splice 5.6|
 M  E  L  G  L  S  W  V  F  L  V  A  I  L  E  G  V  Q  C  E  V  Q  L  V  E  S  G
GGAGGCTTGGTCCAGCCTGGGGGGTCCCTGAGACTCTCCTGTTCAGCCTCTGGATTCACCTTTAGTAGCTTTGGAATGCAC  162
 G  G  L  V  Q  P  G  G  S  L  R  L  S  C  S  A  S  G  F  T  F  S  S  F  G  M  H
TGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTGGCCTACATTAGTAGTGGCAGTAGTACCATCTACTATGGAGAC  243
                          |splice 5.9|
 W  V  R  Q  A  P  G  K  G  L  E  W  V  A  Y  I  S  S  G  S  S  T  I  Y  Y  G  D
ACAGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACTCACTGTTTCTGCAAATGAGCAGCCTGAGAGCCGAG  324
|splice 5.0|
 T  V  K  G  R  F  T  I  S  R  D  N  A  K  N  S  L  F  L  Q  M  S  S  L  R  A  E
GACACGGCCGTGTATTATTGTGCGAGAGAGGGGGGATATTACTACGGTAGGAGTTACTATACTATGGACTACTGGGGCCAA  405
                                        |splice 4.7|
 D  T  A  V  Y  Y  C  A  R  E  G  G  Y  Y  Y  G  R  S  Y  Y  T  M  D  Y  W  G  Q
GGGACCACGGTCACCGTCTCC  426
 G  T  T  V  T  V  S
```

Legend:
Splice donor sites predicted by Lasergene 6.0 GeneQuest analysis, together with their score, using the human_ds_2 matrix with a threshold of 4.2.

Table 30. Mutations in 158RHA removing splice sites in 158RHAss

```
  1 ATGGAATTGGGGCTGAGCTGGGTTTTCCTTGTTGCTATTTTAGAGGGAGT 158RHA
  1 ..........................................A..T..
158RHAss

 51 CCAGTGCGAAGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTG 158RHA
 51 ......T..G........................................
158RHAss

101 GGGGGTCCCTGAGACTCTCCTGTTCAGCCTCTGGATTCACCTTTAGTAGC 158RHA
101 ..................................................
158RHAss

151 TTTGGAATGCACTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAATGGGT 158RHA
151 ............................................G.....
158RHAss

201 GGCCTACATTAGTAGTGGCAGTAGTACCATCTACTATGGAGACACCGTGA 158RHA
201 .............................................A....
158RHAss

251 AGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACTCACTGTTTCTG 158RHA
251 ..................................................
158RHAss

301 CAAATGAGCAGCCTGAGAGCCGAGGACACGGCCGTGTATTATTGTGCGAG 158RHA
301 ..................................................
158RHAss

351 AGAGGGGGGATATTACTACGGAAGGAGTTACTATACTATGGACTACTGGG 158RHA
351 ....................T.............................
158RHAss

401 GCCAAGGGACCACGGTCACCGTCTCC                         158RHA
401 ..........................
158RHAss
```

Legend 158RHA DNA sequence compared to 158RHAss (Table 5.7.2) which contains predicted splice sites. Positions identical to 158RHA are identified as a dot.

Table 31. DNA and protein sequence of 158RHA

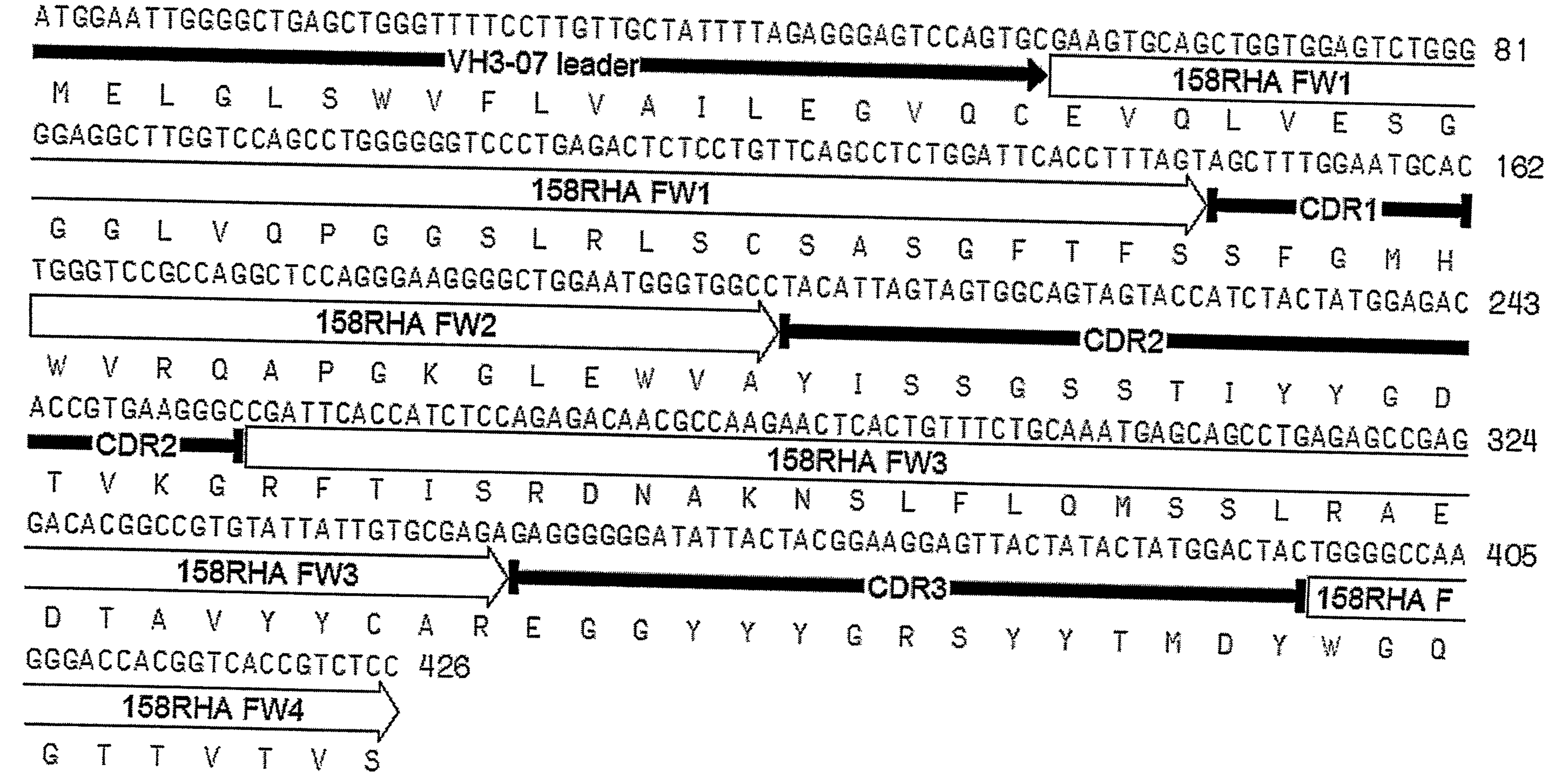


Table 32. Best VCI scores of human VK compared with 158 VK

Kabat Number[6]			2 4 35 36 38 44 46 47 48 49 64 66 68 69 71 87 98
Canonical Residue[8]			1 2 2 1
Vernier Residue[7]			**** ********* *
Interface Residue[9]			IIII II
Sequence	Fw score	VCI score	VCI residues
158 VK	80	17	VMWYQPLLIYGGGTFYF
AB064054	71	17	V................
AB063934	70	17	V................
AB064105	70	17	V................
AY941999	70	17	V................
AX805665	69	17	V................
AB064104	69	17	V................
AY942057	69	17	V................
AB064055	68	17	V................
AX742874	68	17	V................
AY685343	67	17	V................
AY685353	67	17	V................
DQ187506	70	16	I................
DQ187679	70	16	I................
AY043107	69	16	I................
AJ388639	69	16	........V........
AJ388646	69	16	I................
AJ388642	69	16	I................
M74470	69	16	I................
X72466	69	16	I................
U95244	69	16	I................
AAA51016	69	16	I................
X89054	69	16	I................
DQ187505	69	16	I................
DQ187683	69	16	I................
DQ187691	69	16	I................
AX805669	68	16	I................
AF455562	68	16	I................

Legend: Canonical residues are numbered in this table according to which CDR they are associated. FW score and VCI score are the number of residues in the FW or VCI definition respectively, which are identical to their counterpart in 158. Residues identical to 158 VK are indicated by a dot.

Table 33. Sequences of best VCI-scoring human VK, compared with 158 VK

Kabat number [6]	1 2 3 4 5 6 7 8 9 10 12345678901234567890123456 7ABCDEF89012345678901234567890123456789012345678901234567890123456789012345ABCDEF678901234567
Canonical [8]	1 1 1 1 1 2 2 1 3 3 3
Vernier [7]	* * ** **** * * ** * *
Interface [9]	F F F F F F F F F F
VCI	1 * 1 1 1 1F*F F F **** * * ** * F F3F 33 * F3F
Kabat CDR	**************** ******* ***************
158 VK	DVLMTQTPLSLPVSLGDQASISCRSSQSIVHS-NGNTYLEWYLQKPGQSPKLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGIYYCFQGSHVP------PTFGGGTKLEIK
AB064054	DVVMTQSPLSLPVTPGAPASISCRSSQSLLHT-NGVNFLDWYLQKPGQSPKLLIYLASHRASGVPDRFSGSGSGTDFTLRISRVEAEDVGIYYCMQGLQTP------FTFGPGTKLEIK
AB063934	DVVMTQSPLSLPVTPGEPASISCRSSQSLLHS-NGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQTP------LTFGGGTKVEIK
AB064105 scFv	DVVMTQSPLSLPVTPGEPASISCRSSQSLLHS-NGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQTP------YTFGQGTKLEIK
AY941999 scFv	DVVMTQSPLSLPVTPGEPASISCRSSQSLLHS-NGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQTP------YTFGQGTKLEIK
AX805665 patent	DVVMTQSPLSLPVTPGEPASISCRSSQSIVHS-NGNTYLQWYLQKPGQSPQLLIYKVSNRLYGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVP------WTFGQGTKVEIK
AB064104	DVVMTQSPLSLPVTPGEPASISCRSSQSLLHS-NGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDGFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQTP------HTFGQGTKLEIK
AY942057 scFv	DVVMTQSPLSLPVTPGEPASISCRSSQSLLHS-NGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQSP------PTFGRGTKVEIK
AB064055	DVVMTQSPLSLPVTPGEPASISCRSSQSLLHS-NGYNYSDWYLQKPGQSPQLLIYLGSSRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQTP------FTFGPGTKVDIK
AX742874	DVVMTQTPLSLSVTPGQPASISCRSSQSLLHS-DGMTYFSWYLQKPGQPPQLLIYEVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQNIQLP------WTFGQGTKVEIK
AY685343	DVVMTQSPLSLAVTPGEPASISCRSSQSVVFT-NGKNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEADDVGVYYCMHAVQAP------WTFGQGTKVEIK
AY685353	DVVMTQSPLSLAVTPGEPASISCRSSQSVVFT-NGKNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEADDVGVYYCMHAVQAP------WTFGQGTKVEIK
DQ187506	DIVMTQTPLSLPVTPGEPASISCRSSQSLLES-HGYNYLDWYLQKPGQSPQLLIYLASNRPSGVPDRFSGSGSGTDFTLKISRVEAEDVGIYYCMQNLQTP------YSFGQGTKLEIR
DQ187679	DIVMTQTPLSLPVTPGEPASISCRSSQSLLHS-NGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQTP------HSFGQGTKLEIK
AY043107	DIVMTQSPLSLPVTPGEPASISCRSSQSLLHS-NGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQTP------LTFGGGTKVEIK
AJ388639	DVVMTQSPLSLPVTPGEPASISCRSSQSLLHS-NGYNYFAWYLQKPGQSPQLLVYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGIYYCMQVLQTP------YTFGQGTKLEIS
AJ388646	DIVMTQSPLSLPVTPGEPASISCRSSQSLLHS-NGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQTP------LTFGGGTKVEIK
AJ388642	DIVMTQSPLSLPVTPGEPASISCRSSQSLLHS-NGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQTP------PTFGGGTKVEIK
M74470	DIVMTQSPLSLPVTPGEPASISCRSSQSLLHS-NGFNYLHWYLQKPGQSPRLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEADDVGIYYCMQALQSP------YTFGQGTKLEIK
X72466	DIVMTQSPLSLPVTPGEPASISCRSSQSLLHN-NGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQVLQIP------LTFGGGTKVEIK
U95244	DIVMTQSPLSLPVTPGEPASISCRSSQSLLYS-NGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGDYYCMQALQSP------LTFGGGTKVEIK
AAA51016	DIVMTQSPLSLPVTPGEPASISCRSSQSLLHS-NGFNYLHWYLQKPGQSPRLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEADDVGIYYCMQALQSP------YTFGQGTKLEIK
X89054	DIVMTQSPLSLPVTPGEPASISCRSSQSLLHS-NGYNYFDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQTP------LTFGGGTKVEIK
DQ187505	DIVMTQSPLSLPVTPGEPASISCRSSQSLLES-HGYNYLDWYLQKPGQSPQLLIYLASNRPSGVPDRFSGSGSGTDFTLKISRVEAEDVGIYYCMQNLQTP------YSFGQGTKLEIR
DQ187683	DIVMTQTPLSLPVTPGEPASISCRSSQSLLHG-NGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQTP------RTFGQGTKVEIK
DQ187691	DIVMTQTPLSLPVTPGEPASISCRSSQSLLHG-NGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQTP------RTFGQGTKVEIK
AX805669	DIVMTQSPLSLPVTPGEPASISCRSSQSIVHS-NGNTYLQWYLQKPGQSPQLLIYKVSNRLYGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVP------WTFGQGTKVEIK

Table 34. Alignment of 158 VK with the best VCI-scoring human VK

```
         1         2             3         4         5         6         7         8         9         10      Kabat number
1234567890123456789012345678ABCDE8901234567890123456789012345678901234567890123456789012345678901234567890123456789012345678901234567
DVLMTQTPLSLPVSLGDQASISCRSSQSIVHSNGNTYLEWYLQKPGQSPKLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGIYYCFQGSHVPPTFGGGTKLEIK 158K
..V...S......TP.AP.........LL.T..VNF.D...............LA.H.A..................R........V.....M..LQT.F...P....... AB064054
..V...S......TP.EP.........LL....YN..D...........Q...LG...A...........................V.V...M.ALQT.L.......V... AB063934
..V...S......TP.EP.........LL....YN..D...........Q...LG...A...........................V.V...M.ALQT.Y...Q....... AB064105
..V...S......TP.EP.........LL....YN..D...........Q...LG...A...........................V.V...M.ALQT.Y...Q....... AY941999
..V...S......TP.EP...................Q...........Q.......LY...........................V.V.........W...Q...V... AX805665
..V...S......TP.EP.........LL....YN..D...........Q...LG...A.....G.....................V.V...M.ALQT.H...Q....... AB064104
..V...S......TP.EP.........LL....YN..D...........Q...LG...A...........................V.V...M.ALQS.....R...V... AY942057
..V........S.TP.QP.........LL..D.M..FS.........P.Q...LG.S.A...........................V.V...M.ALQT.F...P...VD.. AB064055
..V...S....A.TP.EP.........V.FT..KN..D...........Q...E................................V.V...M.NIQL.W...Q...V... AX742874
..V...S....A.TP.EP.......Y.V.FT..KN..D...........Q...LG...A.........................D.V.V...MHAVQA.W...Q...V... AY685343
..V...S....A.TP.EP.........V.FT..KN..D...........Q...LG...A.........................D.V.V...MHAVQA.W...Q...V... AY685353
.IV..........TP.EP......A..LLE.H.YN..D...........Q...LA...P...........................V.....M.NLQT.YS..Q......R DQ187506
.IV..........TP.EP.........LL....YN..D...........Q...LG...A...........................V.V...M.ALQT.HS..Q....... DQ187679
.IV...S......TP.EP.........LL....YN..D...........Q...LG...A...........................V.V...M.ALQT.L.......V... AY043107
..V...S......TP.EP.........LL....YN.FA...........Q..V.LG...A..........................V.....M.VLQT.Y...Q......S AJ388639
.IV...S......TP.EP.........LL....YN..D...........Q...LG...A...........................V.V...M.ALQT.L.......V... AJ388646
.IV...S......TP.EP.........LL....YN..D...........Q...LG...A...........................V.V...M.ALQT.........V... AJ388642
.IV...S......TP.EP.........LL....FN..H...........R...LG...A.........................D.V.....M.ALQS.Y...Q....... AAA51016
.IV...S......TP.EP.........LL....YN.FD...........Q...LG...A...........................V.V...M.ALQT.L.......V... X89054
.IV...S......TP.EP.....S...LLE.H.YN..D...........Q...LA...P...........................V.....M.NLQT.YS..Q......R DQ187505
.IV..........TP.EP.........LL.G..YN..D...........Q...LG...A...........................V.V...M.ALQT.R...Q...V... DQ187683
.IV..........TP.EP.........LL.G..YN..D...........Q...LG...A...........................V.V...M.ALQT.R...Q...V... DQ187691
.IV...S......TP.EP...................Q...........Q.......LY...........................V.V.........W...Q...V... AX805669
```

Legend: CDR 1, 2 and 3 are grey-shaded.

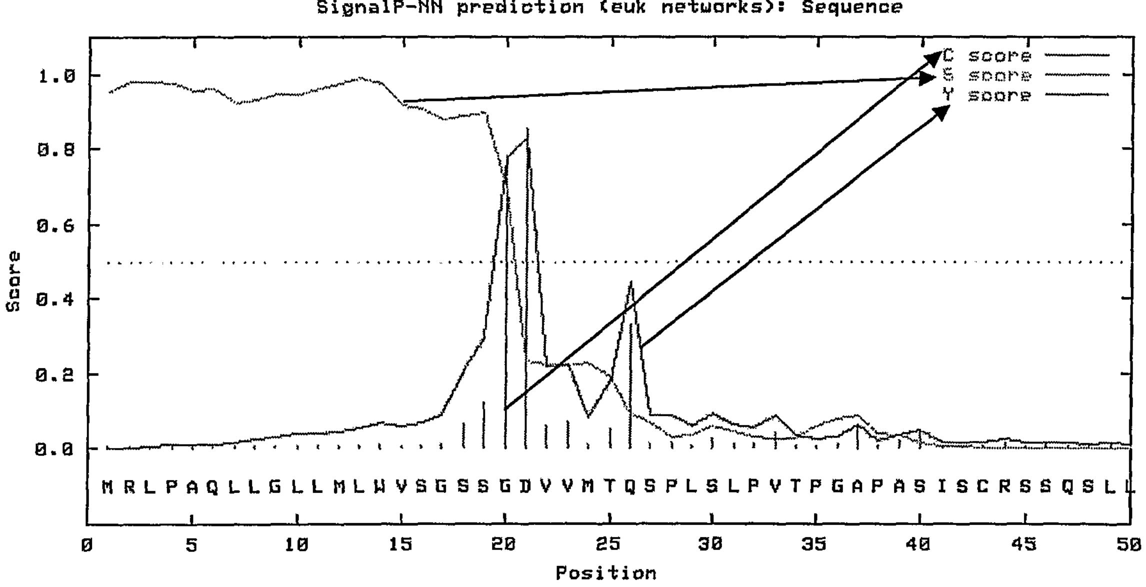
Table 35. VK signal peptide selection - Alignment of 158 VK with human AB064054 and human germline A19

```
DVLMTQTPLSLPVSLGDQASISCRSSQSIVHSNGNTYLEWYLQKPGQSPKLLIYKVSN 158 VK
..V...S......TP.AP..........LL.T..VNF.D...............LA.H AB064054
.IV...S......TP.EP..........LL....YN..D..........Q....LG.. A19

RFSGVPDRFSGSGSGTDFTLKISRVEAEDLGIYYCFQGSHVPPTFGGGTKLEIK     158 VK
.A..................R........V.....M..LQT.F...P......N     AB064054
.A...........................V.V...M.ALQT.                 A19
```

Table 36. Signal peptide of human A19 (VK2-28; X63397) germline VK

	VK A19 leader sequence
DNA	ATGAGGCTCCCTGCTCAGCTCCTGGGGCTGCTAATGCTCTGGGTCTCTGGATCCAGTGGG
protein	MRLPAQLLGLLMLWVSGSSG

Table 37. A19 signal peptide cutting prediction




```
>Sequence                  length = 50
# Measure  Position  Value  Cutoff  signal peptide?
  max. C    21       0.853   0.32   YES
  max. Y    21       0.831   0.33   YES
  max. S    13       0.990   0.87   YES
  mean S     1-20    0.932   0.48   YES
       D     1-20    0.881   0.43   YES
# Most likely cleavage site between pos. 20 and 21: SSG-DV
```

Legend: The signal P algorithm[10] generates the combination score Y ,from the cleavage site score C, and the signal peptide score S.

Table 38. Generation of 158RKA Protein Sequence

Kabat number [6]	1 2 3 4 5 6 7 8 9 10 1234567890123456789012345 67ABCDEF890123456789012345678901234567890123456789012345678901234567890123 45ABCDEF67890123456
CDR	=======L1======== ==L2=== ======L3=======
158 VK	DVLMTQTPLSLPVSLGDQASISCRSSQSIVHS-NGNTYLEWYLQKPGQSPKLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGIYYCFQGSHVP------PTFGGGTKLEI
CDR	RSSQSIVHS-NGNTYLE KVSNRFS FQGSHVP------PT
158RKA	DVVMTQSPLSLPVTPGAPASISCRSSQSIVHS-NGNTYLEWYLQKPGQSPKLLIYKVSNRFSGVPDRFSGSGSGTDFTLRISRVEAEDVGIYYCFQGSHVP------PTFGPGTKLEIK
FW	DVVMTQSPLSLPVTPGAPASISC WYLQKPGQSPKLLIY GVPDRFSGSGSGTDFTLRISRVEAEDVGIYYC FGPGTKLEIK
AB064054	DVVMTQSPLSLPVTPGAPASISCRSSQSLLHT-NGVNFLDWYLQKPGQSPKLLIYLASHRASGVPDRFSGSGSGTDFTLRISRVEAEDVGIYYCMQGLQTP------FTFGPGTKLEIK

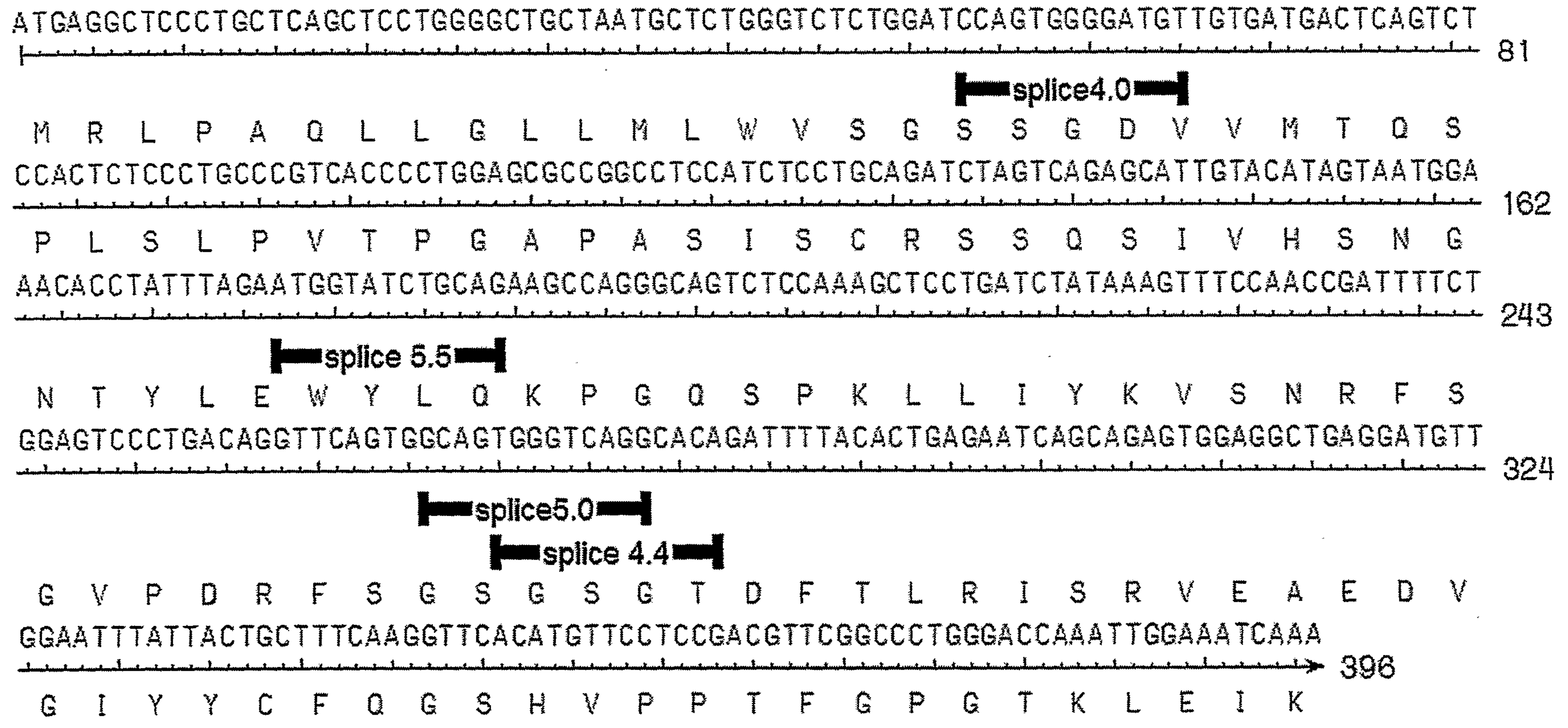
Legend: 158 VK (grey-shaded) CDRs intercalated in human AB064054 FW to generate 158RKA

Table 39 - Generation of 158RKAss DNA Sequence - Intercalation of 158VK into AB064054 FW

Region	DNA	Protein
A19 leader	ATGAGGCTCCCTGCTCAGCTCCTGGGGCTGCTAATGCTCTGGGTCTCTGGATCCAGTGGG	MRLPAQLLGLLMLWVSGSSG
AB064054 FW1	GATGTTGTGATGACTCAGTCTCCACTCTCCCTGCCCGTCACCCCTGGAGCGCCGGCCTCCATCTCCTGC	DVVMTQSPLSLPVTPGAPASISC
158 CDR1	AGATCTAGTCAGAGCATTGTACATAGTAATGGAAACACCTATTTAGAA	RSSQSIVHSNGNTYLE
AB064054 FW2	TGGTATCTGCAGAAGCCAGGGCAGTCTCCAAAGCTCCTGATCTAT	WYLQKPGQSPKLLIY
158 CDR2	AAAGTTTCCAACCGATTTTCT	KVSNRFS
AB064054 FW3	GGAGTCCCTGACAGGTTCAGTGGCAGTGGGTCAGGCACAGATTTTACACTGAGAATCAGCAGAGTGGAGGC TGAGGATGTTGGAATTTATTACTGC	GVPDRFSGSGSGTDFTLRISRVEAEDVGIYYC
158 CDR3	TTTCAAGGTTCACATGTTCCTCCGACG	FQGSHVPPT
AB064054 FW4	TTCGGCCCTGGGACCAAATTGGAAATCAAA	FGPGTKLEIK

Legend: A19 leader and AB064054 FW with intercalated 158 VK (grey-shaded) CDRs to generate 158RKAss

Table 40. 158RKAss DNA sequence



Legend: Splice donor sites predicted by Lasergene 6.0 GeneQuest analysis, together with their score, using the human_ds_2 matrix with a threshold of 4.2.

Table 41. Mutations in 158RKA removing splice sites in 158RKA

```
1   ATGAGGCTCCCTGCTCAGCTCCTGGGGCTGCTAATGCTCTGGGTCTCTGGAAGCAGTGGG 158RKA
1   ...................................................TC....... 158RKAss

61  GATGTTGTGATGACTCAGTCTCCACTCTCCCTGCCCGTCACCCCTGGAGCGCCGGCCTCC 158RKA
61  ............................................................ 158RKAss

121 ATCTCCTGCAGATCTAGTCAGAGCATTGTACATAGTAATGGAAACACCTATTTAGAGTGG 158RKA
121 ........................................................A... 158RKAss

181 TATCTTCAAAAGCCAGGGCAGTCTCCAAAGCTCCTGATCTATAAAGTTTCCAACCGATTT 158RKA
181 .....G..G................................................... 158RKAss

241 TCTGGAGTCCCTGACAGGTTCAGTGGAAGTGGATCAGGCACAGATTTTACACTGAGAATC 158RKA
241 ..........................C.....G........................... 158RKAss

301 AGCAGAGTGGAGGCTGAGGATGTTGGAATTTATTACTGCTTTCAAGGTTCACATGTTCCT 158RKA
301 ............................................................ 158RKAss

361 CCGACGTTCGGCCCTGGGACCAAATTGGAAATCAAA                         158RKA
361 ....................................                         158RKAss
```

Legend: 158RKA DNA sequence compared to 158RKAss (Table 5.13.2) which contains predicted splice sites. Residues identical to 158RKA are identified by a dot.

Table 42. DNA and protein sequence of 158RKA

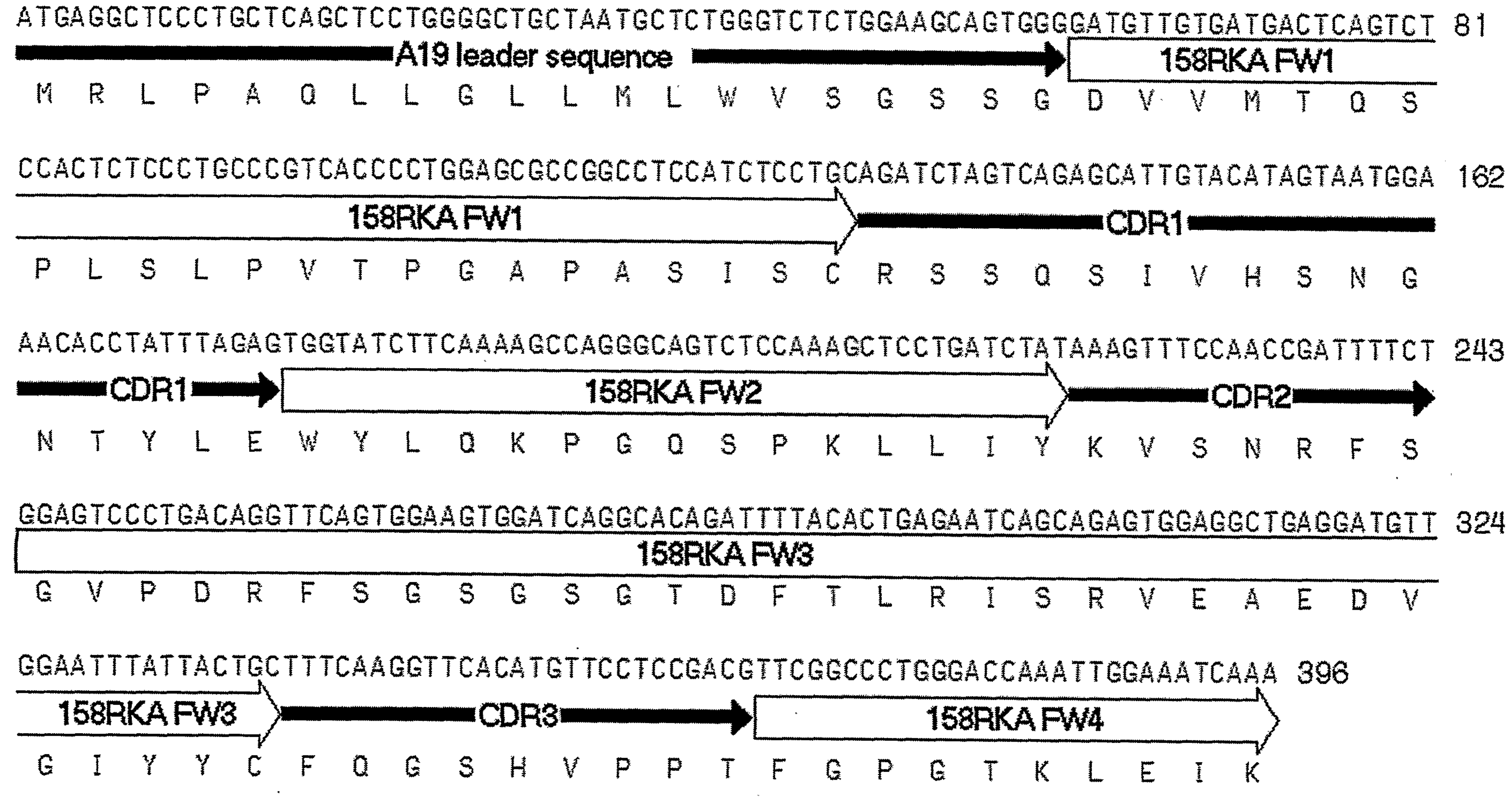


Table 43. Further version of humanized 158VHA (158RHB,158RHC,158RHD)

Kabat number[6]	1 2 3 4 5 6 7 8 9 10 11 12345678901234567890123456789012345678901234567890 12A3456789012345678901234567890 12ABC34567890123456789 0ABCDEFv1234567890123
158RHA	EVQLVESGGGLVQPGGSLRLSCSASGFTFSSFGMHWVRQAPGKGLEWVAYISSGSSTIYYGDTVKGRFTISRDNAKNSLFLQMSSLRAEDTAVYYCAREGGYYYGRSYYTMDYWGQGTTVTVS
158RHB	EVQLVESGGGLVQPGGSLRLSCSASGFTFSSFGMHWVRQAPGKGLEWVAYISSGSSTIYYGDTVKGRFTISRDNAKNSLFLQMSSLRAEDTAVYYCAREGGYYYGRSYYTMDYWGQGTTVTVS
158RHC	EVQLVESGGGLVQPGGSLRLSCSASGFTFSSFGMHWVRQAPGKGLEWVAYISSGSSTIYYGDTVKGRFTISRDNPKNSLFLQMSSLRAEDTAVYYCAREGGYYYGRSYYTMDYWGQGTTVTVS
158RHD	EVQLVESGGGLVQPGGSLRLSCSASGFTFSSFGMHWVRQAPGKGLEWVAYISSGSSTIYYGDTVKGRFTISRDNAKNSLFLQMTSLRAEDTAVYYCAREGGYYYGRSYYTMDYWGQGTTVTVS

Table 44. Further version of humanized 158VKA (158RKB,158RKC)

Kabat number	1 2 3 4 5 6 7 8 9 10 1234567890123456789012345 67ABCDE8901234567890123456789012345678901234567890123456789012345678901234567
158RKA	DVVMTQSPLSLPVTPGAPASISCRSSQSIVHSNGNTYLEWYLQKPGQSPKLLIYKVSNRFSGVPDRFSGSGSGTDFTLRISRVEAEDVGIYYCFQGSHVPPTFGPGTKLEIK
158RKB	DVLMTQSPLSLPVTPGAPASISCRSSQSIVHSNGNTYLEWYLQKPGQSPKLLIYKVSNRFSGVPDRFSGSGSGTDFTLRISRVEAEDVGIYYCFQGSHVPPTFGPGTKLEIK
158RKC	DVVMTQSPLSLPVTPGAPASISCRSSQSIVHSNGNTYLEWYLQKPGQSPKLLIYKVSNRFSGVPDRFSGSGSGTDFTLRISRVEAEDVGIYYCFQGSHVPPTFGGGTKLEIK

Figure 1

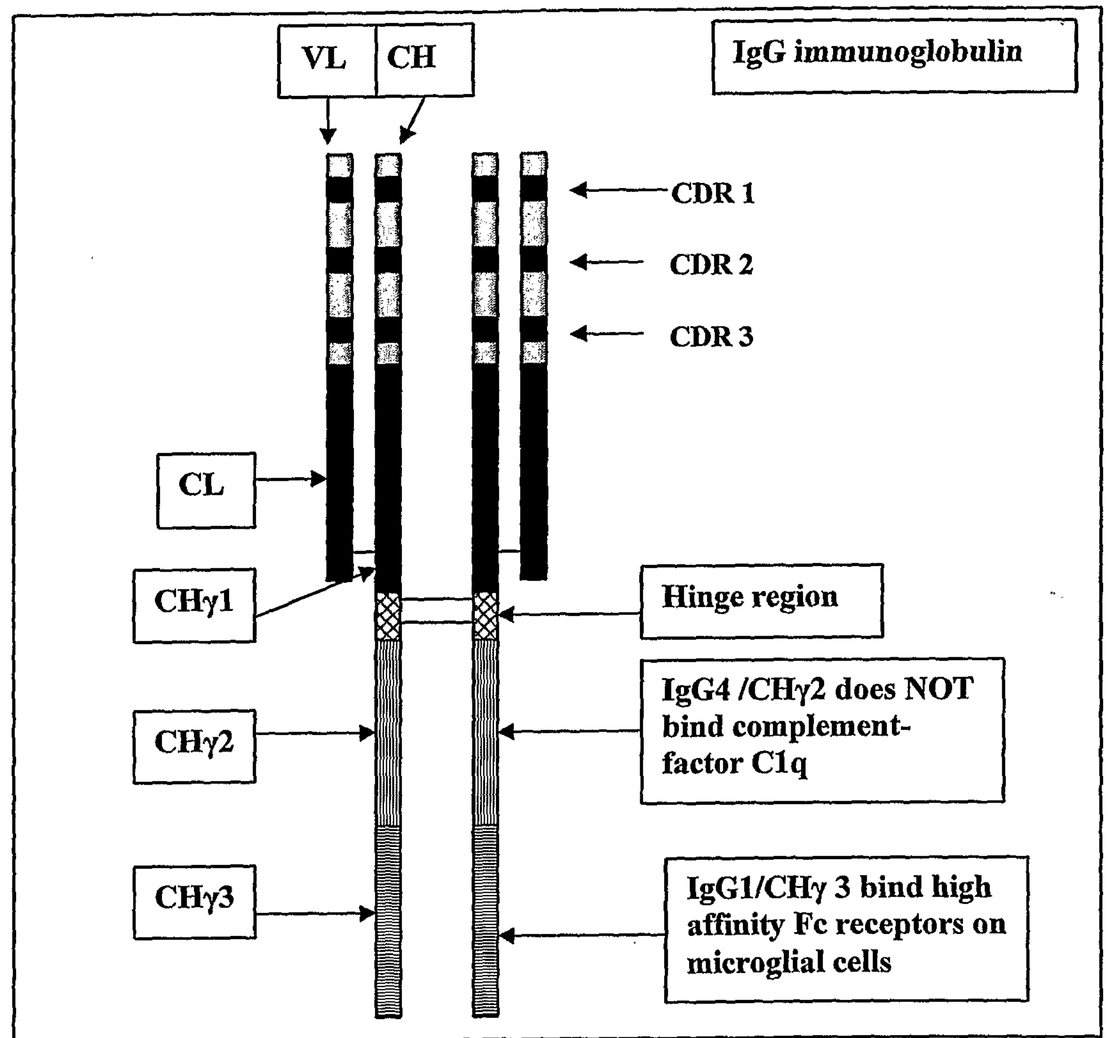
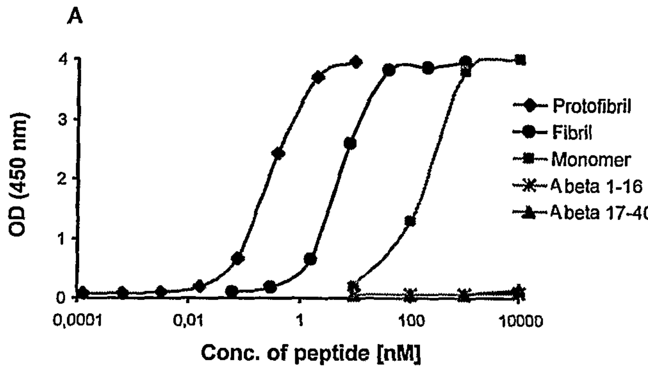


Figure 2: Characterization of a high affinity protofibril selective monoclonal antibody.



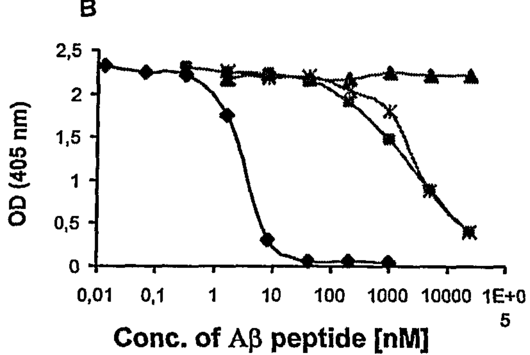


Figure 3. Therapeutic efficacy of a high affinity protofibril selective antibody in transgenic mouse model (APPswe)

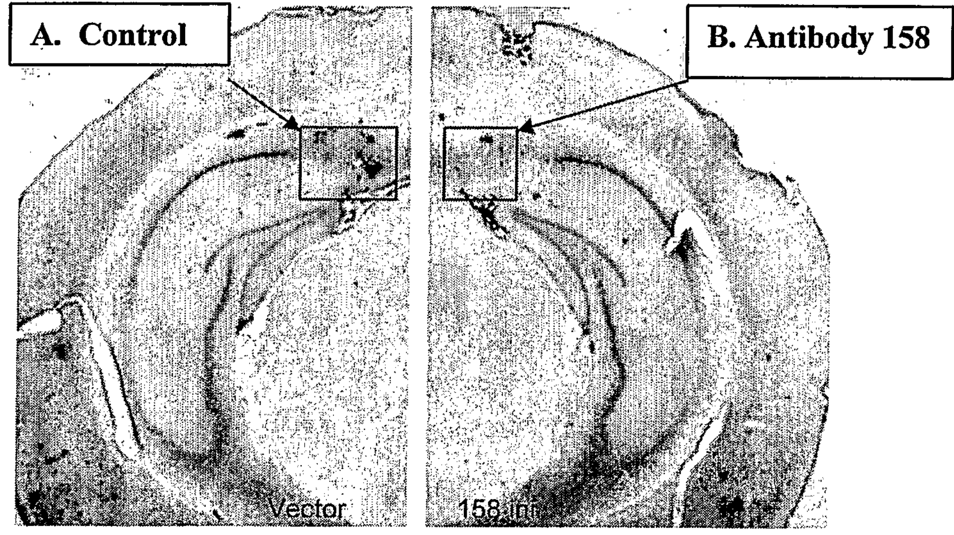


Figure 4
Human Aß protofibrils are measured at pM levels by proximity ligation technique.

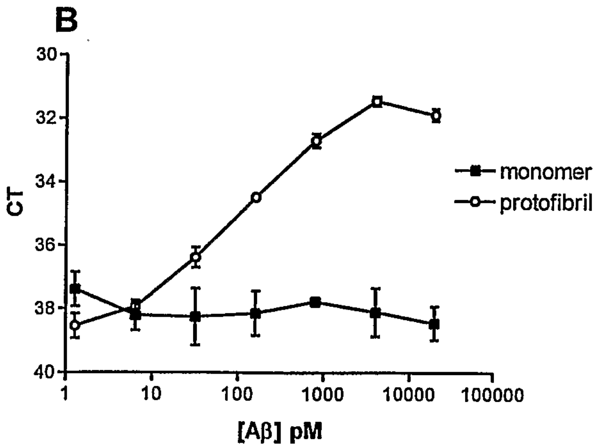


Figure 5

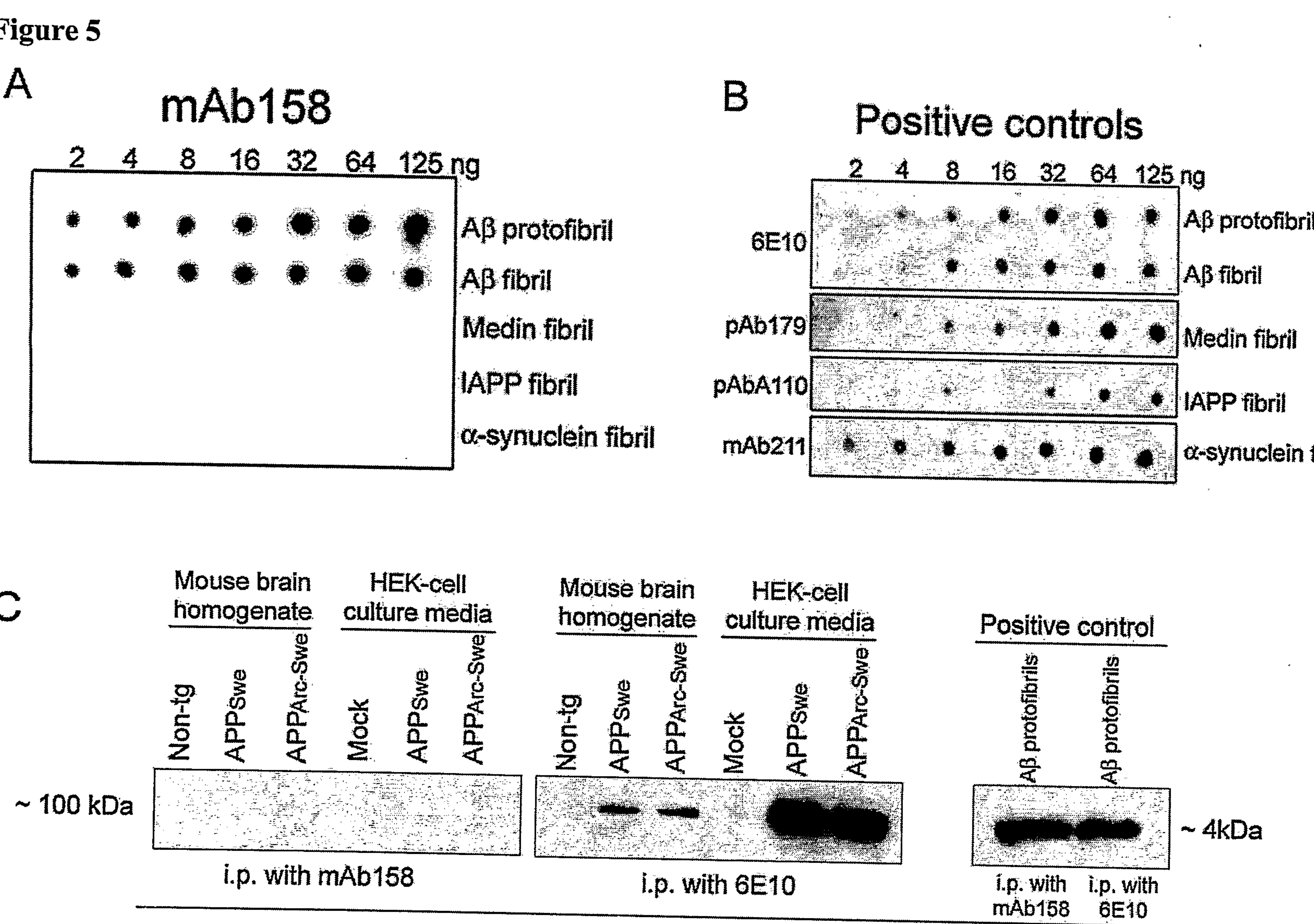


Figure 6

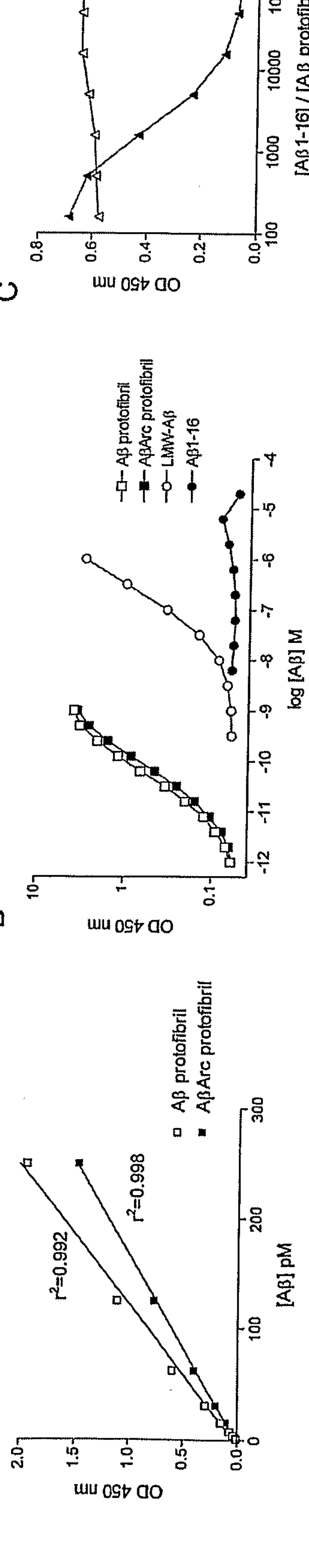
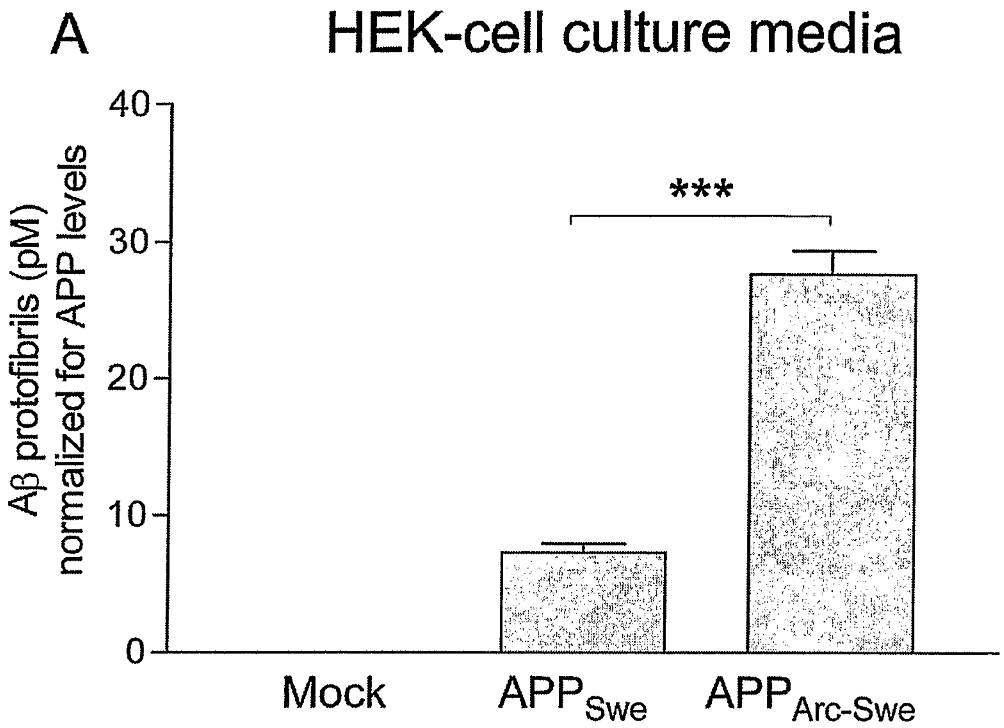


Figure 7



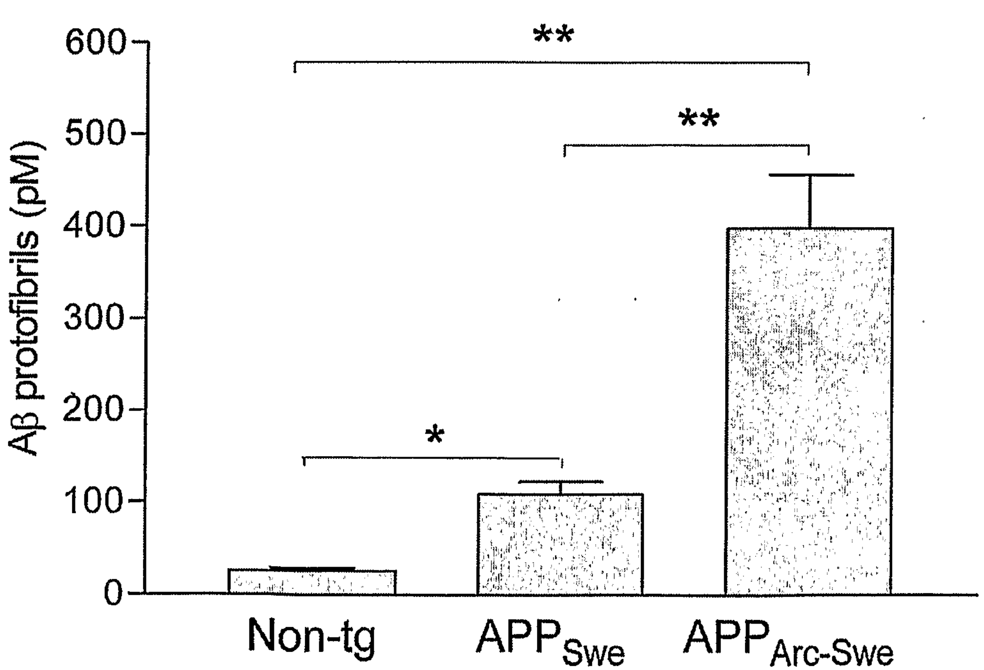


Fig. 8. Aβ protofibril levels in APP_{swearc} transgenic mouse brain TBS extracts after 4 months treatment with either mAb158 or placebo.

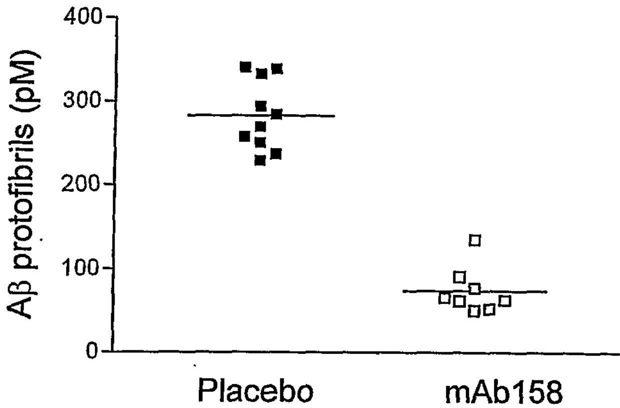


Fig. 9. Total Aβ levels in APP_{swearc} transgenic mouse brain formic acid extracts after 4 months

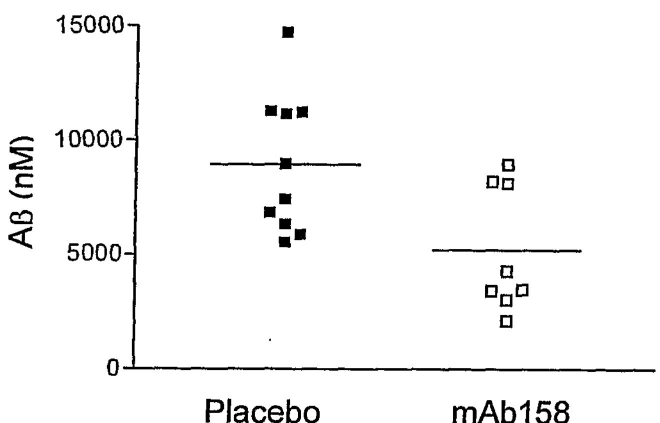


Fig. 10. pKN100 vector

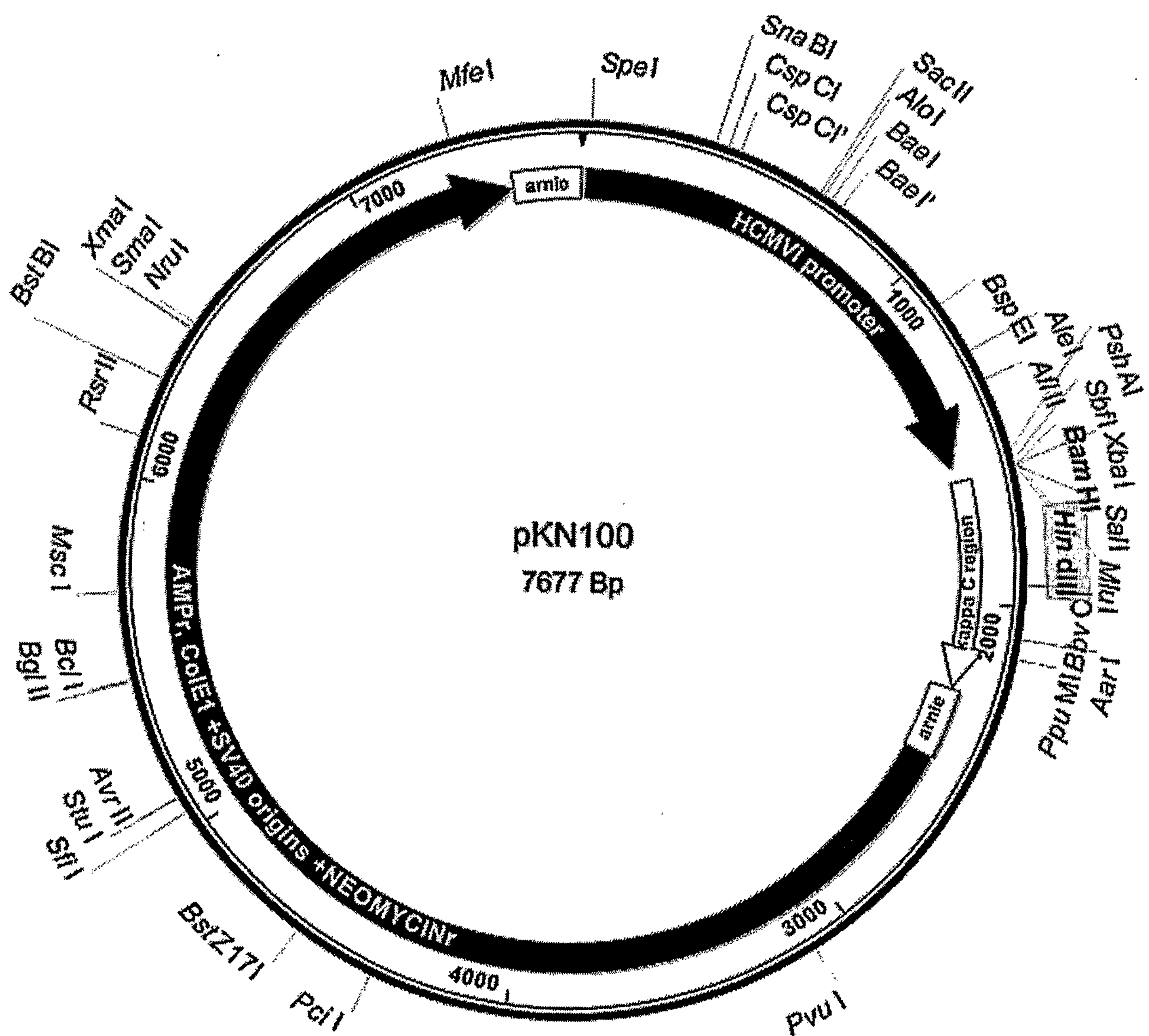


Fig. 11. pG1D200 vector

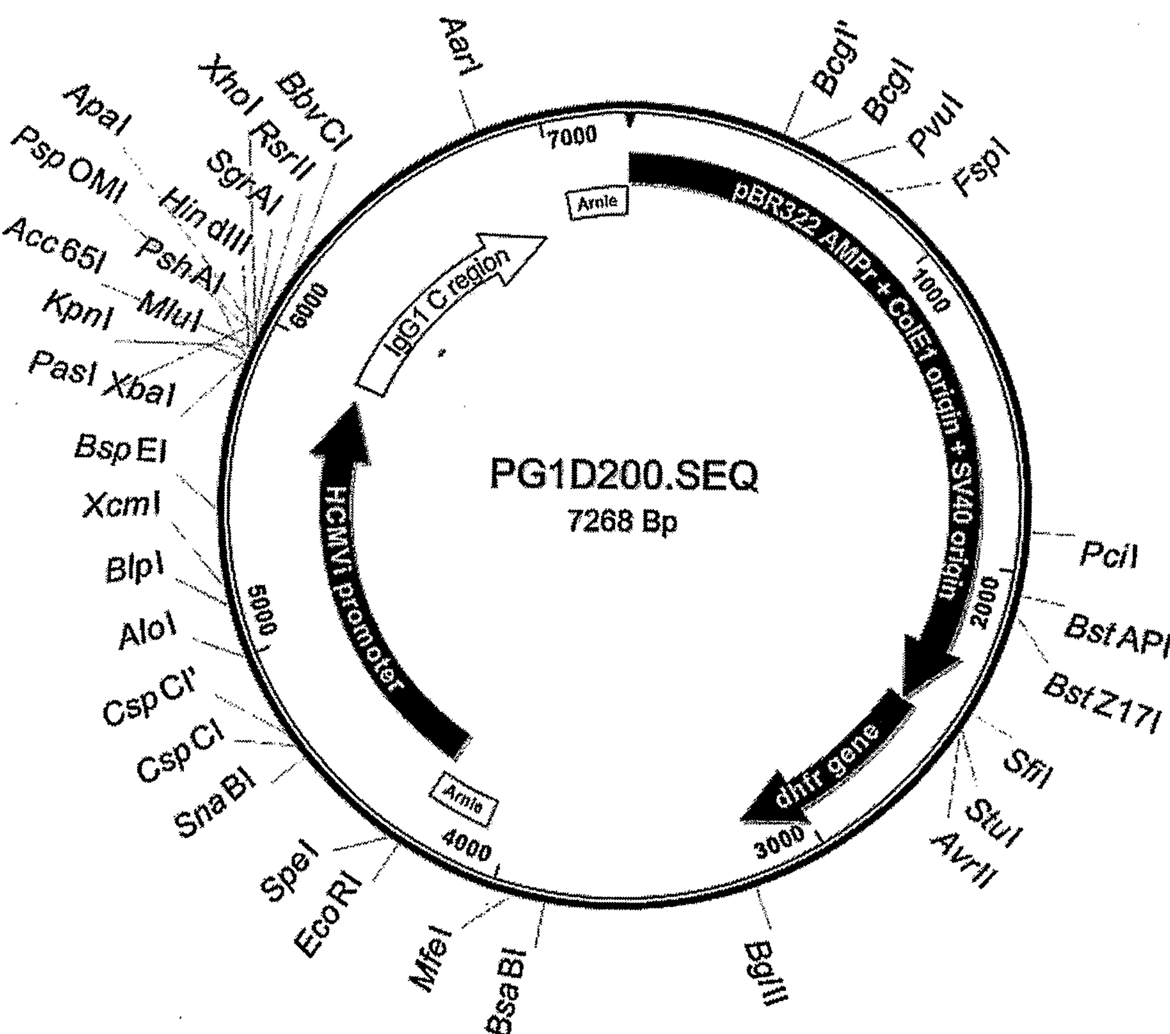
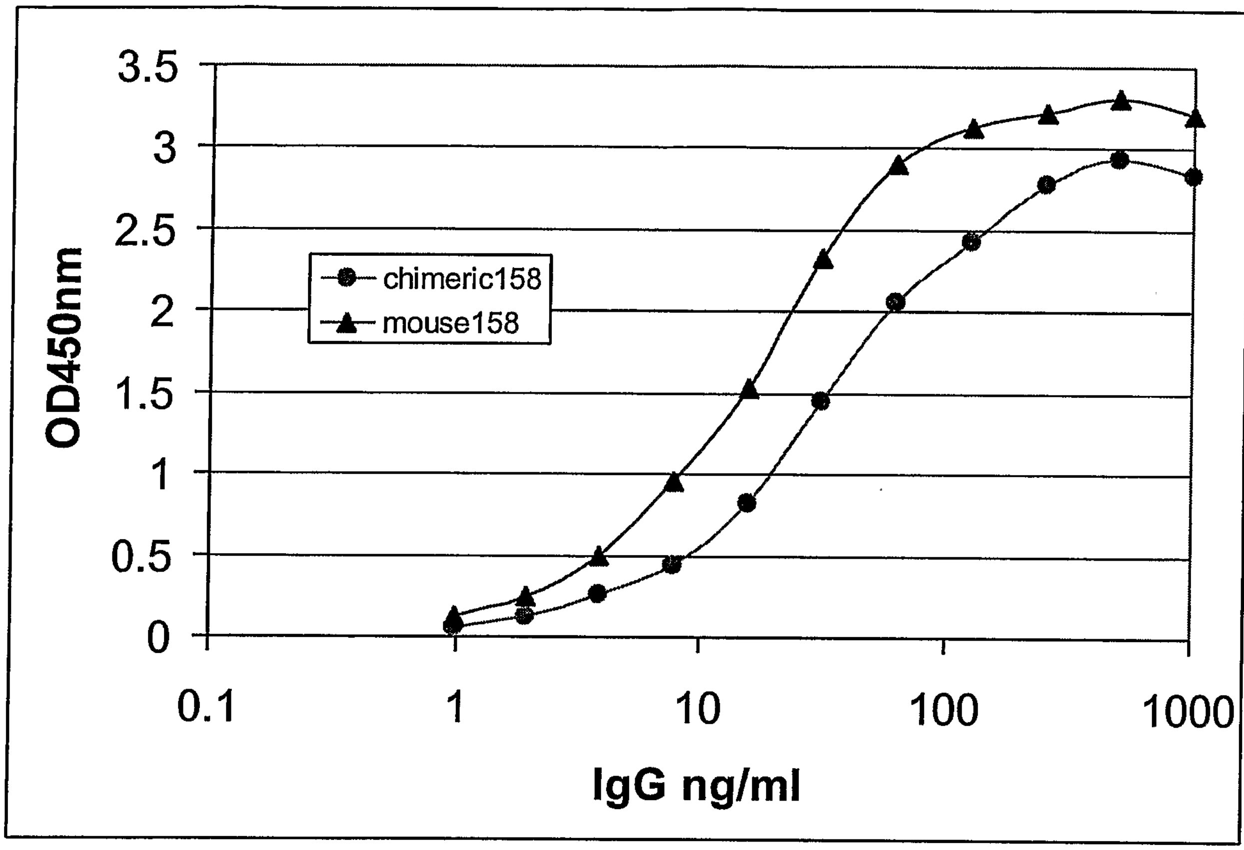
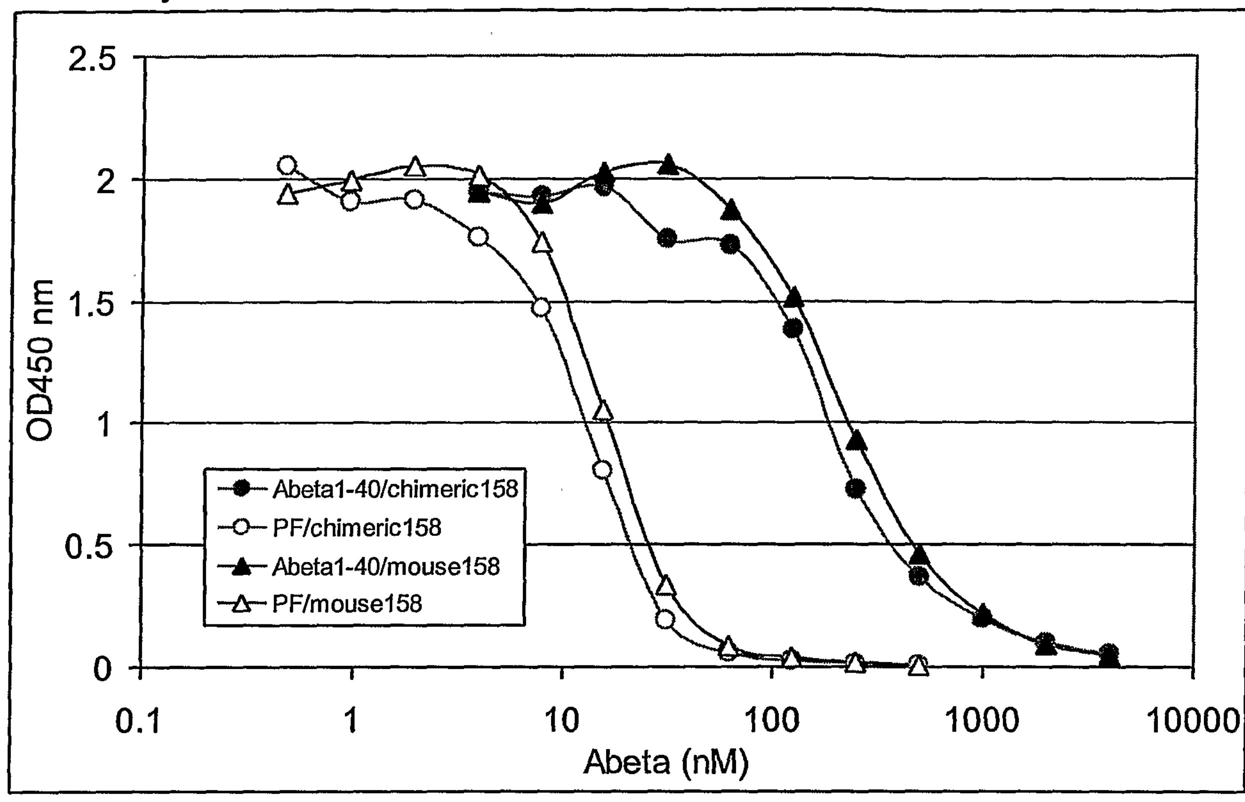


Fig. 12. Aβ monomer binding by chimeric and mouse 158 antibodies



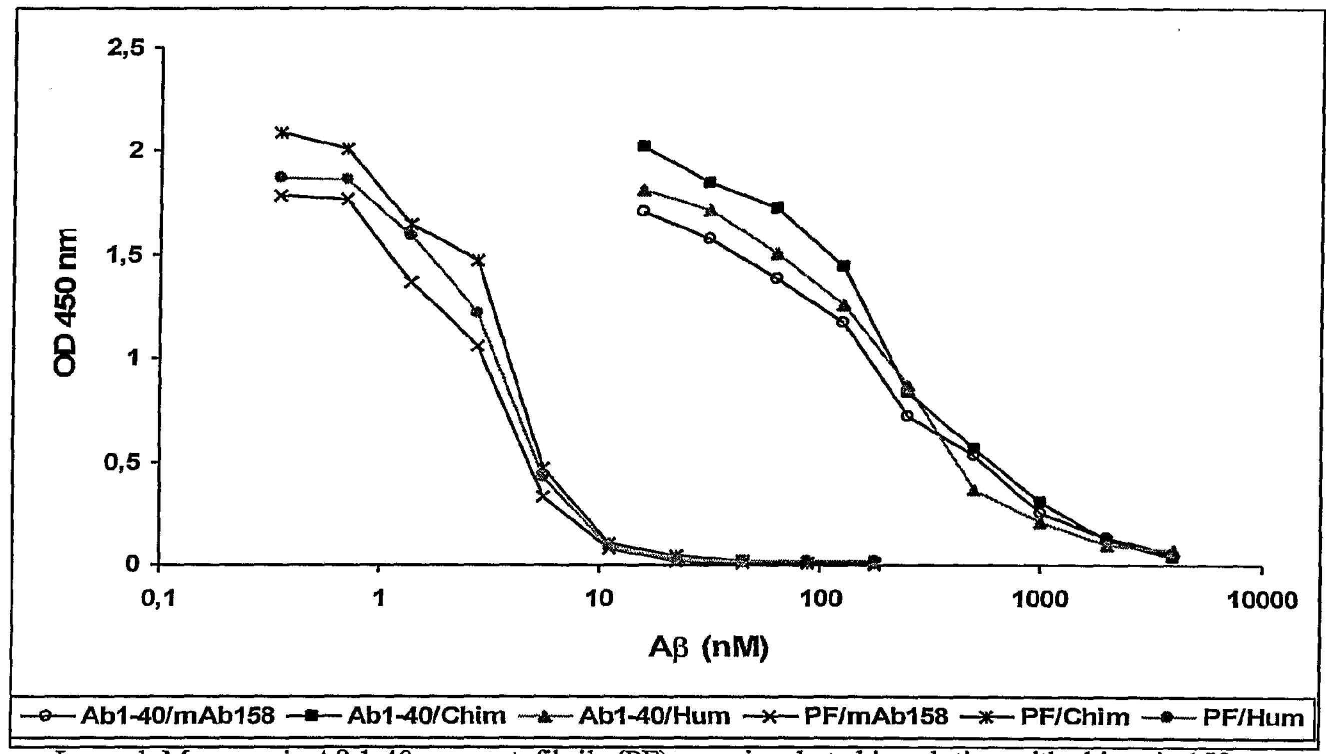
Legend: Direct ELISA coated with Aβ 1-40 (217 ng/well) and using serial dilutions of mAb. Amount of FCS at highest concentration (1 μg/ml) is 2.7%. Detection with anti-mouse k or anti-human k light chain conjugates.

Fig. 13. Competition of monomeric or protofibrillar Aβ for binding to chimeric 158 or mouse 158 antibody



Legend: Monomeric Aβ 1-40 (●▲) or protofibrils (PF) (○△) were incubated in solution with chimeric 158 (○●) or mouse 158 (△▲). The final concentration of FCS was 0.3%. After an incubation for 1 h, the mixture was added to a plate coated with Aβ monomers. The binding of antibody to the plate was detected by anti-mouse κ or anti-human κ light chain conjugates.

Fig. 14. Competition of monomeric or protofibrillar Aβ for binding to chimeric 158, mouse 158 antibody and humanized 158 antibody (BAN2401).



Legend: Monomeric Aβ 1-40 , or protofibrils (PF) were incubated in solution with chimeric 158 antibody (chim), mouse 158 antibody (mAb158) or humanized 158 antibody BAN2401 (Hum). The final concentration of FCS was 0.3%. After an incubation for 1 h, the mixture was added to a plate coated with Aβ monomers. The binding of antibody to the plate was detected by anti-mouse κ or anti-human κ light chain conjugates.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE2007/000292

A. CLASSIFICATION OF SUBJECT MATTER

IPC: see extra sheet
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC: C07K, A61K, A61P, G01N

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

SE,DK,FI,NO classes as above

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

EPO-INTERNAL, WPI DATA, PAJ, BIOSIS, MEDLINE, EMBASE, CHEM ABS DATA, SEQUENCE SEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	US 20060079447 A1 (WETZEL, RONALD B.), 13 April 2006 (13.04.2006), column 5, paragraph (0066); column 6, paragraphs (0074) - (0077); column 13, paragraph (0128), abstract --	1-35
P,X	WO 2006066233 A1 (NEURALAB LIMITED), 22 June 2006 (22.06.2006), page 15, line 29 - page 16, line 9 --	1-35
A	WO 2005123775 A1 (BIOARCTIC NEUROSCIENCE AB), 29 December 2005 (29.12.2005) --	1-35

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

* Special categories of cited documents:
- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier application or patent but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed
- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- "&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 July 2007	20-07-2007

Name and mailing address of the ISA/	Authorized officer
Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Yvonne Siösteen/EÖ Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE2007/000292

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	LANNFELT L., "Genetics, patophysiology and a beta protofibril formation in Alzheimer's disease", Neurobiology of aging, 2004, Vol. 25, Supplement 2, page S308 --	1-35
A	KAYED, RAKEZ et al, "Common Structure of Soluble Amyloid Oligomers Implies Common Mechanism of Pathogenesis", Science, 18 april 2003, page 486 - page 489 --	1-35
A	CHROMY, BRETT A. et al, "Self-Assembly of A Beta 1-42 into Globular Neurotoxins", Biochemistry, 2003, Vol. 42, page 12749 - page 12760 -- --------	1-35

International patent classification (IPC)

C07K 16/18 (2006.01)
A61K 39/395 (2006.01)
A61P 25/28 (2006.01)
G01N 33/68 (2006.01)

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Use the application number as username.
The password is **XACOZTYUVF**.

Paper copies can be ordered at a cost of 50 SEK per copy from PRV InterPat (telephone number 08-782 28 85).

Cited literature, if any, will be enclosed in paper form.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE2007/000292

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

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

1. ☒ Claims Nos.: 26-27, 31-33
because they relate to subject matter not required to be searched by this Authority, namely:

Claims 26-27 and 31-33 relate to a method of treatment of the human or animal body by surgery or by therapy, as well as

.../...

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

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

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

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

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

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

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

☐ No protest accompanied the payment of additional search fees.

Box II.1

Diagnostic methods /Rule 39.1(iv). Nevertheless, a search has been executed for this these claims. The search has been based on the alleged effects of the compound.

INTERNATIONAL SEARCH REPORT
Information on patent family members

30/06/2007

International application No.
PCT/SE2007/000292

US	20060079447	A1	13/04/2006	US	D534464	S	02/01/2007
				US	7154070	B	26/12/2006
				US	20060076342	A	13/04/2006
WO	2006066233	A1	22/06/2006	US	20060240486	A	26/10/2006
WO	2005123775	A1	29/12/2005	AU	2005254928	A	29/12/2005
				CA	2570130	A	29/12/2005
				EP	1781703	A	09/05/2007
				SE	0401601	D	00/00/0000