Immunoglobulins

Field of the Invention

The present invention relates to immunoglobulins, particularly antibodies that bind to NOGO and neutralise the activity thereof, polynucleotides encoding such antibodies, pharmaceutical formulations containing said antibodies and to the use of such antibodies in the treatment and/or prophylaxis of neurological diseases. Other aspects, objects and advantages of the present invention will become apparent from the description below.

Background of the Invention

Stroke is a major cause of death and disability in the Western World. There is no approved therapy for the treatment of stroke other than tissue plasminogen (t-PA) which has to be administered within 3 hours of onset following a computer tomography (CT) scan to exclude haemorrhage. To date most therapeutic agents directed towards the treatment of acute stroke (i.e. neuroprotection), have predominantly involved targeting glutamate receptors and their down stream signalling pathways known to be involved in acute cell death. However to date these strategies have proved unsuccessful in clinical trials and are often associated with dose-limiting side effects (Hill & Hachinski, The Lancet, 352 : (suppl III) 10-14 (1998)). Therefore there is a need for novel approaches directed towards the amelioration of cell death following the cessation of blood flow. Neuroprotection is the ability of a treatment to prevent or ameliorate neuronal cell loss in response to an insult or disease process. This may be achieved by targeting the neurons directly or indirectly by preventing glial (including oligodendrocyte) cell loss.

Following the onset of stroke, some degree of spontaneous functional recovery is observed in many patients, suggesting that the brain has the (albeit limited) ability to repair and/or remodel following injury. Agents that have the potential to enhance this recovery may therefore allow intervention to be made much later (potentially days) following the onset of cerebral ischaemia. Agents which are able to offer both acute neuroprotection and enhance functional

recovery may provide significant advantages over current potential neuroprotective strategies.

Alzheimer's disease (AD) is characterised by the presence of two diagnostic features of pathology. These are amyloid plaques and neurofibrillary tangles composed of aggregated beta-amyloid peptide (Aβ40 and Aβ42) and hyperphosphorylated tau respectively (Dawbarn & Allen 2001 Neurobiology of Alzheimer's Disease OUP).

A comprehensive study has shown a strong link in patients between beta-amyloid accumulation and cognitive decline (Naslund et al, JAMA, March 22/29, 2000, Vol.283, No;12, page 1571-1577). This is consistent with genetic and epidemiological studies that suggest that some mutations in APP and presenilin genes can predispose to early onset AD, which mutations also enhance the levels of Aβ40 and Aβ42 peptide, including the ratio thereof.

Cleavage of the type I transmembrane amyloid precursor protein (APP) by two distinct proteases designated beta- and gamma-secretase is necessary for the formation of beta-amyloid peptide. The molecular identity of beta-secretase as the aspartyl-protease Asp2/BACE1 has been confirmed (Hussain et al Mol.Cell.NeuroSci. 16, 609-619 (2000); Vassar et al, Science (1999), Oct.22; 286 (5440):735-741). The nature of gamma-secretase remains the source of some debate and is likely to consist of a high molecular weight complex consisting of at least the following proteins: presenilins, Aph1, Pen2 and nicastrin (reviewed in Medina & Dotti Cell Signalling 2003 15(9):829-41).

The processing of APP within the CNS is likely to occur within a number of cell-types including neurons, oligodendrocytes, astrocytes and microglia. While the overall rate of APP processing in these cells will be influenced by the relative level of expression of APP, BACE1/Asp2, presenilin-1 and -2, Aph1, Pen2 and nicastrin.

Furthermore, additional factors regulating the subcellular location of APP can also influence its processing as shown by the finding that mutation of the YENP motif in the APP cytoplasmic domain which blocks its endocytosis reduces beta-amyloid production (Perez et al 1999 J Biol Chem 274 (27) 18851-6). Retention of the APP-beta-CTF in the ER by the addition of the KKQN retention motif is sufficient to reduce amyloid production in transfected cells (Maltese et al

2001 J Biol Chem 276 (23) 20267-20279). Conversely, elevation of endocytosis, by overexpression of Rab5 is sufficient to elevate amyloid secretion from transfected cells (Grbovic et al 2003 J Biol Chem 278 (33) 31261-31268).

Consistent with these findings further studies have shown that reduction of cellular cholesterol levels (a well known risk factor for AD) reduced beta-amyloid formation. This change was dependent on altered endocytosis as demonstrated by the use of the dominant negative dynamin mutants (K44A) and overexpression of the Rab5 GTPase activating protein RN-Tre (Ehehalt et al 2003 J Cell Biol 160 (1) 113-123).

Cholesterol rich microdomains or rafts are also an important cellular site of beta-amyloid production and APP, BACE1 and components of the gamma-secretase complex have all been shown to transiently reside within rafts. Antibody cross-linking of APP and BACE1 towards cholesterol rich rafts was able to elevate beta-amyloid production (Ehehalt et al 2003 J Cell Biol 160 (1) 113-123). Expression of GPI-anchored BACE1, which is exclusively targeted to lipid rafts, is similarly able to elevate APP cleavage and beta-amyloid production (Cordy et al 2003 PNAS 100(20) 11735-11740).

The mechanisms underlying functional recovery after a stroke or other neurodamaging event or disease, are currently unknown. The sprouting of injured or non-injured axons has been proposed as one possible mechanism. However, although in vivo studies have shown that treatment of spinal cord injury or stroke with neurotrophic factors results in enhanced functional recovery and a degree of axonal sprouting, these do not prove a direct link between the degree of axonal sprouting and extent of functional recovery (Jakeman, et al. 1998, Exp. Neurol. 154: 170-184, Kawamata et al. 1997, Proc Natl Acad. Sci. USA., 94:8179-8184, Ribotta, et al. 2000, J Neurosci. 20: 5144-5152). Furthermore, axonal sprouting requires a viable neuron. In diseases such as stroke which is associated with extensive cell death, enhancement of functional recovery offered by a given agent post stroke may therefore be through mechanisms other than axonal sprouting such as differentiation of endogenous stem cells, activation of redundant pathways, changes in receptor distribution or excitability of neurons or glia (Fawcett & Asher, 1999, Brain Res. Bulletin, 49: 377-391, Horner & Gage, 2000, Nature 407 963-970).

The limited ability of the central nervous system (CNS) to repair following injury is thought in part to be due to molecules within the CNS environment that have an inhibitory effect on axonal sprouting (neurite outgrowth). CNS myelin is thought to contain inhibitory molecules (Schwab ME and Caroni P (1988) J. Neurosci. 8, 2381-2193). Two myelin proteins, myelin-associated glycoprotein (MAG) and NOGO have been cloned and identified as putative inhibitors of neurite outgrowth (Sato S. et al (1989) Biochem. Biophys. Res. Comm.163,1473-1480; McKerracher L et al (1994) Neuron 13, 805-811; Mukhopadhyay G et al (1994) Neuron 13, 757-767; Torigoe K and Lundborg G (1997) Exp. Neurology 150, 254-262; Schafer et al (1996) Neuron 16, 1107-1113; WO9522344; WO9701352; Prinjha R et al (2000) Nature 403, 383-384; Chen MS et al (2000) Nature 403, 434-439; GrandPre T et al (2000) Nature 403, 439-444; US005250414A; WO200005364A1; WO0031235).

Three forms of human NOGO have been identified: NOGO-A having 1192 amino acid residues (GenBank accession no. AJ251383); NOGO-B, a splice variant which lacks residues 186 to 1004 in the putative extracellular domain (GenBank accession no. AJ251384) and a shorter splice variant, NOGO-C, which also lacks residues 186 to 1004 and also has smaller, alternative amino terminal domain (GenBank accession no. AJ251385) (Prinjha et al (2000) supra).

Inhibition of the CNS inhibitory proteins such as NOGO may provide a therapeutic means to ameliorate neuronal damage and promote neuronal repair and growth thereby potentially assisting recovery from neuronal injury such as that sustained in stroke. Examples of such NOGO inhibitors may include small molecules, peptides and antibodies.

It has been reported that a murine monoclonal antibody, IN-1, that was raised against NI-220/250, a myelin protein which is a potent inhibitor of neurite growth (and subsequently shown to be fragment of NOGO-A), promotes axonal regeneration (Caroni, P and Schwab, ME (1988) Neuron 1 85-96; Schnell, L and Schwab, ME (1990) Nature 343 269-272; Bregman, BS et al (1995) Nature 378 498-501 and Thallmair, M et al (1998) Nature Neuroscience 1 124-131). It has also been reported that NOGO-A is the antigen for IN-1 (Chen et al (2000) Nature 403 434-439). Administration of IN-1 Fab fragment or humanised IN-1 to rats that have undergone spinal cord transection, enhanced recovery (Fiedler, M

et al (2002) Protein Eng 15 931-941; Brosamle, C et al (2000) J. Neuroscience 20 8061-8068).

Monoclonal antibodies which bind to NOGO are described in WO 04/052932 and WO2005028508. WO 04/052932 discloses a murine antibody 11C7 which binds to certain forms of human NOGO with high affinity.

Patent application WO05/061544 also discloses high affinity monoclonal antibodies, including a murine monoclonal antibody 2A10, and generally discloses humanised variants thereof, for example H1L11 (the sequences for the H1 and L11 are provided in SEQ ID NOs. 33 and 34 respectively (VH or VL sequences only)). The antibodies disclosed bind to human NOGO-A with high affinity. The murine 2A10 antibody (and CDR-grafted humanised variants thereof) are characterised by the following complementarity determining region (CDR) sequences (as determined using the Kabat methodology (Kabat et al. (1991) "Sequences of proteins of immunological interest"; Fifth Edition; US Department of Health and Human Services; NIH publication No 91-3242)) within their light and heavy chain variable regions:

Table 1: Antibody 2A10 light chain CDRs

CDR	Sequence
L1	RSSKSLLYKDGKTYLN (SEQ ID NO:4)
L2	LMSTRAS (SEQ ID NO:5)
L3	QQLVEYPLT (SEQ ID NO:6)

Table 2: Antibody 2A10 heavy chain CDRs

CDR	Sequence
H1	SYWMH (SEQ ID NO:1)
H2	NINPSNGGTNYNEKFKS (SEQ ID NO:2)
H3	GQGY (SEQ ID NO:3)

WO05/061544 further discloses “analogues” of the antibodies that comprise the CDRs of Tables 1 and 2 above, such “analogues” the have same

antigen binding specificity and/or neutralizing ability as the donor antibody from which they were derived.

Despite the art providing high affinity anti-NOGO antibodies, it remains a highly desirable goal to isolate and develop alternative, or improved, therapeutically useful monoclonal antibodies that bind and inhibit the activity of human NOGO.

The process of neurodegeneration underlies many neurological diseases/disorders including, but not limited to, acute diseases such as stroke (ischemic or haemorrhagic), traumatic brain injury and spinal cord injury as well as chronic diseases including Alzheimer's disease, fronto-temporal dementias (tauopathies), peripheral neuropathy, Parkinson's disease, Creutzfeldt-Jakob disease (CJD), Schizophrenia, amyotrophic lateral sclerosis (ALS), multiple sclerosis, Huntington's disease, multiple sclerosis and inclusion body myositis. Consequently the anti-NOGO monoclonal antibodies, and the like, of the present invention may be useful in the treatment of these diseases/disorders. Antibodies for the treatment of the above mentioned disease/disorders are provided by the present invention and described in detail below.

Brief Summary of the Invention

The invention provides specific heavy chain variable regions, and antibodies or fragments thereof comprising the said specific heavy chain variable regions and a light chain variable region that allows, when paired with the heavy chain variable regions, the Fv dimer to bind human NOGO-A with high affinity, and thereby neutralise the activity of human NOGO-A.

The heavy chain variable regions of the present invention may be formatted, together with light chain variable regions to allow binding to human NOGO-A, in the conventional immunoglobulin manner (for example, human IgG, IgA, IgM etc.) or in any other fragment thereof or "antibody-like" format that binds to human NOGO-A (for example, single chain Fv, diabodies, Tandabs™ etc (for a summary of alternative "antibody" formats see Holliger and Hudson, Nature Biotechnology, 2005, Vol 23, No. 9, 1126-1136)).

A heavy chain variable region comprising a third CDR consisting essentially of the amino acid residues GQGY wherein the CDR contains at least

one substitution within the GQGY core sequence, the substitutions being selected from the following substitutions: where the G in the first position is replaced by R, I, W or M; the Q in the second position is replaced by D, I, A, L, V or S; the G in the third position is replaced by W, N, Y, S, L or F; and the Y in the fourth position is replaced by W.

In another embodiment, the third heavy chain CDR (CDR H3) only contains one substitutions to yield the following CDR H3: RQGY (SEQ ID NO.75), IQGY (SEQ ID NO.76), MQGY (SEQ ID NO.45), GDGY (SEQ ID NO.77), GIGY (SEQ ID NO.78), GSGY (SEQ ID NO.79), GQNY (SEQ ID NO.80), GQYY (SEQ ID NO.81), GQSY (SEQ ID NO.62), GQLY (SEQ ID NO.82), GQFY (SEQ ID NO.83), GQGW (SEQ ID NO.84), WQGY(SEQ ID NO.86), GAGY(SEQ ID NO.87), GLGY(SEQ ID NO.88), GVGY(SEQ ID NO.89), GQWY(SEQ ID NO.90).

In another embodiment, the heavy chain variable regions above further contain the other CDRs listed in Table 2, i.e. CDR H1 (SEQ ID NO. 1) and CDR H2 (SEQ ID NO.2).

The antibodies of the present invention, or fragments thereof, retain the human NOGO binding activity of antibodies that comprise the CDR H3: GQGY, in terms of their activity as measured in ELISA and Biacore experiments, and in some cases the activity in these experiments is increased.

Human or humanised heavy chain variable regions containing G95M (substitution numbering by Kabat)

In one embodiment of the present invention, the heavy chain variable regions of the present invention comprise the CDRs defined in Table 3 (as defined by Kabat):

Table 3:

CDR	Sequence
H1	SYWMH (SEQ ID NO:1)
H2	NINPSNGGTNYNEKFKS (SEQ ID NO:2)
H3	MQGY (SEQ ID NO:45)

In one embodiment of the present invention there is provided a human or humanised heavy chain variable region comprising each of the CDRs listed in Table 3. In another embodiment of the present invention there is provided a humanised heavy chain variable region comprising the CDRs listed in Table 3 within the larger sequence of a human heavy chain variable region. In yet another embodiment the humanised heavy chain variable region comprises the CDRs listed in Table 3 within an acceptor antibody framework having greater than 40% identity in the framework regions, or greater than 50%, or greater than 60%, or greater than 65% identity to the murine 2A10 donor antibody heavy chain variable region (SEQ ID NO.7).

When the CDRs of Table 3 are all used, in one embodiment the heavy chain variable region sequence is sequence H98 provided as SEQ ID NO. 66 (H98 VH is the equivalent of H1 VH (SEQ ID NO.33) differing only in that the CDR H3 is MQGY in H98 instead of GQGY as found in H1).

In one aspect of the present invention the antibodies comprise a heavy chain variable region having the amino acid sequence of SEQ ID NO. 66 (H98 variable region) further comprising a number of substitutions at one or more of positions 38, 40, 48, 67, 68, 70, 72, 74, and 79; wherein each substituted amino acid residue is replaced with the amino acid residue at the equivalent position in SEQ ID NO 7 (the heavy chain variable region of the donor antibody 2A10) and the number of substitutions is between 1 and 9. In other embodiments the number of substitutions is 1, or 2, or 3, or 4, or 5, or 6, or 7, or 8 or 9.

In this context the substitutions that are described are equivalent in concept to "back-mutations" where the human framework amino acid residues in specific positions within the H98 sequence are back-mutated to the amino acid residues in the equivalent position within the 2A10 donor antibody sequence.

Unless specifically stated otherwise to the contrary herein, when a numerical position of an amino acid residue found within a specific sequence is mentioned in this document, for example "position 12", it is intended that the skilled reader assigns the first amino acid in the sequence the position "1" and counts from position one and identifies the amino acid which is in the desired position, in this example the twelfth amino acid residue in the sequence. The skilled reader will notice that this numbering system does not correspond with the

Kabat numbering system which is often used to define amino acid positions within antibody sequences.

For optimal binding affinity, it was found for the humanisation of the mouse antibody 2A10 (the VH for which is SEQ ID NO. 7) that the pair of amino acid residues in positions 48 and 68, should be I and A respectively (as they exist in 2A10) or M and V respectively (as they exist in H98). It is expected that the above finding is also of relevance to the humanisation of the G95M variant of 2A10.

The following table includes details of three different heavy chain variable (VH) regions which may form part of the antibodies of the present invention. Each of the disclosed VH is based on the H98 VH (SEQ ID NO. 66) further comprising the substitutions mentioned in the table (Table 4) where the H98 residue at the relevant position is substituted with the 2A10 residue at that position (in the table, "-" means that there is no substitution in that position, and so the residue remains as in the sequence of H98):

Table 4,

New VH (SEQ ID NO. X)	Numerical Residue No.	38	40	48	67	68	70	72	74	79
	Kabat No.	38	40	48	66	67	69	71	73	78
	2A10	*K*	*R*	*I*	*K*	*A*	*L*	*V*	*K*	*A*
	H98	*R*	*A*	*M*	*R*	*V*	*M*	*R*	*T*	*V*
H26 (47)		-	-	I	-	A	-	-	-	A
H27 (48)		K	R	I	K	A	L	V	K	A
H28 (49)		-	-	I	K	A	-	-	-	A

In one embodiment of the present invention, therefore, the heavy chain variable regions (VH) of the present invention are H26 VH (SEQ ID NO. 47), H27 VH (SEQ ID NO. 48) and H28 VH (SEQ ID NO. 49)

SEQ ID 47: VH humanised construct H26
QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWMHWVRQAPGQGLEWIGNIN
PSNGGTNYNEKFKSRATMTRDTSTSTAYMELSSLRSEDTAVYYCELMQGYWG
QGTLVTVSS

SEQ ID 48: VH humanised construct H27
QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWMHWVKQRPGQGLEWIGNIN
PSNGGTNYNEKFKSKATLTVDKSTSTAYMELSSLRSEDTAVYYCELMQGYWG
QGTLVTVSS

SEQ ID 49: VH humanised construct H28
QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWMHWVRQAPGQGLEWIGNIN
PSNGGTNYNEKFKSKATMTRDTSTSTAYMELSSLRSEDTAVYYCELMQGYWG
QGTLVTVSS

Human or Humanised heavy chain variable regions including G101S (substitution numbering by Kabat)

In another embodiment of the present invention there is provided a human or humanised heavy chain variable region which comprises CDRs defined in Table 5:

Table 5:

CDR	According to Kabat
H1	SYWMH (SEQ ID NO:1)
H2	NINPSNGGTNYNEKFKS (SEQ ID NO:2)
H3	GQSY (SEQ ID NO:62)

In one embodiment the CDRs of Table 5 are incorporated within a human heavy chain variable region sequence. In another embodiment the humanised heavy chain variable region comprises the CDRs listed in Table 5 within an acceptor antibody framework having greater than 40% identity in the framework regions, or greater than 50%, or greater than 60%, or greater than 65% identity to the murine 2A10 donor antibody heavy chain variable region (SEQ ID NO.7).

In another embodiment the CDRs of Table 5 are inserted into a human heavy chain variable region to give the following sequence (H99):
QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWMHWVRQAPGQGLEWMGNI
NPSNGGTNYNEKFKSRVTMTRDTSTSTVYMELSSLRSEDTAVYYCELGQSYW
GQGTLVTVSS (SEQ ID 61: 2A10 VH humanised construct H99).

In other embodiments, further back mutations are added to the H99 VH sequence in any one of positions (denoted by numerical residue position) 38, 40. 48, 67, 68, 70, 72, 74 or 79; wherein each substituted amino acid residue is replaced with the amino acid residue at the equivalent position in SEQ ID NO 7

(the heavy chain variable region of the donor antibody 2A10) and the number of substitutions is between 1 and 9. In other embodiments the number of substitutions is 1, or 2, or 3, or 4, or 5, or 6, or 7, or 8 or 9. H99 VH is the equivalent of H1 VH (SEQ ID NO.33) differing only in that the CDR H3 is GQSY in H99 instead of GQGY as found in H1.

For optimal binding affinity, it was found for the humanisation of the mouse antibody 2A10 (the VH for which is SEQ ID NO. 7) that the pair of amino acid residues in positions 48 and 68, should be I and A respectively (as they exist in 2A10) or M and V respectively (as they exist in H98). It is expected that the above finding is also of relevance to the humanisation of the G95M variant of 2A10.

In one embodiment the back mutations are located in the positions indicated in Table 6 below where the H99 residue at the relevant position is substituted with the 2A10 residue at that position (in the table, "-" means that there is no substitution in that position, and so the residue remains as in the sequence of H1):

Table 6,

New VH (SEQ ID NO. X)	Numerical Residue No.	38	40	48	67	68	70	72	74	79
	Kabat No.	38	40	48	66	67	69	71	73	78
	2A10	*K*	*R*	*I*	*K*	*A*	*L*	*V*	*K*	*A*
	H99	*R*	*A*	*M*	*R*	*V*	*M*	*R*	*T*	*V*
H100 (63)		-	-	I	-	A	-	-	-	A
H101 (64)		K	R	I	K	A	L	V	K	A
H102 (65)		-	-	I	K	A	-	-	-	A

Antibodies or fragments that comprise the human or humanised heavy chain variable regions and light chain variable regions

The VH constructs of the present invention may be paired with a light chain to form a human NOGO-A binding unit (Fv) in any format, including a conventional IgG antibody format having full length (FL) variable and constant domain heavy chain sequences. Examples of full length (FL) IgG1 heavy chain sequences comprising the VH constructs of the present invention and inactivating

mutations in positions 235 and 237 (EU Index numbering) to render the antibody non-lytic are SEQ ID NOs 53, 54 and 55.

SEQ ID 53: Heavy chain humanised construct H26
MGWSCIILFLVATATGVHSQVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWM
HWVRQAPGQGLEWIGNINPSNGGTNYNEKFKSRATMTRDTSTSTAYMELSSL
RSEDTAVYYCELMQGYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAAL
GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT
QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELAGAPSVFLFPPKPKD
TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRD
ELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKL
TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK.

SEQ ID 54: Heavy chain humanised construct H27
MGWSCIILFLVATATGVHSQVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWM
HWVKQRPGQGLEWIGNINPSNGGTNYNEKFKSKATLTVDKSTSTAYMELSSLR
SEDTAVYYCELMQGYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALG
CLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ
TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELAGAPSVFLFPPKPKDT
LMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV
SVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDE
LTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT
VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID 55: Heavy chain humanised construct H28
MGWSCIILFLVATATGVHSQVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWM
HWVRQAPGQGLEWIGNINPSNGGTNYNEKFKSKATMTRDTSTSTAYMELSSLR
SEDTAVYYCELMQGYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALG
CLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ
TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELAGAPSVFLFPPKPKDT
LMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV
SVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDE
LTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT
VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

The light chain variable region sequence that forms an Fv with the heavy chain variable region sequences of the present invention may be any sequence that allows the Fv to bind to Human NOGO-A.

In one embodiment of the present invention the light chain variable region is the 2A10 light chain (see WO 05/061544), the light chain variable region of which is provided herein as SEQ ID NO. 8 or humanised variants thereof. Humanised variants of the 2A10 light chain preferably contain all of the light

chain variable region CDRs that are described in Table 1 grafted onto a human light chain variable region acceptor framework. In one embodiment the humanised light chain variable regions are L11 (SEQ ID NO.34), L13 (SEQ ID NO.13) or L16 (SEQ ID NO.14). Alternative light chain variable regions that are based on L13 and L16, which comprise specific substitutions in kabat positions 37 and/or 45, are provided in Table 7.

Table7,

Description	SEQ ID NO.	Sequence
L100 (L13+Q37R)	67	DIVMTQSPLSLPVTLGQPASISCRSSKSLLYKDGKTYLNWFRQ RPGQSPQLLIYLMSTRASGVPDRFSGGGSGTDFTLKISRVEA EDVGVYYCQQLVEYPLTFGQGTKLEIK
L101 (L13+Q45R)	68	DIVMTQSPLSLPVTLGQPASISCRSSKSLLYKDGKTYLNWFQQ RPGQSPRLLIYLMSTRASGVPDRFSGGGSGTDFTLKISRVEAE DVGVYYCQQLVEYPLTFGQGTKLEIK
L102 (L13+Q37R/Q45R)	69	DIVMTQSPLSLPVTLGQPASISCRSSKSLLYKDGKTYLNWFRQ RPGQSPRLLIYLMSTRASGVPDRFSGGGSGTDFTLKISRVEAE DVGVYYCQQLVEYPLTFGQGTKLEIK
L103 (L16+Q37R)	70	DIVMTQSPLSNPVTLGQPVSISCRSSKSLLYKDGKTYLNWFRQ RPGQSPQLLIYLMSTRASGVPDRFSGGGSGTDFTLKISRVEA EDVGVYYCQQLVEYPLTFGQGTKLEIK
L104 (L16+Q45R)	71	DIVMTQSPLSNPVTLGQPVSISCRSSKSLLYKDGKTYLNWFLQ RPGQSPRLLIYLMSTRASGVPDRFSGGGSGTDFTLKISRVEAE DVGVYYCQQLVEYPLTFGQGTKLEIK
L105 (L16+Q37R/Q45R)	72	DIVMTQSPLSNPVTLGQPVSISCRSSKSLLYKDGKTYLNWFRQ RPGQSPRLLIYLMSTRASGVPDRFSGGGSGTDFTLKISRVEAE DVGVYYCQQLVEYPLTFGQGTKLEIK

In another embodiment the full length (FL) light chain sequences are L11FL (SEQ ID NO.36), L13 FL (SEQ ID NO.17) or L16 FL (SEQ ID NO.18).

In another embodiment the antibodies, fragments or functional equivalents thereof comprise a VH sequence selected from H26, H27, H28, H100, H101 and H102; in combination with any one of the following VL sequences L11, L13, L16, L100, L101, L102, L103, L104, and L105. It is intended that all possible

combinations of the listed heavy chain variable regions and light chain variable regions be specifically disclosed (e.g. H28L104 et. al.).

In particular embodiments the antibodies, fragments or functional equivalents thereof comprise the following variable region pairs:

H27L16 (SEQ ID NO.48 + SEQ ID NO.14)

H28L13 (SEQ ID NO.49 + SEQ ID NO.13)

H28L16 (SEQ ID NO.49 + SEQ ID NO.14)

In another embodiment the antibodies of the present invention comprise the following full length sequences:

H27FL L16FL (SEQ ID NO. 54 + SEQ ID NO.18)

H28FL L13FL (SEQ ID NO. 55 + SEQ ID NO.17)

H28FL L16FL (SEQ ID NO. 55 + SEQ ID NO.18)

In one embodiment the antibody of the present invention comprises H27L16 (SEQ ID NO.48 + SEQ ID NO.14), or is the full length antibody H28FL L16FL (SEQ ID NO. 55 + SEQ ID NO.18).

In another embodiment, the antibody or fragment thereof binds to the same human NOGO epitope as H28L16, or competes with the binding of H28L16 to human NOGO, characterised in both instances in that the competing antibody, or fragment thereof, is not the murine antibody 2A10 or a human or humanised variant thereof comprising a CDR H3 having the sequence GQGY (SEQ ID NO.3) or a sequence containing one amino acid substitution in the CDR H3.

In particular embodiments the antibodies, fragments or functional equivalents thereof comprise the following variable region pairs:

H100L16 (SEQ ID NO.63 + SEQ ID NO.14)

H101L13 (SEQ ID NO.64 + SEQ ID NO.13)

H102L16 (SEQ ID NO.65 + SEQ ID NO.14)

Epitope Mapping and further antibodies that bind to the same epitope

In another embodiment there is provided an antibody, or fragment thereof, that is capable of binding to human NOGO protein, or fragment thereof such as as a GST-NOGO-A56 protein (SEQ ID NO.32), in an ELISA assay, wherein the

binding of the antibody, or fragment thereof, to the human NOGO protein, or fragment thereof, in the ELISA assay is reduced in the presence of a peptide having the following sequence VLPDIVMEAPLN (SEQ ID NO. 60), and is not reduced in the presence of an irrelevant peptide, for instance a peptide from human NOGO that does not overlap with SEQ ID NO.60 (such as SEQ ID NO. 85, YESIKHEPENPPPYEE), characterised in that the antibody or fragment thereof is not an antibody comprising a heavy chain variable domain having a CDR H3 consisting of the amino acid residues GQGY or analogues thereof having one amino acid substitution in the CDR H3. Alternatively the competing peptide is TPSPVLPDIVMEAPLN (SEQ ID NO. 73) or VLPDIVMEAPLNSAVP (SEQ ID NO. 74). In addition, the antibody that binds to the same epitope as the antibodies, or fragments thereof, may be an antibody that does not comprise all of the CDRs listed in Tables 1 and 2, or any antibody that comprises a set of CDRs that has 80% or greater homology to the CDRs listed in Tables 1 and 2 combined, or Tables 1 or 2 alone.

In another embodiment of the present invention there is provided an antibody or fragment thereof, that is capable of binding in an ELISA assay to a region of human NOGO protein consisting of the polypeptide sequence of VLPDIVMEAPLN (SEQ ID NO. 60), characterised in that the antibody, or fragment thereof is not an antibody comprising a variable heavy domain having CDR H3 consisting of the amino acid residues GQGY or analogues thereof having one amino acid substitution in the CDR H3. Alternatively the antibody or fragment thereof is capable of binding to TPSPVLPDIVMEAPLN (SEQ ID NO. 73) or VLPDIVMEAPLNSAVP (SEQ ID NO. 74). In addition, the antibody that binds to the same epitope as the antibodies, or fragments thereof, may be an antibody that does not comprise all of the CDRs listed in Tables 1 and 2, or any antibody that comprises a set of CDRs that has 80% or greater homology to the CDRs listed in Tables 1 and 2 combined, or Tables 1 or 2 alone.

In another embodiment of the present invention there is provided a method of obtaining an antibody, or binding fragment thereof, that binds to human NOGO epitope VLPDIVMEAPLN (SEQ ID NO. 60), comprising immunising a mammal with said peptide and isolating cells capable of producing an antibody which binds to said peptide. In another embodiment of the present

invention there is provided a method of obtaining an isolated antibody, or binding fragment thereof, that binds to human NOGO epitope VLPDIVMEAPLN (SEQ ID NO. 60) comprising screening a library which comprises a plurality of antibodies or binding fragments thereof, each being isolatable from the library together with a nucleotide sequence that encodes the antibody or binding fragment thereof, by the binding of the antibody, or binding fragment thereof to the NOGO epitope VLPDIVMEAPLN (SEQ ID NO. 60).

Pharmaceutical compositions

A further aspect of the invention provides a pharmaceutical composition comprising an anti-NOGO antibody of the present invention or functional fragment or equivalent thereof together with a pharmaceutically acceptable diluent or carrier.

In a further aspect, the present invention provides a method of treatment or prophylaxis of stroke (particularly ischemic stroke) and other neurological diseases, in particular Alzheimer's disease, and treatment of a patient suffering from a mechanical trauma to the CNS (such as spinal chord injury), in a human which comprises administering to said human in need thereof an effective amount of an anti-NOGO antibody of the invention or functional fragments thereof.

In another aspect, the invention provides the use of an anti-NOGO antibody of the invention or a functional fragment thereof in the preparation of a medicament for treatment or prophylaxis of stroke (particularly ischemic stroke) and other neurological diseases, in particular Alzheimer's disease and treatment of a patient suffering from a mechanical trauma to the CNS (such as spinal chord injury).

In a further aspect, the present invention provides a method of inhibiting neurodegeneration and/or promoting functional recovery in a human patient afflicted with, or at risk of developing, a stroke (particularly ischemic stroke) or other neurological disease, in particular Alzheimer's disease, and treatment of a patient suffering from a mechanical trauma to the CNS (such as spinal chord injury), which comprises administering to said human in need thereof an effective

amount of an anti-NOGO antibody of the invention or a functional fragment thereof.

In a yet further aspect, the invention provides the use of an anti-NOGO antibody of the invention or a functional fragment thereof in the preparation of a medicament for inhibiting neurodegeneration and/or promoting functional recovery in a human patient afflicted with, or at risk of developing, a stroke and other neurological disease, in particular Alzheimer's disease and treatment of a patient suffering from a mechanical trauma to the CNS (such as spinal chord injury).

Other aspects and advantages of the present invention are described further in the detailed description and the preferred embodiments thereof.

Detailed Description of the Invention

The heavy chain variable regions of the invention may be formatted into the structure of a natural antibody or functional fragment or equivalent thereof. The antibody may therefore comprise the VH regions of the invention formatted into a full length antibody, a $(Fab')_2$ fragment, a Fab fragment, or equivalent thereof (such as scFV, bi- tr- or tetra-bodies, Tandabs, etc.), when paired with an appropriate light chain. The antibody may be an IgG1, IgG2, IgG3, or IgG4; or IgM; IgA, IgE or IgD or a modified variant thereof. The constant domain of the antibody heavy chain may be selected accordingly. The light chain constant domain may be a kappa or lambda constant domain. Furthermore, the antibody may comprise modifications of all classes eg IgG dimers, Fc mutants that no longer bind Fc receptors or mediate Clq binding. The antibody may also be a chimeric antibody of the type described in WO86/01533 which comprises an antigen binding region and a non-immunoglobulin region.

The constant region is selected according to the functionality required. Normally an IgG1 will demonstrate lytic ability through binding to complement and/or will mediate ADCC (antibody dependent cell cytotoxicity). An IgG4 will be preferred if a non-cytotoxic blocking antibody is required. However, IgG4 antibodies can demonstrate instability in production and therefore it may be more preferable to modify the generally more stable IgG1. Suggested modifications are described in EP0307434 preferred modifications include at positions 235 and

237. The invention therefore provides a lytic or a non-lytic form of an antibody according to the invention.

In preferred forms therefore the antibody of the invention is a full length (i.e. H2L2 tetramer) non-lytic IgG1 antibody having the heavy chain variable regions described herein.

In a further aspect, the invention provides polynucleotides encoding the heavy chain variable regions as described herein.

"NOGO" refers to any NOGO polypeptide, including variant forms. This includes, but is not limited to, NOGO-A having 1192 amino acid residues (GenBank accession no. AJ251383); NOGO-B, a splice variant which lacks residues 186 to 1004 in the putative extracellular domain (GenBank accession no. AJ251384) and a shorter splice variant, NOGO-C, which also lacks residues 186 to 1004 and also has smaller, alternative amino terminal domain (GenBank accession no. AJ251385) (Prinjha et al (2000) supra). All references to "NOGO" herein is understood to include any and all variant forms of NOGO such as NOGO-A and the splice variants described, unless a specific form is indicated.

"Neutralising" and grammatical variations thereof refers to inhibition, either total or partial, of NOGO function including its binding to neurones and inhibition of neurite growth.

The terms Fv, Fc, Fd, Fab, or $F(ab)_2$ are used with their standard meanings (see, e.g., Harlow et al., Antibodies A Laboratory Manual, Cold Spring Harbor Laboratory, (1988)).

A "chimeric antibody" refers to a type of engineered antibody which contains a naturally-occurring variable region (light chain and heavy chains) derived from a donor antibody in association with light and heavy chain constant regions derived from an acceptor antibody.

A "humanized antibody" refers to a type of engineered antibody having its CDRs derived from a non-human donor immunoglobulin, the remaining immunoglobulin-derived parts of the molecule being derived from one (or more) human immunoglobulin(s). In addition, framework support residues may be altered to preserve binding affinity (see, e.g., Queen et al., Proc. Natl Acad Sci USA, 86:10029-10032 (1989), Hodgson et al., Bio/Technology, 9:421 (1991)). A suitable human acceptor antibody may be one selected from a conventional

database, e.g., the KABAT® database, Los Alamos database, and Swiss Protein database, by homology to the nucleotide and amino acid sequences of the donor antibody (in this case the murine donor antibody 2A10). A human antibody characterized by a homology to the framework regions of the donor antibody (on an amino acid basis) may be suitable to provide a heavy chain constant region and/or a heavy chain variable framework region for insertion of the donor CDRs (see Table 1 for the 2A10 CDRs for insertion into the acceptor framework). A suitable acceptor antibody capable of donating light chain constant or variable framework regions may be selected in a similar manner. It should be noted that the acceptor antibody heavy and light chains are not required to originate from the same acceptor antibody. The prior art describes several ways of producing such humanised antibodies – see for example EP-A-0239400 and EP-A-054951.

The term "donor antibody" refers to a non-human antibody which contributes the amino acid sequences of its variable regions, CDRs, or other functional fragments or analogs thereof to the humanised antibody, and thereby provide the humanised antibody with the antigenic specificity and neutralizing activity characteristic of the donor antibody.

The term "acceptor antibody" refers to an antibody heterologous to the donor antibody, which provides the the amino acid sequences of its heavy and/or light chain framework regions and/or its heavy and/or light chain constant regions to the humanised antibody. The acceptor antibody may be derived from any mammal provided that it is non-immunogenic in humans. Preferably the acceptor antibody is a human antibody.

Alternatively, humanisation maybe achieved by a process of "veneering". A statistical analysis of unique human and murine immunoglobulin heavy and light chain variable regions revealed that the precise patterns of exposed residues are different in human and murine antibodies, and most individual surface positions have a strong preference for a small number of different residues (see Padlan E.A. *et al*; (1991) Mol.Immunol.28, 489-498 and Pedersen J.T. *et al* (1994) J.Mol.Biol. 235; 959-973). Therefore it is possible to reduce the immunogenicity of a non-human Fv by replacing exposed residues in its framework regions that differ from those usually found in human antibodies. Because protein antigenicity can be correlated with surface accessibility,

replacement of the surface residues may be sufficient to render the mouse variable region "invisible" to the human immune system (see also Mark G.E. *et al* (1994) in *Handbook of Experimental Pharmacology vol.113: The pharmacology of monoclonal Antib*odies, Springer-Verlag, pp105-134). This procedure of humanisation is referred to as "veneering" because only the surface of the antibody is altered, the supporting residues remain undisturbed. A further alternative approach is set out in WO04/006955.

"CDRs" are defined as the complementarity determining region amino acid sequences of an antibody which are the hypervariable regions of immunoglobulin heavy and light chains. See, e.g., Kabat et al., Sequences of Proteins of Immunological Interest, 4th Ed., U.S. Department of Health and Human Services, National Institutes of Health (1987). There are three heavy chain and three light chain CDRs (or CDR regions) in the variable portion of an immunoglobulin. Thus, "CDRs" as used herein refers to all three heavy chain CDRs, or all three light chain CDRs (or both all heavy and all light chain CDRs, if appropriate). The structure and protein folding of the antibody may mean that other residues are considered part of the antigen binding region and would be understood to be so by a skilled person. See for example Chothia et al., (1989) Conformations of immunoglobulin hypervariable regions; Nature 342, p877-883.

A bispecific antibody is an antibody having binding specificities for at least two different epitopes. Methods of making such antibodies are known in the art. Traditionally, the recombinant production of bispecific antibodies is based on the coexpression of two immunoglobulin H chain-L chain pairs, where the two H chains have different binding specificities see Millstein *et al*, Nature 305 537-539 (1983), WO93/08829 and Traunecker *et al* EMBO, 10, 1991, 3655-3659. Because of the random assortment of H and L chains, a potential mixture of ten different antibody structures are produced of which only one has the desired binding specificity. An alternative approach involves fusing the variable domains with the desired binding specificities to heavy chain constant region comprising at least part of the hinge region, CH2 and CH3 regions. It is preferred to have the CH1 region containing the site necessary for light chain binding present in at least one of the fusions. DNA encoding these fusions, and if desired the L chain are inserted into separate expression vectors and are then cotransfected into a

suitable host organism. It is possible though to insert the coding sequences for two or all three chains into one expression vector. In one preferred approach, the bispecific antibody is composed of a H chain with a first binding specificity in one arm and a H-L chain pair, providing a second binding specificity in the other arm, see WO94/04690. See also Suresh *et al* Methods in Enzymology 121, 210, 1986.

In one embodiment of the invention there is provided a bispecific therapeutic antibody wherein at least one binding specificity of said antibody binds to human NOGO at the epitope described in SEQ ID NO. 60. In another embodiment of the present invention the bispecific antibody comprises the heavy chain variable region CDR H3 sequence MQGY (SEQ ID NO. 45). In another embodiment the bispecific antibody comprises the following pairs of heavy and light chain variable regions: H27L16 (SEQ ID NO.48 + SEQ ID NO.14), H28L13 (SEQ ID NO.49 + SEQ ID NO.13) or H28L16 (SEQ ID NO.49 + SEQ ID NO.14).

The antibodies of the present invention may be produced by transfection of a host cell with an expression vector comprising the coding sequence for the antibodies of the invention. An expression vector or recombinant plasmid is produced by placing these coding sequences for the antibody in operative association with conventional regulatory control sequences capable of controlling the replication and expression in, and/or secretion from, a host cell. Regulatory sequences include promoter sequences, e.g., CMV promoter, and signal sequences, which can be derived from other known antibodies. Similarly, a second expression vector can be produced having a DNA sequence which encodes a complementary antibody light or heavy chain. Preferably this second expression vector is identical to the first except insofar as the coding sequences and selectable markers are concerned, so to ensure as far as possible that each polypeptide chain is functionally expressed. Alternatively, the heavy and light chain coding sequences for the altered antibody may reside on a single vector.

A selected host cell is co-transfected by conventional techniques with both the first and second vectors (or simply transfected by a single vector) to create the transfected host cell of the invention comprising both the recombinant or synthetic light and heavy chains. The transfected cell is then cultured by conventional techniques to produce the engineered antibody of the invention.

The antibody which includes the association of both the recombinant heavy chain and/or light chain is screened from culture by appropriate assay, such as ELISA or RIA. Similar conventional techniques may be employed to construct other altered antibodies and molecules.

One useful expression system is a glutamate synthetase system (such as sold by Lonza Biologics), particularly where the host cell is CHO or NS0 (see below). Polynucleotide encoding the antibody is readily isolated and sequenced using conventional procedures (e.g. oligonucleotide probes). Vectors that may be used include plasmid, virus, phage, transposons, minichromsomes of which plasmids are a typical embodiment. Generally such vectors further include a signal sequence, origin of replication, one or more marker genes, an enhancer element, a promoter and transcription termination sequences operably linked to the light and/or heavy chain polynucleotide so as to facilitate expression. Polynucleotide encoding the light and heavy chains may be inserted into separate vectors and introduced (e.g. by electroporation) into the same host cell or, if desired both the heavy chain and light chain can be inserted into the same vector for transfection into the host cell. Thus according to one embodiment of the present invention there is provided a process of constructing a vector encoding the light and/or heavy chains of a therapeutic antibody or antigen binding fragment thereof of the invention, which method comprises inserting into a vector, a polynucleotide encoding either a light chain and/or heavy chain of a therapeutic antibody of the invention.

In another embodiment there is provided a polynucletotide encoding a humanised heavy chain variable region having the sequence set forth as SEQ.I.D.NO: 47, 48 or 49.

In another embodiment there is provided a polynucleotide encoding a humanised heavy chain having the sequence set forth as SEQ.I.D.NO: 53, 54 or 55.

It will be immediately apparent to those skilled in the art that due to the redundancy of the genetic code, alternative polynucleotides to those disclosed herein are also available that will encode the polypeptides of the invention.

Suitable vectors for the cloning and subcloning steps employed in the methods and construction of the compositions of this invention may be selected by one of skill in the art. For example, the conventional pUC series of cloning

vectors may be used. One vector, pUC19, is commercially available from supply houses, such as Amersham (Buckinghamshire, United Kingdom) or Pharmacia (Uppsala, Sweden). Additionally, any vector which is capable of replicating readily, has an abundance of cloning sites and selectable genes (e.g., antibiotic resistance), and is easily manipulated may be used for cloning. Thus, the selection of the cloning vector is not a limiting factor in this invention. Other preferable vector sequences include a poly A signal sequence, such as from bovine growth hormone (BGH) and the betaglobin promoter sequence (betaglopro). The expression vectors useful herein may be synthesized by techniques well known to those skilled in this art. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins e.g. ampicillin, neomycin, methotrexate or tetracycline or (b) complement auxiotrophic deficiencies or supply nutrients not available in the complex media. The selection scheme may involve arresting growth of the host cell. Cells, which have been successfully transformed with the genes encoding the therapeutic antibody of the present invention, survive due to e.g. drug resistance conferred by the selection marker. Another example is the so-called DHFR selection marker wherein transformants are cultured in the presence of methotrexate. CHO cells are a particularly useful cell line for the DHFR selection. Methods of selecting transformed host cells and amplifying the cell copy number of the transgene include using the DHFR system see Kaufman R.J. et al J.Mol.Biol. (1982) 159, 601-621, for review, see Werner RG, Noe W, Kopp K,Schluter M," Appropriate mammalian expression systems for biopharmaceuticals", Arzneimittel-Forschung. 48(8):870-80, 1998 Aug. A further example is the glutamate synthetase expression system (Lonza Biologics). A suitable selection gene for use in yeast is the trp1 gene; see Stinchcomb *et al* Nature 282, 38, 1979.

The components of such vectors, e.g. replicons, selection genes, enhancers, promoters, signal sequences and the like, may be obtained from commercial or natural sources or synthesized by known procedures for use in directing the expression and/or secretion of the product of the recombinant DNA in a selected host. Other appropriate expression vectors of which numerous

types are known in the art for mammalian, bacterial, insect, yeast, and fungal expression may also be selected for this purpose.

The present invention also encompasses a cell line transfected with a recombinant plasmid containing the coding sequences of the antibodies or equivalents of the present invention. Host cells useful for the cloning and other manipulations of these cloning vectors are also conventional. However, most desirably, cells from various strains of E. coli are used for replication of the cloning vectors and other steps in the construction of altered antibodies of this invention.

Suitable host cells or cell lines for the expression of the antibody of the invention are preferably mammalian cells such as NS0, Sp2/0, CHO (e.g. DG44), COS, a fibroblast cell (e.g., 3T3), and myeloma cells, and more preferably a CHO or a myeloma cell. Human cells may be used, thus enabling the molecule to be modified with human glycosylation patterns. Alternatively, other eukaryotic cell lines may be employed. The selection of suitable mammalian host cells and methods for transformation, culture, amplification, screening and product production and purification are known in the art. See, e.g., Sambrook et al., cited above.

Bacterial cells may prove useful as host cells suitable for the expression of the antibodies or fragments thereof (such as recombinant Fabs or ScFvs) of the present invention (see, e.g., Plückthun, A., Immunol. Rev., 130:151-188 (1992)). However, due to the tendency of proteins expressed in bacterial cells to be in an unfolded or improperly folded form or in a non-glycosylated form, any recombinant fragment produced in a bacterial cell would have to be screened for retention of antigen binding ability. If the molecule expressed by the bacterial cell was produced in a properly folded form, that bacterial cell would be a desirable host. For example, various strains of E. coli used for expression are well-known as host cells in the field of biotechnology. Various strains of B. subtilis, Streptomyces, other bacilli and the like may also be employed in this method.

Where desired, strains of yeast cells known to those skilled in the art are also available as host cells, as well as insect cells, e.g. Drosophila and Lepidoptera and viral expression systems. See, e.g. Miller et al., Genetic Engineering, 8:277-298, Plenum Press (1986) and references cited therein.

The general methods by which the vectors may be constructed, the transfection methods required to produce the host cells of the invention, and culture methods necessary to produce the antibody of the invention from such host cell are all conventional techniques. Typically, the culture method of the present invention is a serum-free culture method, usually by culturing cells serum-free in suspension. Likewise, once produced, the antibodies of the invention may be purified from the cell culture contents according to standard procedures of the art, including ammonium sulfate precipitation, affinity columns, column chromatography, gel electrophoresis and the like. Such techniques are within the skill of the art and do not limit this invention. For example, preparation of antibodies are described in WO 99/58679 and WO 96/16990.

Yet another method of expression of the antibodies may utilize expression in a transgenic animal, such as described in U. S. Patent No. 4,873,316. This relates to an expression system using the animal's casein promoter which when transgenically incorporated into a mammal permits the female to produce the desired recombinant protein in its milk.

In a further aspect of the invention there is provided a method of producing an antibody of the invention which method comprises the step of culturing a host cell transformed or transfected with a vector encoding the light and/or heavy chain of the antibody of the invention and recovering the antibody thereby produced.

Suitable host cells for cloning or expressing vectors encoding antibodies of the invention are prokaroytic, yeast or higher eukaryotic cells. Suitable prokaryotic cells include eubacteria e.g. enterobacteriaceae such as *Escherichia* e.g. *E.Coli* (for example ATCC 31,446; 31,537; 27,325), *Enterobacter, Erwinia, Klebsiella Proteus, Salmonella* e.g. *Salmonella typhimurium*, Serratia e.g. *Serratia marcescans* and *Shigella* as well as Bacilli such as *B.subtilis* and *B.licheniformis* (see DD 266 710), Pseudomonas such as *P.aeruginosa* and *Streptomyces*. Of the yeast host cells, *Saccharomyces cerevisiae*, *schizosaccharomyces pombe*, Kluyveromyces (e.g. ATCC 16,045; 12,424; 24178; 56,500), yarrowia (EP402, 226), *Pichia Pastoris* (EP183, 070, see also Peng *et al* J.Biotechnol. 108 (2004) 185-192), *Candida, Trichoderma reesia*

(EP244, 234), *Penicillin, Tolypocladium* and *Aspergillus* hosts such as *A.nidulans* and *A.niger* are also contemplated.

Although Prokaryotic and yeast host cells are specifically contemplated by the invention, typically however, host cells of the present invention are vertebrate cells. Suitable vertebrate host cells include mammalian cells such as COS-1 (ATCC No.CRL 1650) COS-7 (ATCC CRL 1651), human embryonic kidney line 293, baby hamster kidney cells (BHK) (ATCC CRL.1632), BHK570 (ATCC NO: CRL 10314), 293 (ATCC NO.CRL 1573), Chinese hamster ovary cells CHO (e.g. CHO-K1, ATCC NO: CCL 61, DHFR-CHO cell line such as DG44 (see Urlaub *et al*, (1986) Somatic Cell Mol.Genet.12, 555-556)), particularly those CHO cell lines adapted for suspension culture, mouse sertoli cells, monkey kidney cells, African green monkey kidney cells (ATCC CRL-1587), HELA cells, canine kidney cells (ATCC CCL 34), human lung cells (ATCC CCL 75), Hep G2 and myeloma or lymphoma cells e.g. NS0 (see US 5,807,715), Sp2/0, Y0.

Thus in one embodiment of the invention there is provided a stably transformed host cell comprising a vector encoding a heavy chain and/or light chain of the therapeutic antibody or antigen binding fragment thereof as described herein. Typically such host cells comprise a first vector encoding the light chain and a second vector encoding said heavy chain.

Host cells transformed with vectors encoding the therapeutic antibodies of the invention or antigen binding fragments thereof may be cultured by any method known to those skilled in the art. Host cells may be cultured in spinner flasks, roller bottles or hollow fibre systems but it is preferred for large scale production that stirred tank reactors are used particularly for suspension cultures. Typically the stirred tankers are adapted for aeration using e.g. spargers, baffles or low shear impellers. For bubble columns and airlift reactors direct aeration with air or oxygen bubbles maybe used. Where the host cells are cultured in a serum free culture media it is preferred that the media is supplemented with a cell protective agent such as pluronic F-68 to help prevent cell damage as a result of the aeration process. Depending on the host cell characteristics, either microcarriers maybe used as growth substrates for anchorage dependent cell lines or the cells maybe adapted to suspension culture (which is typical). The culturing of host cells, particularly vertebrate host cells may utilise a variety of

operational modes such as fed-batch, repeated batch processing (see Drapeau *et al* (1994) cytotechnology 15: 103-109), extended batch process or perfusion culture. Although recombinantly transformed mammalian host cells may be cultured in serum-containing media such media comprising fetal calf serum (FCS), it is preferred that such host cells are cultured in synthetic serum –free media such as disclosed in Keen *et al* (1995) Cytotechnology 17:153-163, or commercially available media such as ProCHO-CDM or UltraCHO™ (Cambrex NJ, USA), supplemented where necessary with an energy source such as glucose and synthetic growth factors such as recombinant insulin. The serum-free culturing of host cells may require that those cells are adapted to grow in serum free conditions. One adaptation approach is to culture such host cells in serum containing media and repeatedly exchange 80% of the culture medium for the serum-free media so that the host cells learn to adapt in serum free conditions (see e.g. Scharfenberg *K et al* (1995) in *Animal Cell technology: Developments towards the 21st century* (Beuvery E.C. *et al* eds), pp619-623, Kluwer Academic publishers).

Antibodies of the invention secreted into the media may be recovered and purified from the media using a variety of techniques to provide a degree of purification suitable for the intended use. For example the use of therapeutic antibodies of the invention for the treatment of human patients typically mandates at least 95% purity, more typically 98% or 99% purity compared to the culture media comprising the therapeutic antibodies. In the first instance, cell debris from the culture media is typically removed using centrifugation followed by a clarification step of the supernatant using e.g. microfiltration, ultrafiltration and/or depth filtration. A variety of other techniques such as dialysis and gel electrophoresis and chromatographic techniques such as hydroxyapatite (HA), affinity chromatography (optionally involving an affinity tagging system such as polyhistidine) and/or hydrophobic interaction chromatography (HIC, see US 5, 429,746) are available. In one embodiment, the antibodies of the invention, following various clarification steps, are captured using Protein A or G affinity chromatography followed by further chromatography steps such as ion exchange and/or HA chromatography, anion or cation exchange, size exclusion chromatography and ammonium sulphate precipitation. Typically, various virus

removal steps are also employed (e.g. nanofiltration using e.g. a DV-20 filter). Following these various steps, a purified (typically monoclonal) preparation comprising at least 75mg/ml or greater e.g. 100mg/ml or greater of the antibody of the invention or antigen binding fragment thereof is provided and therefore forms an embodiment of the invention. Suitably such preparations are substantially free of aggregated forms of antibodies of the invention.

In accordance with the present invention there is provided a method of producing an anti-NOGO antibody of the present invention which specifically binds to and neutralises the activity of human NOGO-A which method comprises the steps of;

(a) providing a first vector encoding a heavy chain of the antibody;

(b) providing a second vector encoding the light chain of the antibody;

(c) tranforming a mammalian host cell (e.g. CHO) with said first and second vectors;

(d) culturing the host cell of step (c) under conditions conducive to the secretion of the antibody from said host cell into said culture media;

(e) recovering the secreted antibody of step (d).

Once expressed by the desired method, the antibody is then examined for in vitro activity by use of an appropriate assay. Presently conventional ELISA assay formats are employed to assess qualitative and quantitative binding of the antibody to NOGO. Additionally, other in vitro assays may also be used to verify neutralizing efficacy prior to subsequent human clinical studies performed to evaluate the persistence of the antibody in the body despite the usual clearance mechanisms.

Other modifications to the antibodies of the present invention include glycosylation variants of the antibodies of the invention. Glycosylation of antibodies at conserved positions in their constant regions is known to have a profound effect on antibody function, particularly effector functioning such as those described above, see for example, Boyd *et al* (1996), Mol.Immunol. 32, 1311-1318. Glycosylation variants of the therapeutic antibodies or antigen binding fragments thereof of the present invention wherein one or more carbonhydrate moiety is added, substituted, deleted or modified are

contemplated. Introduction of an asparagine-X-serine or asparagine-X-threonine motif creates a potential site for enzymatic attachment of carbonhydrate moieties and may therefore be used to manipulate the glycosylation of an antibody. In Raju *et al* (2001) Biochemistry 40, 8868-8876 the terminal sialyation of a TNFR-IgG immunoadhesin was increased through a process of regalactosylation and/or resialylation using beta-1, 4-galactosyltransferace and/or alpha, 2,3 sialyltransferase. Increasing the terminal sialylation is believed to increase the half-life of the immunoglobulin. Antibodies, in common with most glycoproteins, are typically produced in nature as a mixture of glycoforms. This mixture is particularly apparent when antibodies are produced in eukaryotic, particularly mammalian cells. A variety of methods have been developed to manufacture defined glycoforms, see Zhang *et al* Science (2004), 303, 371, Sears *et al*, Science, (2001) 291, 2344, Wacker *et al* (2002) Science, 298 1790, Davis *et al* (2002) Chem.Rev. 102, 579, Hang *et al* (2001) Acc.Chem.Res 34, 727. Thus the invention concerns a plurality of therapeutic (typically monoclonal) antibodies (which maybe of the IgG isotype, e.g. IgG1) as described herein comprising a defined number (e.g. 7 or less, for example 5 or less such as two or a single) glycoform(s) of said antibodies or antigen binding fragments thereof.

The therapeutic agents of this invention may be administered as a prophylactic or following the stroke event/on-set of clinical symptoms, or as otherwise needed. The dose and duration of treatment relates to the relative duration of the molecules of the present invention in the human circulation, and can be adjusted by one of skill in the art depending upon the condition being treated and the general health of the patient. It is envisaged that repeated dosing (e.g. once a week or once every two weeks) over an extended time period (e.g. four to six months) maybe required to achieve maximal therapeutic efficacy.

The mode of administration of the therapeutic agent of the invention may be any suitable route which delivers the agent to the host. The antibodies, and pharmaceutical compositions of the invention are particularly useful for parenteral administration, i.e., subcutaneously (s.c.), intrathecally, intraperitoneally (i.p.), intramuscularly (i.m.), intravenously (i.v.), or intranasally (i.n.).

Therapeutic agents of the invention may be prepared as pharmaceutical compositions containing an effective amount of the antibody of the invention as

an active ingredient in a pharmaceutically acceptable carrier. In the prophylactic agent of the invention, an aqueous suspension or solution containing the engineered antibody, preferably buffered at physiological pH, in a form ready for injection is preferred. The compositions for parenteral administration will commonly comprise a solution of the antibody of the invention or a cocktail thereof dissolved in a pharmaceutically acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers may be employed, e.g., 0.9% saline, 0.3% glycine, and the like. These solutions are sterile and generally free of particulate matter. These solutions may be sterilized by conventional, well known sterilization techniques (e.g., filtration). The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, etc. The concentration of the antibody of the invention in such pharmaceutical formulation can vary widely, i.e., from less than about 0.5%, usually at or at least about 1% to as much as 15 or 20% by weight and will be selected primarily based on fluid volumes, viscosities, etc., according to the particular mode of administration selected.

Thus, a pharmaceutical composition of the invention for intramuscular injection could be prepared to contain 1 mL sterile buffered water, and between about 1 ng to about 100 mg, e.g. about 50 ng to about 30 mg or more preferably, about 5 mg to about 25 mg, of an antibody of the invention. Similarly, a pharmaceutical composition of the invention for intravenous infusion could be made up to contain about 250 ml of sterile Ringer's solution, and about 1 to about 30 and preferably 5 mg to about 25 mg of an engineered antibody of the invention per ml of Ringer's solution. Actual methods for preparing parenterally administrable compositions are well known or will be apparent to those skilled in the art and are described in more detail in, for example, Remington's Pharmaceutical Science, 15th ed., Mack Publishing Company, Easton, Pennsylvania. For the preparation of intravenously administrable antibody formulations of the invention see Lasmar U and Parkins D "The formulation of Biopharmaceutical products", Pharma. Sci.Tech.today, page 129-137, Vol.3 (3rd April 2000), Wang, W "Instability, stabilisation and formulation of liquid protein pharmaceuticals", Int. J. Pharm 185 (1999) 129-188, Stability of Protein

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It is preferred that the therapeutic agent of the invention, when in a pharmaceutical preparation, be present in unit dose forms. The appropriate therapeutically effective dose will be determined readily by those of skill in the art. To effectively treat stroke and other neurological diseases in a human, one dose within the range of 700 to 3500 mg per 70 kg body weight of an antibody of this invention is envisaged to be administered parenterally, preferably s.c., i.v. or i.m. (intramuscularly). Such dose may, if necessary, be repeated at appropriate time intervals selected as appropriate by a physician.

The antibodies described herein can be lyophilized for storage and reconstituted in a suitable carrier prior to use. This technique has been shown to be effective with conventional immunoglobulins and art-known lyophilization and reconstitution techniques can be employed.

In another aspect, the invention provides a pharmaceutical composition comprising anti-NOGO antibody of the present invention or a functional fragment thereof and a pharmaceutically acceptable carrier for treatment or prophylaxis of stroke and other neurological diseases.

In a yet further aspect, the invention provides a pharmaceutical composition comprising the anti-NOGO antibody of the present invention or a functional fragment thereof and a pharmaceutically acceptable carrier for inhibiting neurodegeneration and/or promoting functional recovery in a human patient suffering, or at risk of developing, a stroke or other neurological disease.

The invention further provides a method of treatment or prophylaxis of stroke (particularly ischemic stroke) and other neurological diseases/disorders, in particular Alzheimer's disease, in a human which comprises administering to said human in need thereof an effective amount of an anti-NOGO antibody of the present invention or a functional fragment thereof. Antibodies of the invention may be used in methods of treatment to slow or halt the progression and/or onset of Alzheimer's disease in addition to (or as an alternative to) treating established disease in a human patient.

Further the invention provides the use of an anti-NOGO antibody of the present invention, or a functional fragment thereof, in the preparation of a medicament for treatment or prophylaxis of stroke and other neurological diseases/disorders, in particular Alzheimer's disease.

The invention also provides a method of inhibiting neurodegeneration and/or promoting functional recovery in a human patient suffering, or at risk of developing, a stroke or other neurological disease/disorder, in particular Alzheimer's disease, which comprises administering to said human in need thereof an effective amount of an anti-NOGO antibody of the present invention or a functional fragment thereof.

In addition the invention provides the use of an anti-NOGO antibody of the present invention or a functional fragment thereof in the preparation of a medicament for inhibiting neurodegeneration and/or promoting functional recovery in a human patient afflicted with, or at risk of developing, a stroke and other neurological disease/disorder, in particular Alzheimer's disease.

The invention further provides a method of treating or prophylaxis of stroke or other neurological disease/disorder, in particular Alzheimer's disease, in a human comprising the step of parenteral administration of a therapeutically effective amount of an anti-NOGO antibody of the present invention. Preferably the said anti-NOGO antibody is administered intravenously.

Neurological diseases or disorders as used hereinabove includes, but is not limited to traumatic brain injury, spinal cord injury, fronto-temporal dementias (tauopathies), peripheral neuropathy, Parkinson's disease, Huntington's disease, and in particular Alzheimer's disease, multiple sclerosis or amyotrophic lateral sclerosis (ALS).

The invention also provides a method of promoting axonal sprouting comprising the step of contacting a human axon with an anti-NOGO antibody of the present invention. This method may be performed in-vitro or in-vivo, preferably the method is performed in-vivo.

In a further aspect therefore there is provided a method of treating stroke (particularly ischemic stroke), brain injury, spinal cord injury, fronto-temporal dementias (tauopathies), peripheral neuropathy, Parkinson's disease, Huntington's disease, multiple sclerosis and in particular Alzheimer's disease in a human patient which method comprises the intravenous administration of a therapeutically effective amount of an anti-NOGO antibody of the invention.

In a further aspect of the present invention there is provided a method of promoting axon sprouting of neurons within the central nervous system of a human subject (e.g. patient) which method comprises administering (e.g. intravenously administering) a therapeutically effective amount of an anti-NOGO antibody of the present invention.

In a further aspect of the present invention there is provided the use of an anti-NOGO antibody of the present invention (e.g. an anti-NOGO antibody comprising the CDRs set forth herein) in the manufacture of an intravenously administrable medicament for the treatment of stroke (particularly ischemic stroke), brain injury, spinal cord injury, fronto-temporal dementias (tauopathies), peripheral neuropathy, Parkinson's disease, Huntington's disease, and in particular Alzheimer's disease, multiple sclerosis or amyotrophic lateral sclerosis (ALS) in a human patient.

In a further aspect of the invention there is provided a method of regenerating axon processes in neurons of the central nervous system in a human patient afflicted with (or susceptible to) stroke (particularly ischemic stroke), brain injury, spinal cord injury, fronto-temporal dementias (tauopathies), peripheral neuropathy, Parkinson's disease, Huntington's disease, multiple sclerosis and in particular Alzheimer's disease which method comprises the step of administering (e.g. intravenously) a therapeutically effective amount of an anti-NOGO antibody of the present invention.

In a further aspect of the invention there is provided the use of an anti-NOGO antibody of the present invention in the manufacture of an intravenously

administrable pharmaceutical composition for regenerating axon processes in neurons of the central nervous system in a human patient afflicted with (or susceptible to) stroke (particularly ischemic stroke), brain injury, spinal cord injury, fronto-temporal dementias (tauopathies), peripheral neuropathy, Parkinson's disease, Huntington's disease, multiple sclerosis and in particular Alzheimer's disease.

In a further aspect of the invention there is provided a method of modulating the production of an amyloidogenic peptide comprising contacting a cell which is expressing the precursor from which the amyloidogenic peptide is derived and a NOGO polypeptide (e.g. human NOGO-A) with an anti-NOGO antibody of the present invention. In typical embodiments, the precursor is APP. In further typical embodiments the amyloidogenic peptide is Aβ, most preferably Aβ40, Aβ42 or a combination of both.

As used herein, the term "functional recovery" refers to a motor and/or sensory and/or behavioural improvement in a subject following e.g. an ischemic event or injury or on-set of clinical symptoms. Functional recovery in humans may be evaluated by instruments designed to measure elemental neurological functions such as motor strength, sensation and coordination, cognitive functions such as memory, language and the ability to follow directions, and functional capacities such as basic activities of daily living or instrumental activities. Recovery of elemental neurological function can be measured with instruments such as the NIH Stroke Scale (NIHSS), recovery of cognitive function can be measured with neuropsychological tests such as Boston Naming Test, Trail-making Tests, and California Verbal Learning Test, and activities of daily living may be measured with instruments such as the ADCS/ADL (Alzheimer's Disease Clinical Studies/Activities of Daily Living) scale or the Bristol Activities of Daily Living Scale, all tests and scales known in the art.

The following examples illustrate but do not limit the invention.

Example 1, *Construction and expression of humanised anti-NOGO antibodies*

Humanised V_H and V_L constructs were prepared de novo by build up of overlapping oligonucleotides including restriction sites for cloning into RId and RIn mammalian expression vectors (or any other suitable expression vector for

expression of proteins in mammalian cells) as well as a human signal sequence. Hind III and Spe I restriction sites were introduced to frame the V_H domain containing the CAMPATH-1H signal sequence for cloning into Rld containing the human γ1 mutated constant region to prevent ADCC and CDC activity (L235A and G237A – EU Index numbering system). Hind III and BsiWI restriction sites were introduced to frame the V_L domain containing the CAMPATH-1H signal sequence for cloning into Rln containing the human kappa constant region.

CAMPATH-1H signal sequence: MGWSCIILFLVATATGVHS (SEQ.ID.NO:31)

Plasmids encoding human IgG heavy chain amino acid sequences, wherein the CDR were that described in table 2, were produced. Plasmids encoding human IgG heavy chain amino acid sequences, wherein the CDRs were that described in table 3, were produced from those existing earlier plasmids by introducing
single point mutations, G95M (Kabat numbering), using the Quickchange kit (Stratagene).

The following table discloses which full length heavy chain protein sequences were made in the plasmid vectors and which of the sequences were paired, in the sense that the only difference in the amino acid sequences of the paired full length (FL) heavy chain sequences was a substitution at G95M (kabat numbering) within the CDR H3 of the variable region:
Table 8,

CDRs as defined in Table 2	G95M substitution (to form CDR H3 of Table 3)
H1 FL (SEQ ID NO. 35)	Not done
H6 FL (SEQ ID NO. 15)	H26 FL (SEQ ID NO. 53)
H16 FL (SEQ ID NO. 16)	H27 FL (SEQ ID NO. 54)
H20 FL (SEQ ID NO. 42)	H28 FL (SEQ ID NO. 55)

Plasmids encoding the heavy chains were then co-transfected into CHO cells (for details see example 2) with the one of the following full length light chain sequences: L11 FL (SEQ ID NO. 36), L13 FL (SEQ ID NO. 17), or L16 FL (SEQ ID NO. 18).

In parallel a chimera termed HcLc (which is the chimera of 2A10 (SEQ ID NO. 9 and 10 – the full length chains comprising the 2A10 murine VH (SEQ ID NO. 7) and VL (SEQ ID NO.8) and human IgG constant regions)) was produced.

Example 2, *Antibody expression in CHO cells*

Rld and Rln plasmids (or other vectors suitable for use in mammalian cells) encoding the heavy and light chains respectively were transiently co-transfected into CHO cells and expressed at small scale or large scale to produce antibody. Alternatively the same plasmids were co-transfected into DHFR- CHO cells by electroporation and a stable polyclonal population of cells expressing the appropriate antibody were selected using a nucleoside-free media (Rld contains the DHFR gene, Rln contains a neomycin selection marker). In some assays, antibodies were assessed directly from the tissue culture supernatant. In other assays, recombinant antibody was recovered and purified by affinity chromatography on Protein A sepharose.

Example 3, *Humanised anti-NOGO antibody binds to NOGO*

GST-human NOGO-A56 (see example 5) at 0.05-1µg/ml in PBS was coated onto Nunc Immunosorp plates (100µl per well) at 4°C overnight. Wells were rinsed once with TBS + 0.05% Tween (TBST) then incubated with 2% BSA in TBST to block non-specific binding sites at room temperature for 1hour. Antibodies were diluted in TBST + 2% BSA to 10µg/ml and 1/2 dilutions made from this. Antibodies were added to wells in duplicate and incubated at room temperature for 1hour. Wells were washed three times with TBST then incubated with anti-human kappa peroxidase conjugate (1:2000) for 1hour. The wells were washed three times with TBST and then incubated with 100µl OPD peroxidase substrate (Sigma) per well for 10 minutes. The colour reaction was stopped by the addition of 25µl concentrated H_2SO_4. Optical density at 490nm was measured using a plate reader. Background values read from wells with no antibody were subtracted.

Figures 1-4 illustrate the dose-dependent binding of humanised antibodies in comparison with the chimera (termed HcLc which is the chimera of 2A10 (comprising the 2A10 murine VH (SEQ ID NO. 7) and VL (SEQ ID NO.8) and human IgG constant regions)) to GST-human NOGO-A56 (see Example 5 for details) in an ELISA assay. The Y-axis shows the measured optical density (OD) at 490nm, a quantitative measure of antibody captured in the wells. The X-axis shows the concentration of antibody used (mcg/ml) per well at each data point.

The antibody material used in Figures 1-4 is purified antibody generated by either the polyclonal expression system or large scale transient transfections. In these cases, IgG levels were quantified by ELISA and optical density.

The results from the experiments shown in Figures 1-4 shows that the inclusion of the G95M mutation improves the performance of the antibody. The only exception is H27L16 shown in Figure 3 and 4 which performed very poorly. We believe that this data resulted from an unidentified technical problem with the H27L16 assay, since H27L16 has otherwise consistently performed well in other assays (in ELISA shown in Figures 1 and 2, and in BIAcore assays (Tables 9 and 10)). H27L16 has also been shown to work very well in later experiments (see Figures 11 and 12).

Example 4, *Antibody quantification protocol*

Nunc Immunosorp plates were coated with a goat anti-human IgG chain capture antibody (Sigma #I3382) at 2µg/ml in Bicarbonate buffer (Sigma #C3041) and incubated overnight at 4°C. The plates were washed twice with TBS containing 0.05% Tween20 (TBST) and blocked with 200µl TBST containing 2% (or from 1-3%) BSA (block buffer) for 1hr at room temperature. The plates were washed twice with TBST. Tissue culture supernatants containing antibody were titrated across the plate in 2-fold dilution steps into block buffer and incubated at room temperature for 1hr. The plates were washed three times with TBST. HRP conjugated antibody H23 (goat anti-human kappa chain, Sigma #A7164) was diluted 1:2000 in TBST and 100µl added to each well. The plates were incubated at room temperature for 1hr. The plates were washed three times with TBST and developed with 100µl of Fast-OPD substrate (Sigma #P9187). Colour was allowed to develop for 5-10mins after which time the ELISA was stopped with

25µl 3M H_2SO_4. The absorbance at 490nM was read plate and antibody concentration determined by reference to a standard curve.

Example 5, *Production of NOGO-A Fragment (NOGO-A56, SEQ.ID.NO:32)*

A cDNA sequence encoding a polypeptide comprising amino acids 586-785 and a GST tag(SEQ.I.D.NO:32) of human NOGO-A was created by cloning a cDNA encoding amino acids 586-785 of human NOGO-A into the BamHI-XhoI sites of pGEX-6P1 to generate a GST-tagged fusion protein designated GST-human-NOGO-A56. Plasmid was expressed in BL21 cells in 2XTY medium with 100µg/ml ampicillin following induction with IPTG to 0.5mM at 37C for 3hours. Cell pellets were lysed by sonication and the fusion protein purified using Glutathione-sepharose (Amersham Pharmacia) following manufacturers instructions. Purified protein was eluted using reduced glutathione and extensively dialysed against PBS, quantitated using BSA standards and a BioRad coomassie based protein assay and then stored in aliquots at -80C.

Example 6, *BiaCore Analysis of humanised Anti NOGO Monoclonal Antibodies*

The binding kinetics of the anti-NOGO monoclonal antibody (mAb) to recombinantly expressed GST-human NOGO-A was analysed using the Biacore3000 biosensor or BIAcore T100. The hNOGO-A chip was prepared as follows:

Method

GST-human NOGO-A56 was immobilised to a CM5 chip by primary amine coupling using the Biacore Wizard program designed for targeted immobilisation levels. The CM5 sensor surface was activated by passing a solution of 50mM N-hydroxy-succinimide (NHS) and 200mM N-ethyl-N'-dimethylaminopropyl carbonide (EDC). Then GST-human NOGO-A56in sodium acetate buffer, pH5.0 or pH 4.5, was passed over the chip and immobilised. After immobilisation was complete any still activated esters were blocked by an injection of 1M ethanolamine hydrochloride, pH8.5.

The anti-NOGO mAbs were diluted down in HBS-EP (10mM HEPES, pH 7.4, 150mM NaCl, 3mM EDTA, and 0.005% P-20 surfactant) for the BIAcore 3000 or HBS-EP+ (10mM HEPES, pH 7.4, 150mM NaCl, 3mM EDTA, and 0.05%

P-20 surfactant) in the case of the T100 and binding studies were carried out at range of defined antibody concentrations. All runs were referenced against a blanked sensor surface (one that had been activated and blocked as described earlier but had no addition of ligand). Analysis of binding was carried out using the BIAevaluation kinetic analysis software version 4.1 for the BIAcore 3000 and T100 kinetic analysis software version 1.0. Biacore analysis of other antibodies of the invention essentially followed the same protocol as described herein. Unless otherwise stated, the BIAcore experiments were performed at 25°C.

In the following Results section each data table represents the results obtained from an individual experiment.

Results – Table 9

Antibody	ka (1/Ms)	kd (1/s)	KD (pM)
HcLc	3.19E6	2.49E-3	779
H27L13	6.2E6	1.8E-3	291
H26L13	3.23E6	3.11E-3	963
H28L13	7.26E6	3.3E-3	454
H27L16	6.24E6	1.21E-3	194
H28L16	7.25E6	2.14E-3	296

Results – Table 10

Antibody	ka (1/Ms)	kd (1/s)	KD (nM)
HcLc (25°C)	2.66E6	3.13E-3	1.18
HcLc (37°C)	5.08E6	7.74E-3	1.46
H16L16 (25°C)	3.43E6	3.72E-3	1.08
H16L16 (37°C)	5.31E6	6.16E-3	1.16
H20L16 (25°C)	4.69E6	5.42E-3	1.16
H20L16 (37°C)	7.17E6	1.08E-3	1.51

H27L16 (25°C)	3.94E6	1.50E-3	0.380
H27L16 (37°C)	7.18E6	3.06E-3	0.426
H27L13 (25°C)	3.50E6	2.13E-3	0.606
H27L13 (37°C)	6.58E6	4.22E-3	0.641
H28L16 (25°C)	4.33E6	2.64E-3	0.610
H28L16 (37°C)	7.73E6	5.24E-3	0.678
H28L13 (25°C)	4.16E6	3.89E-3	0.936
H28L13 (37°C)	7.43E6	7.59E-3	1.02

Results – Table 11

Antibodies	**ka (1/Ms)**	**Kd (1/s)**	**KD (nM)**
HcLc	3.17E6	2.33E-3	0.74
H26L13	3.45E6	2.88E-3	0.87
H27L13	6.58E6	1.83E-3	0.28
H28L13	6.97E6	3.17E-3	0.45
H28L16	6.89E6	1.95E-3	0.28

Example 7: *BiaCore Analysis of humanised Anti NOGO Monoclonal Antibodies using off-rate ranking*

The GST-human NOGO-A56 chip was prepared as for kinetic analysis. Cell supernatants where taken directly from transient transfections of CHO-K1 cells. These were passed directly over the sensor surface and the interaction measured. A mock transfected cell supernatant was used for double referencing to remove any artefacts due to the tissue culture media. All runs were referenced against a blanked sensor surface (one that had been activated and blocked as described earlier but had no addition of ligand). Analysis of binding was carried out using the BIAevaluation kinetic analysis software version 4.1.

***Example 8**: Peptide Mapping*

47 overlapping peptides spanning the NOGO-A56 portion of GST-human NOGO-A56 domain (SEQ ID NO. 32) were obtained (from Mimotope™). The

peptides are 16 amino acids in length with a twelve amino acid overlap with the adjacent peptide (each peptide further comprising a biotin-SGSG sequence at the N-terminus) with the exception of the first peptide which has a GSG-biocytin tag at the C-terminus. The peptides were used to epitope map the binding site of 2A10 and H28L16.

Method for epitope mapping:

Streptavidin at 5μg/ml in sterile water was coated onto Nunc immunosorp plates (100 μl per well) at 37°C overnight. The plates were rinsed 3 times with PBS containing 0.05% Tween (PBST) then blocked with 3% BSA in PBST at 4°C overnight. The plates were washed 3 times with PBST. Peptides were then added to the wells at a concentration of approximately 10 μg/ml (diluted in 3% BSA in PBST) and incubated at room temperature for 1 hour. The plates were washed 3 times with PBST then incubated for 1 hour with anti-NOGO antibodies diluted to 5 μg/ml in 3% BSA in PBST. The plates were washed 3 times with PBST then incubated with anti-human or anti-mouse kappa peroxidase conjugate (1:1000, diluted in 3% BSA in PBST) for 1 hour. The plates were washed 3 times with PBST and then incubated with 100 μl OPD peroxidase substrate (Sigma) per well for 10 minutes. The colour reaction was stopped by the addition of 50 μl 3 molar H_2SO_4. Absorbance at 490 nm was measured using a plate reader.

The results are shown in figures 5 (epitope mapping using H28L16), figure 6 (epitope mapping using 2A10). Figures 5 and 6 show the results of the epitope mapping of 2A10 and H28L16, respectively. The data shown indicates that 2A10 and H28L16 bind to peptides 6 and 7 of which the NOGO specific portion is given in SEQ ID NO.73 and SEQ ID NO.74 respectively, both of which contain the sequence VLPDIVMEAPLN. These results indicate that VLPDIVMEAPLN (SEQ ID NO.60) contains the binding epitope of 2A10 and H28L16.

***Example 9**: Comparison of HcLc and HcLc containing the G95M mutation of the CDR H3*

A modified variant of HcLc was constructed from existing expression plasmids by introducing a single point mutation, G95M (Kabat numbering), using

the Quikchange kit (Stratagene). The protein sequence of the variable heavy domain Hc (G95M) protein is given in SEQ ID 59.

Hc(G95M)Lc was expressed in CHO cells as described previously. The antibody was quantified as described in Example 4. Figures 7 and Figure 8 show a comparison of the binding activity of Hc(G95M)Lc and HcLc as determined using a human NOGO-A binding ELISA when NOGO was coated onto Nunc immunosorp plates at 0.05 (Figure 7) and 1 µg/ml (Figure 8). Table below shows a comparison of the binding affinities of Hc(G95M)Lc and HcLc.

Table 12, Off-rate measured by Biacore ranking based on one experiment

Antibody	Sequence ID of heavy chain variable region	Off-rate kd (1/s)
H6L13	11	1.38E-2
H6(G95M)L13	47	4.31E-3
HcLc	7	2.66E-3
Hc(G95M)Lc	59	6.14E-4

The data demonstrates that the G95M substitution within CDR H3 not only increases the binding activity of the humanised antibodies (H6L13), but also the murine donor antibody 2A10 (HcLc).

***Example 10**: Construction and testing of NOGO antibodies containing substitutions in CDR H3*

A panel of 90 heavy chain variable regions was created by single point mutations in the residues contained in the CDR H3, or the preceding Leucine. Specifically, vectors encoding a heavy chain (based on H6FL, SEQ ID NO. 15) were made encoding heavy chain variable regions where each amino acid residue in CDR H3 and the preceding Leucine was substituted (using the Quikchange kit (Stratagene)) with all other naturally occurring amino acids, excluding cysteine, and expressed in conjunction with a light chain (L13FL, SEQ ID NO. 17) to give 90 different antibodies. These antibodies were assayed for binding to NOGO in ELISA and Biacore experiments.

Figures 9 and 10 show a comparison of the binding activity of the variants of H6FL in comparison to H6FL L13FL. Tables 14 and 15 show a comparison of the off-rate kinetics as measured by Biacore – only the results for those antibodies that had a measurable off rate in the Biacore assay and had comparable binding activity to H6L13 in ELISA are shown.

Table 14

Parent Antibody	VH CDR3	kd (1/s)
H6L13	MQGY	4.85E-03
HcLc	GQGY	5.58E-03
H6L13	GQNY	9.66E-03
H6L13	GQLY	1.32E-02
H6L13	IQGY	1.72E-02
H6L13	RQGY	1.75E-02
H6L13	GQSY	1.86E-02
H6L13	GQGY	1.98E-02
H6L13	GSGY	2.07E-02
H6L13	GDGY	2.12E-02
H6L13	GQGW	2.16E-02
H6L13	GIGY	2.57E-02
H6L13	GQYY	3.28E-02
H6L13	GQFY	3.35E-02
H6L13	WQGY	1.98E-02
H6L13	GAGY	3.15E-02
H6L13	GLGY	1.90E-02
H6L13	GVGY	1.78E-02
H6L13	GQWY	1.77E-02

Conclusions

The results indicate that the antibodies which retain the binding properties of the murine 2A10, and the GQGY containing antibody H6L13, are those containing following CDR H3: RQGY, IQGY, MQGY, GDGY, GIGY, GSGY, GQNY, GQYY, GQSY, GQLY, GQFY, GQGW, WQGY, GAGY, GLGY, GVGY, GQWY.

Example 11, *Comparison of GQGY containing mab (H20L16) with G95M variant mabs (H27L16 and H28L13 and H28L16)*

The antibodies listed in Table 15 were manufactured as described above.

Table 15 – humanised 2A10 anti-Nogo-A antibodies giving the total number of back-mutations for the whole antibody (2x heavy chain + 2x light chain).

Antibody	Total number of back-mutations per whole antibody/tetramer
H20L16	22
H28L16	22
H28L13	16
H27L16	32

In vitro binding characteristics

In an attempt to rank the antibodies, their binding properties were investigated in a range of assays including ELISA, reverse format ELISA, competition ELISA, Biacore and by flow cytometry.

11.1 Binding to recombinant human NOGO-A in ELISA

The ability of the antibodies to bind recombinant human Nogo-A (GST-human Nogo-A 56) was investigated by various related ELISA assays (performed in a related, but slightly different, protocol as that described in Example 3). In the first assay, the recombinant Nogo-A is directly coated to the plate at various different antigen concentrations. The results of the direct binding ELISA when the antigen is loaded at 1mcg/ml or 0.05mcg/ml are shown in Figure 11A and Figure 11B respectively. The data confirms that all the antibodies show comparable binding activity to recombinant human Nogo-A when compared with the chimeric form of the parental antibody (HcLc). At higher antigen coating concentrations, all antibodies yield a similar EC50 value. In contrast, at a lower antigen coating concentration the assay was able to discriminate between the antibodies. Although saturation curves were not obtained, a trend analysis on the lines revealed the following rank order: H27L16>H28L16, H28L13, H20L16.

In a parallel experiment, the format of the assay was reversed. In this format, the antibody is captured on to the plate and the binding of the recombinant human Nogo-A (GST-human Nogo-A-56) detected using the GST tag. The results of the reverse format ELISA are shown in Figure 12. The data

confirms that all the antibodies show comparable binding activity to recombinant human Nogo-A when compared with the chimeric form of the parental antibody (HcLc). This format of the binding ELISA did not distinguish between the antibodies.

11.2 Competition ELISA

The ability of the antibodies to compete directly with the parental antibody for the same epitope on human Nogo-A was assessed using a competition ELISA. The recombinant human Nogo-A (GST-human Nogo-A 56) was coated onto the plates. The parental antibody 2A10 and the humanised antibodies were pre-mixed prior to adding to the plates. The binding of 2A10 was quantified using an anti-mouse IgG-HRP conjugate (Dakocytomation, #P0260). The results shown in Figure 13 confirm that all four antibodies can compete with 2A10. This suggests that the humanised antibodies and parental antibody recognise an overlapping epitope on human Nogo-A. Furthermore, the activity of the humanised antibodies is comparable or better than the chimera HcLc. The results indicate that H27L16, H28L16 and H28L13 are more potent than H20L16.

11.3 Biacore Affinity measurements

Biacore was used to determine affinities and rank antibodies using two different methodologies. In the first approach, the recombinant Nogo-A was coupled to the surface of the chip and anti-Nogo-A antibodies passed over this surface. In the second approach, Protein A was used to capture the antibody onto the surface of the chip over which the recombinant GST-human Nogo-A56 was passed. The results shown in Table 16 were obtained by coupling the antigen to the surface and confirm that all four antibodies show comparable/better affinity than the parental antibody (HcLc). Based on the average of six independent runs, the antibodies rank in the following order in terms of overall affinity: H27L16>H28L16>H28L13>H20L16, consistent with the rank order of the direct binding ELISA (Figure 11B). In the case of H27L16 and H28L16, the humanised antibodies demonstrate 2-3x higher affinity that the parental antibody (HcLc).

Table 16 *– Binding kinetics of the anti-Nogo-A humanised antibodies to recombinant human Nogo-A (GST-human Nogo-A 56) as determined using the Biacore T100. The antigen was bound to the CM5 chip by primary amine coupling. The antibodies were flowed over a various concentrations (0.125-8nM). The values show the mean and standard deviation (in brackets) of six independent runs carried out in duplicate. Each completed data set was analysed independently prior to the calculation of mean and standard deviation. **Only 11 sets of data analysed for H20L16 as one set could not be analysed.*

Antibody	***Ka***	***kd***	**KD (nM)**
H20L16**	5.37E6 (7.65E5)	9.70E-3 (2.65E-3)	1.80 (0.31)
H27L16	3.96E6 (9.93E5)	2.30E-3 (1.11E-3)	0.56 (0.15)
H28L13	8.13E6 (1.35E6)	9.10E-3 (2.65E-3)	1.11 (0.18)
H28L16	6.97E6 (6.62E5)	4.43E-3 (1.18E-3)	0.64 (0.15)
HcLc	3.80E6 (7.11E5)	7.09E-3 (2.22E-3)	1.86 (0.32)

In a similar manner to the ELISA, the kinetics of antibody binding to recombinant human Nogo-A (GST-human Nogo-A 56) was also assessed in a reverse format (see Example 11.1). In this assay, the humanised antibodies were captured onto the CM5 chip by Protein A. The averaged results for six independent runs are shown in Table 17. Consistent with the reverse format ELISA, all the humanised Nogo-A antibodies show similar binding kinetics to the chimera (HcLc) in the reverse format Biacore.

Table 17 – Reverse format binding kinetics of the anti-Nogo-A humanised antibodies to recombinant human Nogo-A (GST Nogo-A 5+6) as determined using the Biacore T100. Protein A was immobilised to approximately 4000RUs by primary amine and used to capture 200-300RUs of the sample antibodies. Recombinant human Nogo-A was passed over at various concentrations (0.125-8nM). The values show the mean and standard deviation (in brackets) of three independent runs in duplicate. Each data set was independently analysed prior to the calculation of the mean and standard deviation.

Antibody	***Ka***	***kd***	**KD (nM)**
H20L16	1.01E6(1.35E5)	3.13E-4(2.79E-5)	0.31(0.036)
H27L16	9.93E5(2.02E4)	3.04E-4(1.83E-5)	0.31(0.019)
H28L13	1.12E6(1.21E5)	3.84E-4(3.24E-5)	0.34(0.015)
H28L16	1.18E6(8.32E4)	4.01E-4(2.48E-5)	0.34(0.032)
HcLc	1.38E6(3.70E5)	5.69E-4(1.54E-4)	0.41(0.062)

11.4 Binding to native human NOGO

To demonstrate that the humanised antibodies bind to native human Nogo-A with a profile comparable to the parental antibody, two flow cytometry based assays were developed. In the first assay, a CHO-K1-based cell line expressing human Nogo-A extracellular domain on the cell surface was generated. Binding of the humanised anti-Nogo-A antibodies was assessed by flow cytometry using a PE-labelled anti-human IgG (Sigma, #P8047). Figure 14 below shows a typical profile for the anti-Nogo-A antibodies on the CHO-Nogo-A cell line. Whilst the assay is not sensitive enough to distinguish between the antibodies, the results confirm that all four antibodies can recognise cell surface expressed human Nogo-A at levels comparable to that of the chimera. None of the antibodies recognise the parental cell line (CHO-K1 – data not shown).

In the second assay, the ability of the humanised antibodies to bind native Nogo-A was assessed using a human neuroblastoma cell line - IMR32. This cell line is characterised by high intracellular/low cell surface levels of Nogo-A protein. In an attempt to increase the binding signal, the assay was set-up to

detect intracellular Nogo-A (ER-resident). IMR32 cells were permeabilised and fixed prior to staining with the anti-Nogo-A humanised antibodies. Binding of the antibodies to Nogo-A was detected using an anti-human IgG-PE labelled secondary (Sigma, #P8047). The results, shown in Figure 15 below, confirm that all the antibodies bind to intracellular Nogo-A at levels comparable or higher than the parental antibody HcLc. These data, in conjunction with the results from the CHO-Nogo-A cell line, confirm that the humanised antibodies can recognise a more native form of the Nogo-A protein at levels comparable or better than the chimera, HcLc. The assays are not sufficiently sensitive to rank the antibody panel.

11.5 Neurite-outgrowth assays

Humanised anti-Nogo-A antibodies were tested for their ability to neutralise neurite-outgrowth (NO) inhibitory activity of Nogo-A in an assay that is based on quantifying NO as described previously. Antibodies tested in the assay were selected on the basis of their binding kinetics for Nogo-A. High affinity humanised antibodies namely, H28L16, H27L16, H20L16 and for reference their parental antibodies 2A10 (mouse monoclonal) and HcLc (human mouse chimera) were tested for Nogo-A neutralisation. For comparison, antibody 11C7 (see Example 13) was also tested in the assay.

In order to test the neutralising activity of selected humanised antibodies, wells coated with human recombinant GST-human Nogo-A56 and treated with varying concentrations of antibodies at 37°C for 1h prior to the addition of cerebellum granular neurons (CGNs). Control wells were treated with HBSS. Average neurite length per neurite was measured for each well. Figure 16 shows the results for the humanised antibodies tested in the assay. A panel of control antibodies (control IgG, purified mouse IgG; Campath and another irrelevant humanised antibodies) used to confirm the specificity of the activity. As a further control, the same humanised antibodies were titrated onto GST coated plates. The results confirm that H28L16, H27L16 and H20L16 reverse Nogo-A-mediated inhibition of neurite outgrowth to a similar degree observed for the parental antibodies (2A10 and HcLc). The effects appear to be robust and stable and were seen with H28L16 in eight out of eleven independent neurite-outgrowth

experiments. In contrast, the humanised antibodies do not increase neurite-outgrowth on GST coated plates and the panel of control antibodies do not show any dose dependent reversal of inhibition, confirming that the effect of the humanised antibodies is specific for Nogo-A-mediated inhibition. The data presented for the neurtite outgrowth is selected from number of repeat experiments. Whilst a number of the repeats which are not shown appeared to be variable in nature, it is believed that the data shown reflects a true activity of the antibodies of the present invention in reducing the inhibitory effect of NOGO in the neurite outgrowth assay.

Example 12, *Further characterisation of H28L16*

12.1 Binding to full-length recombinant Nogo-A

The ability of the antibodies to bind full-length extracellular domain recombinant human Nogo-A (GST-human Nogo-A-ECD) was investigated by a direct binding ELISA assay. In this case the ECD was a splice variant falling within the region of approximately position 186-1004 of human NOGO A (the portion beginning DETFAL (SEQ ID NO.95) and ending with ELSKTS (SEQ ID NO.96)).

The recombinant GST-human Nogo-A-ECD was directly coated to the plate at 1µg/ml. The data shown in Figure 17 confirms that H28L16 can recognise GST-human Nogo-A-ECD as levels comparable or better than the parental (HcLc) or H20L16.

12.2 Inhibition of Fc functionality

To improve the safety profile of the candidate, residues L235 and G237 within the CH2 domain of the heavy chain constant region (EU Index system) were mutated to alanine residues thus reducing the likelihood of triggering antibody-mediated immunological effector functions. Reduced human C1q binding was used as a surrogate for inhibition of Fc functionality. Figure 18 below shows that H28L16 has significantly reduced C1q binding activity, compared to Campath-IgG1 (wild-type) and comparable to a Campath IgG1 construct bearing the same mutations (Fc-mutated antibody (Fc-)) and Campath

IgG4. These data suggest that the CH2-domain mutations present in H28L16 will significantly reduce the likelihood of triggering Fc mediated effector functions.

12.3 Orthologue binding

To confirm that H28L16 shows binding activity to various orthologues of Nogo-A, comparable to that of the parental antibody (HcLc), a series of binding assays were performed. Figure 19 A-D below shows the results of a direct binding ELISA to recombinant NOGO (GST-human Nogo-A 56) from rat (SEQ ID NO.94), cynomolgus (SEQ ID NO. 92), marmoset (SEQ ID NO. 93) and squirrel monkey respectively (SEQ ID NO. 91). In all cases, H28L16 shows activity comparable or better than the chimeric antibody (HcLc). The calculated EC50 values are very sirnilar to those calculated for binding to human recombinant Nogo-A.

The kinetics of binding of H28L16 to the various orthologues of Nogo-A in comparison to HcLc and 11C7 was determined using the Biacore. Table 18 and Table 19 below show the kinetics of binding in two different formats of the assay. Where the recombinant Nogo-A was coupled directly to the CM5 chip (Table 18), the binding kinetics for rat, cynomolgus monkey, squirrel monkey and marmoset are very similar to that for human (range = 0.33-0.67nM). When the format of the assay was reversed and the antibodies are captured onto the chip using Protein A (Table 19), the binding affinity of H28L16 to rat Nogo-A is approximately 4-fold lower than for human Nogo-A. A similar trend is observed for cynomolgus Nogo-A (8.5x lower affinity than human) and the other primate orthologues (12-17x lower affinity than human). The chimeric antibody HcLc shows a similar profile of binding to the orthologues of Nogo-A in both orientations of the assay. Since it is unclear which assay format best represents the in vivo situation, the primary conclusions that can be drawn from this study are 1) H28L16 has retained the orthologue cross-reactivity profile associated with the chimeric antibody HcLc and 2) the affinity of HcLc for rat and cynomolgus Nogo-A is within 4-fold and 8.5-fold of the affinity for human Nogo-A and under certain conditions may be very similar.

Table 18 *- Binding kinetics of H28L16, 11C7 and HcLc to the recombinant orthologues of human Nogo-A as determined using the Biacore T100. Approximately 140-180RUs of the various Nogo-A orthologues were captured to the CM5 chip by primary amine coupling. The antibodies were flowed over a various concentrations (0.125-8nM). The values show the mean and standard deviation (in brackets) of 1-2 independent runs carried out in duplicate with each data set independently analysed prior to calculation of the mean and standard deviation. *One set of curves was discarded due to uninterpretable curves for antibody 11C7.*

	H28L16			11C7			HcLc		
Orthologue	*Ka*	*Kd*	KD (nM)	*Ka*	*Kd*	KD (nM)	*Ka*	*Kd*	KD (nM)
Cynomolgus (2 runs)*	4.65E6 (7.47E5)	3.07E-3 (2.37E-4)	0.67 (0.06)	1.47E6 (1.67E5)	3.40E-4 (4.45E-5)	0.23 (0.01)	2.94E6 (7.13E5)	4.78E-3 (6.34E-4)	1.68 (0.35)
Rat (2 runs)	4.64E6 (2.34E5)	1.54E-3 (3.06E-5)	0.33 (0.01)	8.36E5 (5.58E5)	1.20E-4 (2.14E-5)	0.11 (0.03)	2.53E6 (5.32E4)	2.83E-3 (2.30E-5)	1.12 (0.03)
Marmoset (1 run)	4.2E6 (2.47E4)	3.02E-3 (5.09E-5)	0.626 (0.000)	1.16E6 (5.37E4)	2.80E-4 (6.15E-6)	0.24 (0.006)	3.13E6 (2.76E4)	4.44E-3 (1.41E-4)	1.419 (0.03)
Squirrel Monkey (1 run)	4.46E6 (6.08E4)	2.73E-3 (4.95E-6)	0.61 (0.000)	1.10E6 (3.25E4)	2.86E-4 (1.87E-5)	0.26 (0.010)	3.04E6 (1.64E5)	4.68E-3 (2.11E-4)	1.54 (0.15)
Human	6.97E6 (6.62E5)	4.43E-3 (1.18E-3)	0.64 (0.15)	1.58E6 (6.42E5)	2.64E-4 (5.57E-5)	0.19 (7.96E-2)	3.80E6 (7.11E5)	7.09E-3 (2.22E-3)	1.86 (0.32)

Table 19 - Reverse format binding kinetics of H28L16, 11C7 and HcLc to the recombinant orthologues of human Nogo-A as determined on the Biacore T100. Protein A was immobilised on the surface at about 4000RUs and anti-Nogo-A antibodies were captured at approximately 300-400RUs. The recombinant proteins (GST- NOGO-A56) were flowed over a various concentrations (0.125-64nM) dependent on the construct. All the runs were done in duplicates. The values show the mean and standard deviation (in brackets) of 1-3 independent runs with each run done in duplicate and each data set analysed independently prior to calculation of the mean and standard deviation.

	H28L16			11C7			HcLc		
Orthologue	*Ka*	*Kd*	KD (nM)	*Ka*	*Kd*	KD (nM)	*Ka*	*Kd*	KD (nM)
Cynomolgus (3 runs)	3.26E5 (4.06E3)	1.11E-3 (2.23E-5)	3.41 (0.05)	4.02E5 (6.85E4)	2.97E-4 (1.11E-5)	0.76 (0.12)	3.03E5 (4.58E3)	1.41E-3 (2.84E-5)	4.66 (0.08)
Rat (3 runs)	3.80E5 (5.68E3)	6.69E-4 (1.24E-5)	1.76 (0.03)	2.83E5 (4.66E4)	1.77E-4 (1.34E-5)	0.64 (0.09)	5.47E5 (1.20E4)	1.10E-3 (2.86E-5)	2.01 (0.07)
Marmoset (1 run)	2.22E5 (3.61E3)	1.09E-3 (7.35E-5)	4.89 (0.25)	1.91E5 (2.90E3)	2.54E-4 (3.46E-6)	1.33 (0.00)	3.02E5 (9.90E2)	1.36E-3 (7.92E-5)	4.51 (0.28)
Squirrel Monkey (1 run)	1.57E5 (2.69E3)	1.08E-3 (5.02E-5)	6.86 (0.20)	1.03E5 (2.12E3)	2.78E-4 (3.61E-6)	2.69 (0.02)	1.74E5 (2.19E3)	1.29E-3 (7.64E-5)	7.45 (0.34)
Human (1 run)	1.20E6 (8.49E4)	4.75E-4 (9.97E-6)	0.40 (0.02)	2.64E5 (3.32E3)	1.49E-4 (1.61E-5)	0.57 (0.07)	1.32E6 (2.71E5)	7.00E-4 (3.18E-5)	0.54 (0.09)

12.4 Physical properties

The physicochemical properties of H28L16 and H20L16 were assessed by SEC-HPLC and SDS-PAGE. SEC-HPLC was carried out at 1.0ml/minute using 100mM sodium phosphate, 400mM sodium chloride pH 6.8 and a TSK G3000 SW xl 30cm x 7.8mm stainless steel column with detection at 214nm and 280nm. SDS-PAGE was carried out on a 4-20% Novex Tris-HCL gel loading 10µg product and staining with Sypro Ruby. C-IEF was carried out on a Beckman MDQ using pH 3.5 -10 ampholines.

The following results were obtained:

Table 20 – Size exclusion chromatography (SEC) HPLC analysis of the anti-Nogo-A antibodies. The values shown are percentages of the antibody assigned to each of the three different species.

Antibody	Aggregate %	Monomer %	Fragment %
H28L16	0.50	99.50	0.00
H20L16	14.21	85.75	0.05

Table 21 – SDS-PAGE analysis of the anti-Nogo-A antibodies. The values shown are percentages of the antibody found in the major bands.

Antibody	Non-reduced	Reduced
H28L16	82.4%	HC:67.2% LC:27.7% H+L: 94.9%
H20L16	84.6%	HC: 69.3% LC: 26.4% H+L: 95.7%

The SEC-HPLC data suggests that H20L16 is more susceptible to aggregation than H28L16 (H28L16). If the data reported here were to be repeated at large scale, this could impact the ability of the manufacturing process to produce material of acceptable quality for clinical use (>95% monomer). The SDS-PAGE data shows both candidates are acceptable with both showing a typical profile.

Example 13, *Comparison of H28L16 with 11C7*

A murine anti-Nogo-A antibody designated 11C7 is described in WO2004052932, which was raised to a peptide epitope. A chimeric 11C7 was made based on the sequence information provided in WO2004052932. To compare the binding epitopes of 2A10 and 11C7, a competition ELISA was

established to investigate if 11C7 and 2A10 recognise an overlapping epitope on Nogo-A. As shown in Figure 20 below, HcLc (the chimeric form of 2A10) was able to compete with 2A10 for binding to human recombinant Nogo-A whereas 11C7 showed no competition with 2A10, even at concentrations of up to 100mcg/ml.

Example 14: *Competition ELISA to demonstrate the ability of peptides to compete directly with human NOGO-5+6 for binding to NOGO H28L16*

Method for competition ELISA

The ability of peptides to compete directly with NOGO-A (GST-human Nogo-A56) for binding to NOGO H28L16 was assessed using a competition ELISA. Rabbit anti-human IgG (Sigma, #I-9764) at 5g/ml in bicarbonate buffer was coated onto Nunc immunosorp plates (100 **ul** per well) at 4°C overnight. The plates were rinsed 3 times with TBS containing 0.05% Tween (TBST) then blocked with 1% BSA in TBST at room temperature for 1 hour. H28L16 was then captured onto the plate (1ug/ml, diluted in 1% BSA in TBST, 50ul per well) at room temperature for 1 hour. The plates were washed 3 times with TBST. Peptides (from 0 to 100g/ml) and GST-human NOGO-A56 at a concentration of 1**u**g/ml (diluted in 1% BSA in TBST) were pre-mixed prior to addition into the wells and incubated at room temperature for 1 hour. The plates were washed 3 times with TBST then incubated for 1 hour with rabbit anti-GST peroxidase conjugate (Sigma, #A7340, 1:2000, diluted in 1% BSA in TBST) for 1 hour. The plates were washed 3 times with TBST and then incubated with 50 l OPD peroxidase substrate (Sigma) per well for 10 minutes. The colour reaction was stopped by the addition of 25 l concentrated H_2SO_4. Absorbance at 490 nm was measured using a plate reader.

The results shown in Figure 21 confirm that peptides 6 and 7, which were positive in the epitope mapping ELISA (Example 8) can compete with GST-human NOGO-A56 binding to H28L16. This suggests that the peptides which were positive in the epitope mapping study contain an epitope for H28L16 binding. Peptides 16 and 17 (which contain NOGO peptides, but not overlapping

with peptides 6 or 7), which do not contain the proposed epitope, do not compete with NOGO-5+6.

Example 15: *ELISA analysis of a humanised Anti NOGO Monoclonal Antibody based on the NOGO antibody variant G101S/Q37R*

G101S (also known as H100 (SEQ ID NO.63)), a modified variant of the heavy chain variable region of H6 (SEQ ID NO.11) was generated by introducing a single substitution, G101S (Kabat numbering) into CDR H3 as described above. Similarly, Q37R, a modified variant of the light chain variable region of L13 (SEQ ID NO. 13) were generated by introducing a single substitution (Kabat numbering Q37R) into the framework region (to form L100). The protein sequence of the variable light domain Q37R is given in SEQ ID NO. 67.

Genes encoding full length versions of the heavy and light chains containing the G101S/Q37R substitutions were expressed in CHO cells as described previously and assayed in a direct binding ELISA as described previously.

The results of the direct binding ELISA when the antigen is loaded at 0.05ug/ml are shown in Figure 22. The data confirms that antibody H100L100 shows comparable binding activity to recombinant GST-human NOGO-A56 when compared with H27L16 and that H100L100 has an improved binding profile when compared to H6L13. Corresponding EC50 values are shown in the table below:

Table 22: *EC50 measurements for the G101S/Q37R variant in comparison with H6L13 and H27L16*

Antibody	EC50 value
H6L13	0.086
H27L16	0.052
H100/L100	0.048

Example 16: *BiaCore Analysis of humanised Anti NOGO Monoclonal Antibodies based on the CDR H3 variant G101S*

H100, A modified variant of the heavy chain variable region of H6 (SEQ ID NO.11) was generated by introducing a single substitution, G101S (Kabat numbering) into CDR H3. The protein sequence of the variable heavy domain H100 protein is given in SEQ ID NO.63. Similarly, L100 and L101, modified variants of the light chain variable region of L13 (SEQ ID NO. 13) were generated by introducing a single substitution (Kabat numbering Q37R and Q45R respectively) into the framework region. The protein sequences of the variable light domains L100 and L101 proteins are given in SEQ ID NO.67 and SEQ ID NO.68 respectively.

Full length versions of H100L100 and H100L101 were expressed in CHO cells as described previously. Table 23 shows a comparison of the binding affinities of H6L13 with H100L100 and H100L101 and indicates that H100L100 and H100L101 have an improved binding affinity when compared with H6L13. In this example, the method was performed essentially as described in Example 6 where the CM5 chip was activated by passing the NHS and EDC solutions over the chip at 5µ l /ml for 7 minutes and the NOGO was suspended in 10nM sodium acetate buffer (pH 4.5) before passing over the chip.

Table 23 – Biacore measurements for the G101S variants of the H6 variable heavy chain in combination with variants of the L13 variable light chain in comparison with H6L13.

Antibody	On rate ka (1/Ms)	Off-rate kd (1/s)	Affinity (KD, nM)
H6L13	1.04E+06	7.22E-03	6.97
H100L100	1.28E+07	5.07E-03	0.396
H100L101	1.30E+07	4.29E-03	0.329

NOGO antibody sequences Summary (Table 24)

Description	Sequence identifier (SEQ.I.D.NO)	
	amino acid sequence	Polynucleotide sequence
2A10, CDR-H1	1	-
2A10, CDR-H2	2	-
2A10, CDR-H3	3	-
2A10, CDR-L1	4	-
2A10, CDR-L2	5	-
2A10, CDR-L3	6	-
2A10, VH (murine)	7	19
2A10, VL (murine)	8	20
Chimeric heavy chain Hc	9	21
Chimeric light chain Lc	10	22
2A10 VH humanised construct H6	11	23
2A10 VH humanised construct H16	12	24
2A10 VL humanised construct L13	13	25
2A10 VL humanised construct L16	14	26
2A10 heavy chain humanised construct H6	15	27
2A10 heavy chain humanised construct H16	16	28
2A10 light chain humanised construct L13	17	29
2A10 light chain humanised construct L16	18	30
Campath leader sequence	31	-
Amino acids 586-785 of human NOGO A (NOGO-A56) fused to GST	32	-
2A10 VH humanised construct H1	33	37
2A10 VL humanised construct L11	34	38
2A10 heavy chain humanised construct H1	35	39
2A10 light chain humanised construct L11	36	40
2A10 VH humanised construct H20	41	43
2A10 heavy chain humanised construct H20	42	44
2A10, CDR-H3 (G95M)	45	

Sequence of Marmoset NOGO-A fragment	46	
VH humanised construct H26	47	50
VH humanised construct H27	48	51
VH humanised construct H28	49	52
Heavy chain humanised construct H26	53	56
Heavy chain humanised construct H27	54	57
Heavy chain humanised construct H28	55	58
Chimeric heavy chain Hc (G95M)	59	
Epitope	60	
2A10 VH humanised construct H99	61	
CDR (G101S)	62	
VH humanised construct H100	63	
VH humanised construct H101	64	
VH humanised construct H102	65	
VH humanised construct H98	66	
L100 (L13+Q37R)	67	
L101 (L13+Q45R)	68	
L102 (L13+Q37R/Q45R)	69	
L103 (L16+Q37R)	70	
L104 (L16+Q45R)	71	
L105 (L16+Q37R/Q45R)	72	
Peptide	73	
peptide	74	
CDR H3 analogue	75	
CDR H3 analogue	76	
CDR H3 analogue	77	
CDR H3 analogue	78	
CDR H3 analogue	79	
CDR H3 analogue	80	
CDR H3 analogue	81	
CDR H3 analogue	82	
CDR H3 analogue	83	
CDR H3 analogue	84	
NOGO peptide	85	
CDR H3 analogue	86	
CDR H3 analogue	87	

CDR H3 analogue	88	
CDR H3 analogue	89	
CDR H3 analogue	90	
Squirrel monkey NOGO (A56) plus GST tag	91	
Cynomolgus monkey NOGO (A56) plus GST tag	92	
Marmoset NOGO (A56) plus GST tag	93	
Rat NOGO (A56) plus GST tag	94	
Human NOGO peptide	95	
Human NOGO peptide	96	

Sequences

SEQ ID NO. 1: 2A10 CDR-H1
SYWMH

SEQ ID NO. 2: 2A10 CDR-H2
NINPSNGGTNYNEKFKS

SEQ ID NO. 3: 2A10 CDR-H3
GQGY

SEQ ID NO. 4: 2A10 CDR-L1
RSSKSLLYKDGKTYLN

SEQ ID NO. 5: 2A10 CDR-L2
LMSTRAS

SEQ ID NO. 6: 2A10 CDR-L3
QQLVEYPLT

SEQ ID NO. 7: 2A10, VH (murine)
QVQLQQPGTELVKPGASVKLSCKASGYTFTSYWMHWVKQRPGQGLEWIGNINPSNGGTNYNEKFKSKATLTV
DKSSSTAYMQLSSLTSEDSAVYYCELGQGYWGQGTTLTVSS

SEQ ID NO. 8: 2A10, VL (murine)
DIVITQDELSNPVTSGESVSISCRSSKSLLYKDGKTYLNWFLQRPGQSPQLLIYLMSTRASGVSDRFSGSGS
GTDFTLEISRVKAEDVGVYYCQQLVEYPLTFGAGTKLELK

SEQ ID NO. 9: Chimeric heavy chain Hc
MGWSCIILFLVAAATGVHSQVQLQQPGTELVKPGASVKLSCKASGYTFTSYWMHWVKQRPGQGLEWIGNINP
SNGGTNYNEKFKSKATLTVDKSSSTAYMQLSSLTSEDSAVYYCELGQGYWGQGTLVTVSSASTKGPSVFPLA
PSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICN
VNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELAGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV
KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE
PQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO.10: Chimeric light chain Lc
MRCSLQFLGVLMFWISGVSGDIVITQDELSNPVTSGESVSISCRSSKSLLYKDGKTYLNWFLQRPGQSPQLL
IYLMSTRASGVSDRFSGSGSGTDFTLEISRVKAEDVGVYYCQQLVEYPLTFGAGTKLELKRTVAAPSVFIFP
PSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKV
YACEVTHQGLSSPVTKSFNRGEC

SEQ ID 11: 2A10 VH humanised construct H6
QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWMHWVRQAPGQGLEWIGNINPSNGGTNYNEKFKSRATMTR
DTSTSTAYMELSSLRSEDTAVYYCELGQGYWGQGTLVTVSS

SEQ ID NO.12 2A10 VH humanised construct H16
QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWMHWVKQRPGQGLEWIGNINPSNGGTNYNEKFKSKATLTV
DKSTSTAYMELSSLRSEDTAVYYCELGQGYWGQGTLVTVSS

SEQ ID NO. 13: 2A10 VL humanised construct L13
DIVMTQSPLSLPVTLGQPASISCRSSKSLLYKDGKTYLNWFQQRPGQSPQLLIYLMSTRASGVPDRFSGGGS
GTDFTLKISRVEAEDVGVYYCQQLVEYPLTFGQGTKLEIK

SEQ ID NO. 14: 2A10 VL humanised construct L16
DIVMTQSPLSNPVTLGQPVSISCRSSKSLLYKDGKTYLNWFLQRPGQSPQLLIYLMSTRASGVPDRFSGGGS
GTDFTLKISRVEAEDVGVYYCQQLVEYPLTFGQGTKLEIK

SEQ ID NO. 15: 2A10 heavy chain humanised construct H6
MGWSCIILFLVATATGVHSQVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWMHWVRQAPGQGLEWIGNINP
SNGGTNYNEKFKSRATMTRDTSTSTAYMELSSLRSEDTAVYYCELGQGYWGQGTLVTVSSASTKGPSVFPLA
PSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICN
VNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELAGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV
KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE
PQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO. 16: 2A10 heavy chain humanised construct H16
MGWSCIILFLVATATGVHSQVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWMHWVKQRPGQGLEWIGNINP
SNGGTNYNEKFKSKATLTVDKSTSTAYMELSSLRSEDTAVYYCELGQGYWGQGTLVTVSSASTKGPSVFPLA
PSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICN
VNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELAGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV
KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE
PQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO. 17: 2A10 light chain humanised construct L13
MGWSCIILFLVATATGVHSDIVMTQSPLSLPVTLGQPASISCRSSKSLLYKDGKTYLNWFQQRPGQSPQLLI
YLMSTRASGVPDRFSGGGSGTDFTLKISRVEAEDVGVYYCQQLVEYPLTFGQGTKLEIKRTVAAPSVFIFPP
SDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVY
ACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO. 18: 2A10 light chain humanised construct L16
MGWSCIILFLVATATGVHSDIVMTQSPLSNPVTLGQPVSISCRSSKSLLYKDGKTYLNWFLQRPGQSPQLLI
YLMSTRASGVPDRFSGGGSGTDFTLKISRVEAEDVGVYYCQQLVEYPLTFGQGTKLEIKRTVAAPSVFIFPP
SDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVY
ACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO. 19: PN encoding 2A10, VH (murine) SEQ ID: 7
CAGGTCCAACTGCAGCAGCCTGGGACTGAACTGGTGAAGCCTGGGGCTTCAGTGAAGCTGTCCTGCAAGGCT
TCTGGCTACACCTTCACCAGCTACTGGATGCACTGGGTGAAGCAGAGGCCTGGACAAGGCCTTGAGTGGATT
GGAAATATTAATCCTAGCAATGGTGGTACTAACTACAATGAGAAGTTCAAGAGCAAGGCCACACTGACTGTA
GACAAATCCTCCAGCACAGCCTACATGCAGCTCAGCAGCCTGACATCTGAGGACTCTGCGGTCTATTATTGT
GAACTGGGACAGGGCTACTGGGGCCAAGGCACCACTCTCACAGTCTCCTCA

SEQ ID NO. 20: PN encoding 2A10, VL (murine) SEQ ID: 8
GATATTGTGATAACCCAGGATGAACTCTCCAATCCTGTCACTTCTGGAGAATCAGTTTCCATCTCCTGCAGG
TCTAGTAAGAGTCTCCTATATAAGGATGGGAAGACATACTTGAATTGGTTTCTGCAGAGACCAGGACAATCT
CCTCAGCTCCTGATCTATTTGATGTCCACCCGTGCATCAGGAGTCTCAGACCGGTTTAGTGGCAGTGGGTCA
GGAACAGATTTCACCCTGGAAATCAGTAGAGTGAAGGCTGAGGATGTGGGTGTGTATTACTGTCAACAACTT
GTAGAGTATCCGCTCACGTTCGGTGCTGGGACCAAGCTGGAGCTGAAA

SEQ ID NO. 21: PN encoding Chimeric heavy chain Hc SEQ ID: 9
ATGGGATGGAGCTGTATCATCCTCTTTTTGGTAGCAGCAGCTACAGGTGTCCACTCCCAGGTCCAACTGCAG
CAGCCTGGGACTGAACTGGTGAAGCCTGGGGCTTCAGTGAAGCTGTCCTGCAAGGCTTCTGGCTACACCTTC
ACCAGCTACTGGATGCACTGGGTGAAGCAGAGGCCTGGACAAGGCCTTGAGTGGATTGGAAATATTAATCCT
AGCAATGGTGGTACTAACTACAATGAGAAGTTCAAGAGCAAGGCCACACTGACTGTAGACAAATCCTCCAGC
ACAGCCTACATGCAGCTCAGCAGCCTGACATCTGAGGACTCTGCGGTCTATTATTGTGAACTGGGACAGGGC
TACTGGGGCCAAGGCACACTAGTCACAGTCTCCTCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCA
CCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCG
GTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCA
GGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAAC
GTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACTCACACA
TGCCCACCGTGCCCAGCACCTGAACTCGCGGGGGCACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGAC
ACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTC
AAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAAC
AGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGC
AAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAA
CCACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTC
AAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACC
ACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGG
CAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTC
TCCCTGTCTCCGGGTAAATGA

SEQ ID NO. 22: PN encoding Chimeric light chain Lc SEQ ID: 10
ATGAGGTGCTCTCTTCAGTTTCTGGGGGTGCTTATGTTCTGGATCTCTGGAGTCAGTGGGGATATTGTGATA
ACCCAGGATGAACTCTCCAATCCTGTCACTTCTGGAGAATCAGTTTCCATCTCCTGCAGGTCTAGTAAGAGT
CTCCTATATAAGGATGGGAAGACATACTTGAATTGGTTTCTGCAGAGACCAGGACAATCTCCTCAGCTCCTG
ATCTATTTGATGTCCACCCGTGCATCAGGAGTCTCAGACCGGTTTAGTGGCAGTGGGTCAGGAACAGATTTC
ACCCTGGAAATCAGTAGAGTGAAGGCTGAGGATGTGGGTGTGTATTACTGTCAACAACTTGTAGAGTATCCG
CTCACGTTCGGTGCTGGGACCAAGCTGGAGCTGAAACGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCG
CCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAG
GCCAAAGTACAGTGGAAGGTGGACAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGAC
AGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTC
TACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAG

SEQ ID NO. 23: PN encoding 2A10 VH humanised construct H6 SEQ ID: 11
CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTTTCCTGCAAGGCA
TCTGGATACACCTTCACCAGCTACTGGATGCACTGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTGGATC
GGAAATATTAATCCTAGCAATGGTGGTACTAACTACAATGAGAAGTTCAAGAGCAGAGCCACCATGACCAGG
GACACGTCCACGAGCACAGCCTACATGGAGCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTATTACTGT
GAACTGGGACAGGGCTACTGGGGCCAGGGAACACTAGTCACAGTCTCCTCA

SEQ ID NO. 24: PN encoding 2A10 VH humanised construct H16 SEQ ID: 12
CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTTTCCTGCAAGGCA
TCTGGATACACCTTCACCAGCTACTGGATGCACTGGGTGAAACAGCGACCTGGACAAGGGCTTGAGTGGATC
GGAAATATTAATCCTAGCAATGGTGGTACTAACTACAATGAGAAGTTCAAGAGCAAAGCCACCCTCACCGTC
GACAAATCCACGAGCACAGCCTACATGGAGCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTATTACTGT
GAACTGGGACAGGGCTACTGGGGCCAGGGAACACTAGTCACAGTCTCCTCA

SEQ ID NO. 25: PN encoding 2A10 VL humanised construct L13 SEQ ID: 13
GATATTGTGATGACCCAGTCTCCACTCTCCCTGCCCGTCACCCTTGGACAGCCGGCCTCCATCTCCTGCAGG
TCTAGTAAGAGTCTCCTATATAAGGATGGGAAGACATACTTGAATTGGTTTCAGCAGAGGCCAGGCCAATCT
CCACAGCTCCTAATTTATTTGATGTCCACCCGTGCATCTGGGGTCCCAGACAGATTCAGCGGCGGTGGGTCA

GGCACTGATTTCACACTGAAAATCAGCAGGGTGGAGGCTGAGGATGTTGGGGTTTATTACTGCCAACAACTT
GTAGAGTATCCGCTCACGTTTGGCCAGGGGACCAAGCTGGAGATCAAA

SEQ ID NO. 26: PN encoding 2A10 VL humanised construct L16 SEQ ID: 14
GATATTGTGATGACCCAGTCTCCACTCTCCAACCCCGTCACCCTTGGACAGCCGGTCTCCATCTCCTGCAGG
TCTAGTAAGAGTCTCCTATATAAGGATGGGAAGACATACTTGAATTGGTTTCTCCAGAGGCCAGGCCAATCT
CCACAGCTCCTAATTTATTTGATGTCCACCCGTGCATCTGGGGTCCCAGACAGATTCAGCGGCGGTGGGTCA
GGCACTGATTTCACACTGAAAATCAGCAGGGTGGAGGCTGAGGATGTTGGGGTTTATTACTGCCAACAACTT
GTAGAGTATCCGCTCACGTTTGGCCAGGGGACCAAGCTGGAGATCAAA

SEQ ID NO. 27: PN encoding 2A10 heavy chain humanised construct H6 SEQ ID: 15
ATGGGATGGAGCTGTATCATCCTCTTCTTGGTAGCAACAGCTACAGGTGTCCACTCCCAGGTGCAGCTGGTG
CAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTTTCCTGCAAGGCATCTGGATACACCTTC
ACCAGCTACTGGATGCACTGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTGGATCGGAAATATTAATCCT
AGCAATGGTGGTACTAACTACAATGAGAAGTTCAAGAGCAGAGCCACCATGACCAGGGACACGTCCACGAGC
ACAGCCTACATGGAGCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTATTACTGTGAACTGGGACAGGGC
TACTGGGGCCAGGGAACACTAGTCACAGTCTCCTCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCA
CCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCG
GTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCA
GGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAAC
GTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACTCACACA
TGCCCACCGTGCCCAGCACCTGAACTCGCGGGGGCACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGAC
ACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTC
AAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAAC
AGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGC
AAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAA
CCACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTC
AAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACC
ACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGG
CAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTC
TCCCTGTCTCCGGGTAAATGA

SEQ ID NO. 28: PN encoding 2A10 heavy chain humanised construct H16 SEQ ID: 16
ATGGGATGGAGCTGTATCATCCTCTTCTTGGTAGCAACAGCTACAGGTGTCCACTCCCAGGTGCAGCTGGTG
CAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTTTCCTGCAAGGCATCTGGATACACCTTC
ACCAGCTACTGGATGCACTGGGTGAAACAGCGACCTGGACAAGGGCTTGAGTGGATCGGAAATATTAATCCT
AGCAATGGTGGTACTAACTACAATGAGAAGTTCAAGAGCAAAGCCACCCTCACCGTCGACAAATCCACGAGC
ACAGCCTACATGGAGCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTATTACTGTGAACTGGGACAGGGC
TACTGGGGCCAGGGAACACTAGTCACAGTCTCCTCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCA
CCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCG
GTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCA
GGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAAC
GTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACTCACACA
TGCCCACCGTGCCCAGCACCTGAACTCGCGGGGGCACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGAC
ACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTC
AAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAAC
AGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGC
AAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAA
CCACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTC
AAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACC
ACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGG
CAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTC
TCCCTGTCTCCGGGTAAATGA

SEQ ID NO. 29: PN encoding 2A10 light chain humanised construct L13 SEQ ID: 17
ATGGGATGGAGCTGTATCATCCTCTTCTTGGTAGCAACAGCTACAGGTGTCCACTCCGATATTGTGATGACC
CAGTCTCCACTCTCCCTGCCCGTCACCCTTGGACAGCCGGCCTCCATCTCCTGCAGGTCTAGTAAGAGTCTC

CTATATAAGGATGGGAAGACATACTTGAATTGGTTTCAGCAGAGGCCAGGCCAATCTCCACAGCTCCTAATT
TATTTGATGTCCACCCGTGCATCTGGGGTCCCAGACAGATTCAGCGGCGGTGGGTCAGGCACTGATTTCACA
CTGAAAATCAGCAGGGTGGAGGCTGAGGATGTTGGGGTTTATTACTGCCAACAACTTGTAGAGTATCCGCTC
ACGTTTGGCCAGGGGACCAAGCTGGAGATCAAACGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCA
TCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCC
AAAGTACAGTGGAAGGTGGACAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGC
AAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTAC
GCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAG

SEQ ID NO. 30: PN encoding 2A10 light chain humanised construct L16 SEQ ID: 18
ATGGGATGGAGCTGTATCATCCTCTTCTTGGTAGCAACAGCTACAGGTGTCCACTCCGATATTGTGATGACC
CAGTCTCCACTCTCCAACCCCGTCACCCTTGGACAGCCGGTCTCCATCTCCTGCAGGTCTAGTAAGAGTCTC
CTATATAAGGATGGGAAGACATACTTGAATTGGTTTCTCCAGAGGCCAGGCCAATCTCCACAGCTCCTAATT
TATTTGATGTCCACCCGTGCATCTGGGGTCCCAGACAGATTCAGCGGCGGTGGGTCAGGCACTGATTTCACA
CTGAAAATCAGCAGGGTGGAGGCTGAGGATGTTGGGGTTTATTACTGCCAACAACTTGTAGAGTATCCGCTC
ACGTTTGGCCAGGGGACCAAGCTGGAGATCAAACGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCA
TCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCC
AAAGTACAGTGGAAGGTGGACAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGC
AAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTAC
GCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAG

SEQ ID NO. 31: Campath leader sequence
MGWSCIILFLVATATGVHS

SEQ ID NO. 32: Amino acids 586-785 of human NOGO A (NOGO-A56)fused to GST
MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVKLTQSMAII
RYIADKHNMLGGCPKERAEISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN
GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHP
PKSDLEVLFQGPLGSMQESLYPAAQLCPSFEESEATPSPVLPDIVMEAPLNSAVPSAGASVIQPSSSPLEAS
SVNYESIKHEPENPPPYEEAMSVSLKKVSGIKEEIKEPENINAALQETEAPYISIACDLIKETKLSAEPAPD
FSDYSEMAKVEQPVPDHSELVEDSSPDSEPVDLFSDDSIPDVPQKQDETVMLVKESLTETSFESMIEYENKE
LERPHRD

SEQ ID NO. 33: 2A10 VH humanised construct H1
QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWMHWVRQAPGQGLEWMGNINPSNGGTNYNEKFKSRVTMTR
DTSTSTVYMELSSLRSEDTAVYYCELGQGYWGQGTLVTVSS

SEQ ID NO. 34: 2A10 VL humanised construct L11
DIVITQSPLSLPVTLGQPASISCRSSKSLLYKDGKTYLNWFQQRPGQSPQLLIYLMSTRASGVPDRFSGGGS
GTDFTLKISRVEAEDVGVYYCQQLVEYPLTFGQGTKLEIK

SEQ ID NO. 35: 2A10 heavy chain humanised construct H1
MGWSCIILFLVATATGVHSQVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWMHWVRQAPGQGLEWMGNINP
SNGGTNYNEKFKSRVTMTRDTSTSTVYMELSSLRSEDTAVYYCELGQGYWGQGTLVTVSSASTKGPSVFPLA
PSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICN
VNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELAGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV
KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE
PQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO. 36: 2A10 light chain humanised construct L11
MGWSCIILFLVATATGVHSDIVITQSPLSLPVTLGQPASISCRSSKSLLYKDGKTYLNWFQQRPGQSPQLLI
YLMSTRASGVPDRFSGGGSGTDFTLKISRVEAEDVGVYYCQQLVEYPLTFGQGTKLEIKRTVAAPSVFIFPP
SDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVY
ACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO. 37: PN encoding 2A10 VH humanised construct H1 SEQ ID: 33
CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTTTCCTGCAAGGCA
TCTGGATACACCTTCACCAGCTACTGGATGCACTGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTGGATG
GGAAATATTAATCCTAGCAATGGTGGTACTAACTACAATGAGAAGTTCAAGAGCAGAGTCACCATGACCAGG
GACACGTCCACGAGCACAGTCTACATGGAGCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTATTACTGT
GAACTGGGACAGGGCTACTGGGGCCAGGGAACACTAGTCACAGTCTCCTCA

SEQ ID NO. 38: PN encoding 2A10 VL humanised construct L11 SEQ ID: 34
GATATTGTGATAACCCAGTCTCCACTCTCCCTGCCCGTCACCCTTGGACAGCCGGCCTCCATCTCCTGCAGG
TCTAGTAAGAGTCTCCTATATAAGGATGGGAAGACATACTTGAATTGGTTTCAGCAGAGGCCAGGCCAATCT
CCACAGCTCCTAATTTATTTGATGTCCACCCGTGCATCTGGGGTCCCAGACAGATTCAGCGGCGGTGGGTCA
GGCACTGATTTCACACTGAAAATCAGCAGGGTGGAGGCTGAGGATGTTGGGGTTTATTACTGCCAACAACTT
GTAGAGTATCCGCTCACGTTTGGCCAGGGGACCAAGCTGGAGATCAAA

SEQ ID NO. 39: PN encoding 2A10 humanised heavy chain H1 SEQ ID: 35
ATGGGATGGAGCTGTATCATCCTCTTCTTGGTAGCAACAGCTACAGGTGTCCACTCCCAGGTGCAGCTGGTG
CAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTTTCCTGCAAGGCATCTGGATACACCTTC
ACCAGCTACTGGATGCACTGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTGGATGGGAAATATTAATCCT
AGCAATGGTGGTACTAACTACAATGAGAAGTTCAAGAGCAGAGTCACCATGACCAGGGACACGTCCACGAGC
ACAGTCTACATGGAGCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTATTACTGTGAACTGGGACAGGGC
TACTGGGGCCAGGGAACACTAGTCACAGTCTCCTCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCA
CCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCG
GTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCA
GGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAAC
GTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACTCACACA
TGCCCACCGTGCCCAGCACCTGAACTCGCGGGGGCACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGAC
ACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTC
AAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAAC
AGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGC
AAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAA
CCACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTC
AAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACC
ACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGG
CAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTC
TCCCTGTCTCCGGGTAAATGA

SEQ ID NO. 40: PN encoding 2A10 humanised light chain construct L11 SEQ ID: 36
ATGGGATGGAGCTGTATCATCCTCTTCTTGGTAGCAACAGCTACAGGTGTCCACTCCGATATTGTGATAACC
CAGTCTCCACTCTCCCTGCCCGTCACCCTTGGACAGCCGGCCTCCATCTCCTGCAGGTCTAGTAAGAGTCTC
CTATATAAGGATGGGAAGACATACTTGAATTGGTTTCAGCAGAGGCCAGGCCAATCTCCACAGCTCCTAATT
TATTTGATGTCCACCCGTGCATCTGGGGTCCCAGACAGATTCAGCGGCGGTGGGTCAGGCACTGATTTCACA
CTGAAAATCAGCAGGGTGGAGGCTGAGGATGTTGGGGTTTATTACTGCCAACAACTTGTAGAGTATCCGCTC
ACGTTTGGCCAGGGGACCAAGCTGGAGATCAAACGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCA
TCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCC
AAAGTACAGTGGAAGGTGGACAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGC
AAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTAC
GCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAG

SEQ ID NO. 41: 2A10 VH humanised construct H20
QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWMHWVRQAPGQGLEWIGNINPSNGGTNYNEKFKSKATMTR
DTSTSTAYMELSSLRSEDTAVYYCELGQGYWGQGTLVTVSS

SEQ ID NO. 42: 2A10 heavy chain humanised construct H20
MGWSCIILFLVATATGVHSQVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWMHWVRQAPGQGLEWIGNINP
SNGGTNYNEKFKSKATMTRDTSTSTAYMELSSLRSEDTAVYYCELGQGYWGQGTLVTVSSASTKGPSVFPLA
PSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICN
VNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELAGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV
KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE

PQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO. 43: PN encoding 2A10 VH humanised construct H20 SEQ ID: 41
CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTTTCCTGCAAGGCA
TCTGGATACACCTTCACCAGCTACTGGATGCACTGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTGGATC
GGAAATATTAATCCTAGCAATGGTGGTACTAACTACAATGAGAAGTTCAAGAGCAAGGCCACCATGACCAGG
GACACGTCCACGAGCACAGCCTACATGGAGCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTATTACTGT
GAACTGGGACAGGGCTACTGGGGCCAGGGAACACTAGTCACAGTCTCCTCA

SEQ ID NO. 44: PN encoding 2A10 heavy chain humanised construct H20 SEQ ID: 42
ATGGGATGGAGCTGTATCATCCTCTTCTTGGTAGCAACAGCTACAGGTGTCCACTCCCAGGTGCAGCTGGTG
CAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTTTCCTGCAAGGCATCTGGATACACCTTC
ACCAGCTACTGGATGCACTGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTGGATCGGAAATATTAATCCT
AGCAATGGTGGTACTAACTACAATGAGAAGTTCAAGAGCAAGGCCACCATGACCAGGGACACGTCCACGAGC
ACAGCCTACATGGAGCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTATTACTGTGAACTGGGACAGGGC
TACTGGGGCCAGGGAACACTAGTCACAGTCTCCTCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCA
CCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCG
GTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCA
GGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAAC
GTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACTCACACA
TGCCCACCGTGCCCAGCACCTGAACTCGCGGGGGCACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGAC
ACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTC
AAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAAC
AGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGC
AAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAA
CCACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTC
AAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACC
ACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGG
CAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTC
TCCCTGTCTCCGGGTAAATGA

SEQ ID NO. 45: 2A10 CDR-H3 (G95M)
MQGY

SEQ ID NO. 46: Amino acid sequence of Marmoset NOGO-A fragment
VQDSLCPVAQLCPSFEESEATPSPVLPDIVMEAPLNSAVPSAGASAVQPSSSPLEASSVNFESVKHEPENPP
PYEEAMNVSRKKVSGIKEEIKEPESINAAVQETEAPYISIACDLIKETKLSAEPTPDFSSYSEMAKVEQPLP
DHSELVEDSSPDSEPVDLFSDDSIPDVPQKQDEAVILVKETLTETSFESMIEHENK

SEQ ID NO. 47: VH humanised construct H26
QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWMHWVRQAPGQGLEWIGNINPSNGGTNYNEKFKSRATMTR
DTSTSTAYMELSSLRSEDTAVYYCELMQGYWGQGTLVTVSS

SEQ ID NO. 48: VH humanised construct H27
QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWMHWVKQRPGQGLEWIGNINPSNGGTNYNEKFKSKATLTV
DKSTSTAYMELSSLRSEDTAVYYCELMQGYWGQGTLVTVSS

SEQ ID NO. 49: VH humanised construct H28
QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWMHWVRQAPGQGLEWIGNINPSNGGTNYNEKFKSKATMTR
DTSTSTAYMELSSLRSEDTAVYYCELMQGYWGQGTLVTVSS

SEQ ID NO. 50: PN encoding VH humanised construct H26 SEQ ID: 47
CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTTTCCTGCAAGGCA
TCTGGATACACCTTCACCAGCTACTGGATGCACTGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTGGATC
GGAAATATTAATCCTAGCAATGGTGGTACTAACTACAATGAGAAGTTCAAGAGCAGAGCCACCATGACCAGG
GACACGTCCACGAGCACAGCCTACATGGAGCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTATTACTGT
GAACTGATGCAGGGCTACTGGGGCCAGGGAACACTAGTCACAGTCTCCTCA

SEQ ID NO. 51: PN encoding VH humanised construct H27 SEQ ID: 48
CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTTTCCTGCAAGGCA
TCTGGATACACCTTCACCAGCTACTGGATGCACTGGGTGAAACAGCGACCTGGACAAGGGCTTGAGTGGATC
GGAAATATTAATCCTAGCAATGGTGGTACTAACTACAATGAGAAGTTCAAGAGCAAAGCCACCCTCACCGTC
GACAAATCCACGAGCACAGCCTACATGGAGCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTATTACTGT
GAACTGATGCAGGGCTACTGGGGCCAGGGAACACTAGTCACAGTCTCCTCA

SEQ ID NO. 52: PN encoding VH humanised construct H28 SEQ ID: 49
CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTTTCCTGCAAGGCA
TCTGGATACACCTTCACCAGCTACTGGATGCACTGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTGGATC
GGAAATATTAATCCTAGCAATGGTGGTACTAACTACAATGAGAAGTTCAAGAGCAAGGCCACCATGACCAGG
GACACGTCCACGAGCACAGCCTACATGGAGCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTATTACTGT
GAACTGATGCAGGGCTACTGGGGCCAGGGAACACTAGTCACAGTCTCCTCA

SEQ ID NO. 53: Heavy chain humanised construct H26
MGWSCIILFLVATATGVHSQVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWMHWVRQAPGQGLEWIGNINP
SNGGTNYNEKFKSRATMTRDTSTSTAYMELSSLRSEDTAVYYCELMQGYWGQGTLVTVSSASTKGPSVFPLA
PSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICN
VNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELAGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV
KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE
PQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPGK.

SEQ ID NO. 54: Heavy chain humanised construct H27
MGWSCIILFLVATATGVHSQVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWMHWVKQRPGQGLEWIGNINP
SNGGTNYNEKFKSKATLTVDKSTSTAYMELSSLRSEDTAVYYCELMQGYWGQGTLVTVSSASTKGPSVFPLA
PSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICN
VNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELAGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV
KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE
PQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO. 55: Heavy chain humanised construct H28
MGWSCIILFLVATATGVHSQVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWMHWVRQAPGQGLEWIGNINP
SNGGTNYNEKFKSKATMTRDTSTSTAYMELSSLRSEDTAVYYCELMQGYWGQGTLVTVSSASTKGPSVFPLA
PSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICN
VNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELAGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV
KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE
PQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO. 56: PN encoding Heavy chain humanised construct H26 SEQ ID: 53
ATGGGATGGAGCTGTATCATCCTCTTCTTGGTAGCAACAGCTACAGGTGTCCACTCCCAGGTGCAGCTGGTG
CAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTTTCCTGCAAGGCATCTGGATACACCTTC
ACCAGCTACTGGATGCACTGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTGGATCGGAAATATTAATCCT
AGCAATGGTGGTACTAACTACAATGAGAAGTTCAAGAGCAGAGCCACCATGACCAGGGACACGTCCACGAGC
ACAGCCTACATGGAGCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTATTACTGTGAACTGATGCAGGGC
TACTGGGGCCAGGGAACACTAGTCACAGTCTCCTCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCA
CCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCG
GTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCA
GGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAAC
GTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACTCACACA
TGCCCACCGTGCCCAGCACCTGAACTCGCGGGGGCACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGAC
ACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTC
AAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAAC
AGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGC
AAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAA
CCACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTC
AAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACC

ACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGG
CAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTC
TCCCTGTCTCCGGGTAAATGA

SEQ ID NO. 57: PN encoding Heavy chain humanised construct H27 SEQ ID: 54

ATGGGATGGAGCTGTATCATCCTCTTCTTGGTAGCAACAGCTACAGGTGTCCACTCCCAGGTGCAGCTGGTG
CAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTTTCCTGCAAGGCATCTGGATACACCTTC
ACCAGCTACTGGATGCACTGGGTGAAACAGCGACCTGGACAAGGGCTTGAGTGGATCGGAAATATTAATCCT
AGCAATGGTGGTACTAACTACAATGAGAAGTTCAAGAGCAAAGCCACCCTCACCGTCGACAAATCCACGAGC
ACAGCCTACATGGAGCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTATTACTGTGAACTGATGCAGGGC
TACTGGGGCCAGGGAACACTAGTCACAGTCTCCTCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCA
CCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCG
GTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCA
GGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAAC
GTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACTCACACA
TGCCCACCGTGCCCAGCACCTGAACTCGCGGGGGCACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGAC
ACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTC
AAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAAC
AGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGC
AAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAA
CCACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTC
AAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACC
ACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGG
CAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTC
TCCCTGTCTCCGGGTAAATGA

SEQ ID NO. 58: PN encoding Heavy chain humanised construct H28 SEQ ID: 55

ATGGGATGGAGCTGTATCATCCTCTTCTTGGTAGCAACAGCTACAGGTGTCCACTCCCAGGTGCAGCTGGTG
CAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTTTCCTGCAAGGCATCTGGATACACCTTC
ACCAGCTACTGGATGCACTGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTGGATCGGAAATATTAATCCT
AGCAATGGTGGTACTAACTACAATGAGAAGTTCAAGAGCAAGGCCACCATGACCAGGGACACGTCCACGAGC
ACAGCCTACATGGAGCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTATTACTGTGAACTGATGCAGGGC
TACTGGGGCCAGGGAACACTAGTCACAGTCTCCTCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCA
CCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCG
GTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCA
GGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAAC
GTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACTCACACA
TGCCCACCGTGCCCAGCACCTGAACTCGCGGGGGCACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGAC
ACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTC
AAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAAC
AGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGC
AAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAA
CCACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTC
AAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACC
ACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGG
CAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTC
TCCCTGTCTCCGGGTAAATGA

SEQ ID NO. 59: Heavy chain Hc (G95M)

MGWSCIILFLVAAATGVHSQVQLQQPGTELVKPGASVKLSCKASGYTFTSYWMHWVKQRPGQGLEWIGNINP
SNGGTNYNEKFKSKATLTVDKSSSTAYMQLSSLTSEDSAVYYCELMQGYWGQGTLVTVSSASTKGPSVFPLA
PSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICN
VNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELAGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV
KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE
PQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID 60: Epitope
VLPDIVMEAPLN

SEQ ID 61: 2A10 VH humanised construct H99
QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWMHWVRQAPGQGLEWMGNINPSNGGTNYNEKFKSRVTMTR
DTSTSTVYMELSSLRSEDTAVYYCELGQSYWGQGTLVTVSS

SEQ ID NO. 62: CDR H3
GQSY

SEQ ID NO. 63: VH humanised construct H100
QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWMHWVRQAPGQGLEWIGNINPSNGGTNYNEKFKSRATMTR
DTSTSTAYMELSSLRSEDTAVYYCELGQSYWGQGTLVTVSS

SEQ ID NO. 64: VH humanised construct H101
QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWMHWVKQRPGQGLEWIGNINPSNGGTNYNEKFKSKATLTV
DKSTSTAYMELSSLRSEDTAVYYCELGQSYWGQGTLVTVSS

SEQ ID NO. 65: VH humanised construct H102
QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWMHWVRQAPGQGLEWIGNINPSNGGTNYNEKFKSKATMTR
DTSTSTAYMELSSLRSEDTAVYYCELGQSYWGQGTLVTVSS

SEQ ID NO. 66 2A10 VH humanised construct H98
QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWMHWVRQAPGQGLEWMGNINPSNGGTNYNEKFKSRVTMTR
DTSTSTVYMELSSLRSEDTAVYYCELMQGYWGQGTLVTVSS

SEQ ID NO. 67
DIVMTQSPLSLPVTLGQPASISCRSSKSLLYKDGKTYLNWFRQRPGQSPQLLIYLMSTRASGVPDRFSGGGS
GTDFTLKISRVEAEDVGVYYCQQLVEYPLTFGQGTKLEIK

SEQ ID NO. 68
DIVMTQSPLSLPVTLGQPASISCRSSKSLLYKDGKTYLNWFQQRPGQSPRLLIYLMSTRASGVPDRFSGGGS
GTDFTLKISRVEAEDVGVYYCQQLVEYPLTFGQGTKLEIK

SEQ ID NO. 69
DIVMTQSPLSLPVTLGQPASISCRSSKSLLYKDGKTYLNWFRQRPGQSPRLLIYLMSTRASGVPDRFSGGGS
GTDFTLKISRVEAEDVGVYYCQQLVEYPLTFGQGTKLEIK

SEQ ID NO. 70
DIVMTQSPLSNPVTLGQPVSISCRSSKSLLYKDGKTYLNWFRQRPGQSPQLLIYLMSTRASGVPDRFSGGGS
GTDFTLKISRVEAEDVGVYYCQQLVEYPLTFGQGTKLEIK

SEQ ID NO. 71
DIVMTQSPLSNPVTLGQPVSISCRSSKSLLYKDGKTYLNWFLQRPGQSPRLLIYLMSTRASGVPDRFSGGGS
GTDFTLKISRVEAEDVGVYYCQQLVEYPLTFGQGTKLEIK

SEQ ID NO. 72
DIVMTQSPLSNPVTLGQPVSISCRSSKSLLYKDGKTYLNWFRQRPGQSPRLLIYLMSTRASGVPDRFSGGGS
GTDFTLKISRVEAEDVGVYYCQQLVEYPLTFGQGTKLEIK

SEQ ID NO. 73
TPSPVLPDIVMEAPLN

SEQ ID NO. 74
VLPDIVMEAPLNSAVP

SEQ ID NO.75
RQGY

SEQ ID NO.76
IQGY

SEQ ID NO.77
GDGY

SEQ ID NO.78
GIGY

SEQ ID NO.79
GSGY

SEQ ID NO.80
GQNY

SEQ ID NO.81
GQYY

SEQ ID NO.82
GQLY

SEQ ID NO.83
GQFY

SEQ ID NO.84
GQGW

SEQ ID NO.85
YESIKHEPENPPPYEE

SEQ ID NO.86
WQGY

SEQ ID NO.87
GAGY

SEQ ID NO.88
GLGY

SEQ ID NO.89
GVGY

SEQ ID NO.90
GQWY

SEQ ID NO. 91
MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVKLTQSMAII
RYIADKHNMLGGCPKERAEISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN
GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHP
PKSDLEVLFQGPLGSMQESLYPVAQLCPSFEESEATPSPVLPDIVMEAPLNSAVPSAVASAVQPSLSPLEAS
SVNYESVKHEPENPPPYEEAMNVSLKKVSGIKEEIKEPESIKAAVQETEAPYISIACDLIKETKLSAEPTPD
FSNYSEMAKVEQPLPDHSEIVEDSSPDSEPVDLFSDDSIPDVPQKQDEAVILVKENLTETSFESMIEHENKL
ERPHRD

SEQ ID NO. 92
MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVKLTQSMAII
RYIADKHNMLGGCPKERAEISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN
GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHP
PKSDLEVLFQGPLGSKMDLVQTSEVMQESLYPAAQLCPSFEESEATPSPVLPDIVMEAPLNSAVPSAGASAV
QPSSSPLEASSVNYESIIHEPENPPPYEEAMSVSLKKVSGIKEEIKEPESINAAVQETEAPYISIACDLIKE

TKLSAEPTPDFSDYSEMAKVEQPVPDHSELVEDSSPDSEPVDLFSDDSIPDVPQKQDEAVMLVKENLPETSF
ESMIEHENKEKLSALPPEGGSSGRIVTD

SEQ ID NO. 93
MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVKLTQSMAII
RYIADKHNMLGGCPKERAEISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN
GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHP
PKSDLEVLFQGPLGSVQDSLCPVAQLCPSFEESEATPSPVLPDIVMEAPLNSAVPSAGASAVQPSSSPLEAS
SVNFESVKHEPENPPPYEEAMNVSRKKVSGIKEEIKEPESINAAVQETEAPYISIACDLIKETKLSAEPTPD
FSSYSEMAKVEQPLPDHSELVEDSSPDSEPVDLFSDDSIPDVPQKQDEAVILVKETLTETSFESMIEHENKL
ERPHRD

SEQ ID NO. 94
MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVKLTQSMAII
RYIADKHNMLGGCPKERAEISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN
GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHP
PKSDLEVLFQGPLGSIQESLYPTAQLCPSFEEAEATPSPVLPDIVMEAPLNSLLPSAGASVVQPSVSPLEAP
PPVSYDSIKLEPENPPPYEEAMNVALKALGTKEGIKEPESFNAAVQETEAPYISIACDLIKETKLSTEPSPD
FSNYSEIAKFEKSVPEHAELVEDSSPESEPVDLFSDDSIPEVPQTQEEAVMLMKESLTEVSETVAQHKEERL

SEQ ID NO.95
DETFAL

SEQ ID NO.96
ELSKTS

CLAIMS

1. A heavy chain variable region comprising a third CDR consisting essentially of the amino acid residues GQGY wherein the CDR contains at least one substitution within the GQGY core sequence, the substitutions being selected from the following substitutions: where the G in the first position is replaced by R, I, W or M; the Q in the second position is replaced by D, I, A, L, V or S; the G in the third position is replaced by W, N, Y, S, L or F; and the Y in the fourth position is replaced by W.

2. A heavy chain variable region as claimed in claim 1 wherein there is a single substitution to the GQGY sequence to yield one of the following CDR H3: RQGY (SEQ ID NO.75), IQGY (SEQ ID NO.76), MQGY (SEQ ID NO.45), GDGY (SEQ ID NO.77), GIGY (SEQ ID NO.78), GSGY (SEQ ID NO.79), GQNY (SEQ ID NO.80), GQYY (SEQ ID NO.81), GQSY (SEQ ID NO.62), GQLY (SEQ ID NO.82), GQFY (SEQ ID NO.83), GQGW (SEQ ID NO.84), WQGY(SEQ ID NO.86), GAGY(SEQ ID NO.87), GLGY(SEQ ID NO.88), GVGY(SEQ ID NO.89), GQWY(SEQ ID NO.90).

3. A heavy chain variable region as claimed in claim 2 wherein the CDR H3 is MQGY or GQSY.

4. A heavy chain variable region as claimed in any one of claims 1 to 3, wherein the heavy chain variable region comprises the sequence SYWMH as CDR H1 (SEQ ID NO. 1) and NINPSNGGTNYNEKFKS as CDR H2 (SEQ ID NO.2).

5. A heavy chain variable region as claimed in any one of claims 1 to 4 wherein the heavy chain variable region is a humanised sequence.

6. A heavy chain variable region as claimed in claim 5 wherein the acceptor heavy chain variable region sequence has at least 40% identity in the framework regions to the 2A10 donor antibody heavy chain variable region sequence given in SEQ ID NO.7.

7. A heavy chain variable region as claimed in claim 6 wherein the heavy chain variable region has an amino acid sequence of SEQ ID NO. 66 (H98 variable region) or SEQ ID NO. 61 (H99 variable region), further comprising a number of substitutions at one or more of numerical positions 38, 40, 67, 68, 70,

72, 74, and 79; wherein each substituted amino acid residue is replaced with the amino acid residue at the equivalent position in SEQ ID NO 7.

8. A heavy chain variable region as claimed in any one of claims 1 to 7, having the amino acid sequence given in SEQ ID NO.47 (H26), SEQ ID NO.48 (H27), SEQ ID NO.49 (H28), SEQ ID NO. 63 (H100), SEQ ID NO. 54 (H101), SEQ ID NO. 65 (H102).

9. An isolated antibody, or fragment thereof, capable of binding to human NOGO-A comprising a heavy chain variable region as claimed in any one of claims 1 to 8 and a light chain variable region.

10. An isolated antibody or fragment thereof as claimed in claim 7 comprising the following heavy and light chain variable region pairs: H27L16 (SEQ ID NO.48 + SEQ ID NO.14), H28L13 (SEQ ID NO.49 + SEQ ID NO.13), H28L16 (SEQ ID NO.49 + SEQ ID NO.14).

11. An isolated antibody comprising the following heavy and light chain sequences H27FL L16FL (SEQ ID NO. 54 + SEQ ID NO.18), H28FL L13FL (SEQ ID NO. 55 + SEQ ID NO.17), H28FL L16FL (SEQ ID NO. 55 + SEQ ID NO.18).

12. A pharmaceutical composition comprising an anti-NOGO antibody or fragment thereof comprising the heavy chain variable regions claimed in any one of claims 1 to 8 or the antibodies or fragments thereof as claimed in claims 9 to 11, together with a pharmaceutically acceptable diluent or carrier.

13. Use of an anti-NOGO antibody or fragment thereof as claimed in any one of claims 1 to 8 or the antibodies or fragments thereof as claimed in claims 9 to 11, in the preparation of a medicament for treatment or prophylaxis of stroke and other neurological diseases/disorders or for the treatment of a patient suffering from a mechanical trauma to the central or peripheral nervous system.

14. A method for the treatment or prophylaxis of stroke or other neurological disease/disorder or for the treatment of a patient suffering from a mechanical trauma to the central or peripheral nervous system, in a human comprising the step of parenteral administration of a therapeutically effective amount of an anti-NOGO antibody or fragment thereof as claimed in any one of claims 1 to 8 or the antibodies or fragments thereof as claimed in claims 9 to 11.

15. An antibody or fragment thereof that is capable of binding to a region of human NOGO protein consisting of the polypeptide sequence of VLPDIVMEAPLN (SEQ ID NO. 60), characterised in that the antibody, or fragment thereof is not an antibody comprising a variable heavy domain having CDR H3 consisting of the amino acid residues GQGY or analogues thereof having one amino acid substitution therein.

16. A polynucletotide that encodes the polypeptides having the sequence set forth in SEQ ID NOs. 47, 48, 49, 53, 54 or 55.

17. An antibody, or fragment thereof, that is capable of binding to human NOGO protein in an ELISA assay, wherein the binding of the antibody, or fragment thereof, to human NOGO protein is reduced in the presence of a peptide having the following sequence VLPDIVMEAPLN (SEQ ID NO. 60), and is not reduced in the presence of an irrelevant peptide, characterised in that the antibody or fragment thereof is not an antibody comprising a heavy chain variable domain having a CDR H3 consisting of the amino acid residues GQGY or analogues thereof having one amino acid substitution therein.