USE OF DR6 AND P75 ANTAGONISTS TO PROMOTE SURVIVAL OF CELLS OF THE NERVOUS SYSTEM

BACKGROUND OF THE INVENTION

Field of the Invention

[0001] This invention relates to neurobiology, neurology and pharmacology. More particularly, it relates to methods for promoting survival of cells of the nervous system using Death Receptor-6 (DR6) antagonists, optionally in combination with p75 antagonists. The invention also relates to methods of treating neurodegenerative conditions by the administration of a DR6 antagonist, optionally in combination with a p75 antagonist. The invention also relates to methods of preventing the interaction of DR6 and p75 using DR6 and/or p75 antagonists.

Background

[0002] Apoptosis (*i.e.* programmed cell death) has been shown to play an important role in numerous diseases of the nervous system including both acute and chronic injuries. For example, the role of apoptosis has been demonstrated in Alzheimer's disease, Parkinson's disease, Huntington's disease, motor neuron disease (*e.g.* amyotrophic lateral sclerosis, which is also called ALS or Lou Gehrig's disease), multiple sclerosis, neuronal trauma and cerebral ischemia (*e.g.* stroke).

[0003] Many studies have been directed to understanding the molecular mechanisms of apoptosis, and these studies have led to the discovery of a family of receptors called the death receptors. Eight death receptors, which are characterized by a cytoplasmic death domain, have been identified thus far. The death receptors have been grouped into two different families. Members of the first family recruit a death inducing signaling complex (DISC), which promotes apoptotic signaling. Members of the second family recruit a different set of molecules to transduce apoptotic signals. Interestingly, members of the second family also transduce cell survival signals.

[0004] Death receptor 6 (DR6) is a member of the second family of death receptors. DR6 is widely expressed, but appears to function differently in different cell types. DR6 mRNA has been observed in heart, brain, placental, pancreas, lymph node, thymus and prostate tissues. Lower levels have been observed in other cell types including skeletal muscle, kidney and testes,

but little or no expression has previously been observed in adult liver or any lines of hematopoeitic origin. Interestingly, it has been observed that DR6 is capable of inducing apoptosis in only a subset of cells tested. For example, overexpression of DR6 in HeLa S3 cervical carcinoma cells resulted in apoptosis in a death-domain-dependent manner (Pan *et al. FEBS 431*:351-356 (1998)). In addition, Nikoleav *et al.* (*Nature 457*:981-990 (2009)) have shown that beta-amyloid precursor protein (APP) is a DR6 ligand and suggested that the binding of an APP fragment to DR6 triggers degeneration of neuronal cell bodies and axons. In contrast, DR6 did not induce cell death in MCF7 (a human breast adenocarcinoma line) cells (Pan *et al. FEBS 431*:351-356 (1998)). The characteristics that differentiate a cell's response to DR6 expression and signaling have not yet been identified.

[0005] Drugs that can specifically modulate apoptosis may be useful for treating diseases involving neuronal cell death, for example, because neurons may have less capacity to regenerate than other cell types. However, currently available anti-apoptotic drugs have low specificity and selectivity and as a result, produce undesirable side effects. Such side effects might be reduced or avoided by for example, targeting anti-apoptotic drugs specifically to the desired site of action. Alternatively, characterization of death receptors such as DR6 that specifically act in a particular subset of cell types has the potential to provide a more specific therapeutic effect.

BRIEF SUMMARY OF THE INVENTION

[0006] The present invention is based on the discovery that DR6 is specifically able to induce apoptosis in cells of the nervous system and that p75 is a ligand for DR6. It has also been discovered that antagonists of DR6 and p75, including anti-DR6 antibodies, are able to inhibit the interaction of DR6 and p75 and to inhibit death of cells of the nervous system. Accordingly, antagonists of DR6 and/or p75 can be useful for therapy in which modulation of DR6 expression or activity is advantageous.

[0007] Based on the discoveries described herein, an isolated antibody or antigen-binding fragment thereof that can specifically bind to a DR6 polypeptide, such that the antibody promotes survival of cells of the nervous system is described. Also described is an isolated antibody or antigen-binding fragment thereof that specifically binds to DR6, wherein the antibody promotes proliferation, differentiation or survival of oligodendrocytes. DR6 antibodies also include an isolated antibody or antigen-binding fragment thereof which specifically binds to DR6, wherein the antibody promotes myelination. In some embodiments, the DR6 antibody inhibits binding of

DR6 to p75. In some embodiments, the DR6 antibody inhibits binding of DR6 to p75 but does not inhibit binding of DR6 to beta-amyloid precursor protein (APP).

[0008] In some embodiments, the DR6 antibody is an isolated antibody or antigen-binding fragment thereof that specifically binds to the same DR6 epitope as a reference monoclonal Fab antibody fragment selected from the group consisting of M50-H01, M51-H09, M53-E04, M53-F04, M62-B02, M63-E10, M66-B03, M67-G02, M72-F03, and M73-C04 or a reference monoclonal antibody selected from the group consisting of 1P1D6.3, 1P2F2.1, and 1P5D10.2.

[0009] In some embodiments, the DR6 antibody an isolated antibody or antigen-binding fragment thereof which specifically binds to DR6, wherein said antibody or fragment thereof competitively inhibits a reference monoclonal Fab antibody fragment selected from the group consisting of M50-H01, M51-H09, M53-E04, M53-F04, M62-B02, M63-E10, M66-B03, M67-G02, M72-F03, and M73-C04 or a reference monoclonal antibody selected from the group consisting of 1P1D6.3, 1P2F2.1, and 1P5D10.2 from binding to DR6.

[0010] In some embodiments, the DR6 antibody is an isolated antibody or antigen-binding fragment thereof that specifically binds to DR6, wherein said antibody or fragment thereof is comprises an antigen binding domain identical to that of a monoclonal Fab antibody fragment selected from the group consisting of M50-H01, M51-H09, M53-E04, M53-F04, M62-B02, M63-E10, M66-B03, M67-G02, M72-F03, and M73-C04 or a reference monoclonal antibody selected from the group consisting of 1P1D6.3, 1P2F2.1, and 1P5D10.2.

[0011] In some embodiments, the DR6 antibody is an isolated antibody or fragment thereof that specifically binds to DR6, wherein the heavy chain variable region (VH) of said antibody or fragment thereof comprises an amino acid sequence at least 90% identical to a reference amino acid sequence selected from the group consisting of: SEQ ID NO:7, SEQ ID NO:17, SEQ ID NO:27, SEQ ID NO:37, SEQ ID NO:47, SEQ ID NO:57, SEQ ID NO:67, SEQ ID NO:77, SEQ ID NO:87, SEQ ID NO: 97, SEQ ID NO:107, SEQ ID NO:117, and SEQ ID NO:127.

[0012] In some embodiments, the DR6 antibody is an isolated antibody or fragment thereof that specifically binds to DR6, wherein the light chain variable region (VL) of said antibody or fragment thereof comprises an amino acid sequence at least 90% identical to a reference amino acid sequence selected from the group consisting of: SEQ ID NO:12, SEQ ID NO:22, SEQ ID NO:32, SEQ ID NO:42, SEQ ID NO:52, SEQ ID NO:62, SEQ ID NO:72, SEQ

ID NO:82, SEQ ID NO:92, SEQ ID NO:102 SEQ ID NO:112, SEQ ID NO:122, and SEQ ID NO:132.

[0013] In some embodiments, the DR6 antibody is an isolated antibody or fragment thereof that specifically binds to DR6, wherein the VH of said antibody or fragment thereof comprises an amino acid sequence identical, except for 20 or fewer conservative amino acid substitutions, to a reference amino acid sequence selected from the group consisting of: SEQ ID NO:7, SEQ ID NO:17, SEQ ID NO:27, SEQ ID NO:37, SEQ ID NO:47, SEQ ID NO:57, SEQ ID NO:67, SEQ ID NO:77, SEQ ID NO:87, SEQ ID NO: 97, SEQ ID NO:107, SEQ ID NO:117, and SEQ ID NO:127.

[0014] In some embodiments, the DR6 antibody is an isolated antibody or fragment thereof that specifically binds to DR6, wherein the VL of said antibody or fragment thereof comprises an amino acid sequence identical, except for 20 or fewer conservative amino acid substitutions, to a reference amino acid sequence selected from the group consisting of: SEQ ID NO:12, SEQ ID NO:22, SEQ ID NO:32, SEQ ID NO:42, SEQ ID NO:52, SEQ ID NO:62, SEQ ID NO:72, SEQ ID NO:82, SEQ ID NO:92, SEQ ID NO:102 SEQ ID NO:112, SEQ ID NO:122, and SEQ ID NO:132.

[0015] In some embodiments, the DR6 antibody is an isolated antibody or fragment thereof that specifically binds to DR6, wherein the VH of said antibody or fragment thereof comprises an amino acid sequence selected from the group consisting of: SEQ ID NO:7, SEQ ID NO:17, SEQ ID NO:27, SEQ ID NO:37, SEQ ID NO:47, SEQ ID NO:57, SEQ ID NO:67, SEQ ID NO:77, SEQ ID NO:87, SEQ ID NO: 97, SEQ ID NO:107, SEQ ID NO:117, and SEQ ID NO:127.

[0016] In some embodiments, the DR6 antibody is an isolated antibody or fragment thereof that specifically binds to DR6, wherein the VL of said antibody or fragment thereof comprises an amino acid sequence selected from the group consisting of: SEQ ID NO:12, SEQ ID NO:22, SEQ ID NO:32, SEQ ID NO:42, SEQ ID NO:52, SEQ ID NO:62, SEQ ID NO:72, SEQ ID NO:82, SEQ ID NO:92, SEQ ID NO:102 SEQ ID NO:112, SEQ ID NO:122, and SEQ ID NO:132.

[0017] In some embodiments, the DR6 antibody is an isolated antibody or fragment thereof that specifically binds to DR6, wherein the VH and VL of said antibody or fragment thereof comprise, respectively, amino acid sequences at least 90% identical to reference amino acid sequences selected from the group consisting of: SEQ ID NO:7 and SEQ ID NO:12; SEQ ID

NO:17 and SEQ ID NO:22; SEQ ID NO:27 and SEQ ID NO:32; SEQ ID NO:37 and SEQ ID NO:42; SEQ ID NO:47 and SEQ ID NO:52; SEQ ID NO:57 and SEQ ID NO:62; SEQ ID NO:67 and SEQ ID NO:72; SEQ ID NO:77 and SEQ ID NO:82; SEQ ID NO:87 and SEQ ID NO:92; SEQ ID NO:97 and SEQ ID NO:102; SEQ ID NO:107 and SEQ ID NO:112; SEQ ID NO117 and SEQ ID NO:122; and SEQ ID NO:127 and SEQ ID NO:132.

[0018] In some embodiments, the DR6 antibody is an isolated antibody or fragment thereof that specifically binds to DR6, wherein the VH and VL of said antibody or fragment thereof comprise, respectively, amino acid sequences identical, except for 20 or fewer conservative amino acid substitutions each, to reference amino acid sequences selected from the group consisting of: SEQ ID NO:7 and SEQ ID NO:12; SEQ ID NO:17 and SEQ ID NO:22; SEQ ID NO:27 and SEQ ID NO:32; SEQ ID NO:37 and SEQ ID NO:42; SEQ ID NO:47 and SEQ ID NO:52; SEQ ID NO:57 and SEQ ID NO:62; SEQ ID NO:67 and SEQ ID NO:72; SEQ ID NO:77 and SEQ ID NO:82; SEQ ID NO:87 and SEQ ID NO:92; SEQ ID NO:97 and SEQ ID NO:102; SEQ ID NO:107 and SEQ ID NO:112; SEQ ID NO117 and SEQ ID NO:122; and SEQ ID NO:127 and SEQ ID NO:132.

[0019] In some embodiments, the DR6 antibody is an isolated antibody or fragment thereof that specifically binds to DR6, wherein the VH and VL of said antibody or fragment thereof comprise, respectively, amino acid sequences selected from the group consisting of: SEQ ID NO:7 and SEQ ID NO:12; SEQ ID NO:17 and SEQ ID NO:22; SEQ ID NO:27 and SEQ ID NO:32; SEQ ID NO:37 and SEQ ID NO:42; SEQ ID NO:47 and SEQ ID NO:52; SEQ ID NO:57 and SEQ ID NO:62; SEQ ID NO:67 and SEQ ID NO:72; SEQ ID NO:77 and SEQ ID NO:82; SEQ ID NO:87 and SEQ ID NO:92; SEQ ID NO:97 and SEQ ID NO:102; SEQ ID NO:107 and SEQ ID NO:112; SEQ ID NO117 and SEQ ID NO:122; and SEQ ID NO:127 and SEQ ID NO:132.

[0020] In some embodiments, the DR6 antibody is an isolated antibody or fragment thereof that specifically binds to DR6, wherein the VH of said antibody or fragment thereof comprises a Kabat heavy chain complementarity determining region-1 (VH-CDR1) amino acid sequence identical, except for two or fewer amino acid substitutions, to a reference VH-CDR1 amino acid sequence selected from the group consisting of: SEQ ID NO: 8, SEQ ID NO: 18, SEQ ID NO: 28, SEQ ID NO: 38, SEQ ID NO: 48, SEQ ID NO: 58, SEQ ID NO: 68, SEQ ID NO: 78, SEQ ID NO: 88, SEQ ID NO: 98, SEQ ID NO: 108 SEQ ID NO: 118, and SEQ ID NO: 128. In one embodiment, the VH-CDR1 amino acid sequence is selected from the group

consisting of: SEQ ID NO: 8, SEQ ID NO: 18, SEQ ID NO: 28, SEQ ID NO: 38, SEQ ID NO: 48, SEQ ID NO: 58, SEQ ID NO: 68, SEQ ID NO: 78, SEQ ID NO: 88, SEQ ID NO: 98, SEQ ID NO: 108 SEQ ID NO: 118, and SEQ ID NO: 128.

[0021] In some embodiments, the DR6 antibody is an isolated antibody or fragment thereof that specifically binds to DR6, wherein the VH of said antibody or fragment thereof comprises a Kabat heavy chain complementarity determining region-2 (VH-CDR2) amino acid sequence identical, except for four or fewer amino acid substitutions, to a reference VH-CDR2 amino acid sequence selected from the group consisting of: SEQ ID NO: 9, SEQ ID NO: 19, SEQ ID NO: 29, SEQ ID NO: 39, SEQ ID NO: 49, SEQ ID NO: 59, SEQ ID NO: 69, SEQ ID NO: 79, SEQ ID NO: 89, SEQ ID NO: 99, SEQ ID NO: 109, SEQ ID NO: 119 and SEQ ID NO: 129. In one embodiment, the VH-CDR2 amino acid sequence is selected from the group consisting of: SEQ ID NO: 9, SEQ ID NO: 19, SEQ ID NO: 29, SEQ ID NO: 39, SEQ ID NO: 49, SEQ ID NO: 59, SEQ ID NO: 69, SEQ ID NO: 79, SEQ ID NO: 89, SEQ ID NO: 99, SEQ ID NO: 109, SEQ ID NO: 119 and SEQ ID NO: 129.

[0022] In some embodiments, the DR6 antibody is an isolated antibody or fragment thereof that specifically binds to DR6, wherein the VH of said antibody or fragment thereof comprises a Kabat heavy chain complementarity determining region-3 (VH-CDR3) amino acid sequence identical, except for four or fewer amino acid substitutions, to a reference VH-CDR3 amino acid sequence selected from the group consisting of: SEQ ID NO: 10, SEQ ID NO: 20, SEQ ID NO: 30, SEQ ID NO: 40, SEQ ID NO: 40, SEQ ID NO: 60, SEQ ID NO: 70, SEQ ID NO: 80, SEQ ID NO: 90, SEQ ID NO: 100, SEQ ID NO: 110, SEQ ID NO: 120 and SEQ ID NO:130. In one embodiment, the VH-CDR3 amino acid sequence is selected from the group consisting of: SEQ ID NO: 10, SEQ ID NO: 20, SEQ ID NO: 30, SEQ ID NO: 40, SEQ ID NO: 40, SEQ ID NO: 60, SEQ ID NO: 70, SEQ ID NO: 80, SEQ ID NO: 90, SEQ ID NO: 100, SEQ ID NO: 110, SEQ ID NO: 120 and SEQ ID NO:130.

[0023] In some embodiments, the DR6 antibody is an isolated antibody or fragment thereof that specifically binds to DR6, wherein the VL of said antibody or fragment thereof comprises a Kabat light chain complementarity determining region-1 (VL-CDR1) amino acid sequence identical, except for four or fewer amino acid substitutions, to a reference VL-CDR1 amino acid sequence selected from the group consisting of: SEQ ID NO: 13, SEQ ID NO: 23, SEQ ID NO: 33, SEQ ID NO: 43, SEQ ID NO: 53, SEQ ID NO: 63, SEQ ID NO: 73, SEQ ID NO: 83, SEQ ID NO: 93, SEQ ID NO: 103, SEQ ID NO: 113, SEQ ID NO: 123 and SEQ ID

NO: 133. In one embodiment, the VL-CDR1 amino acid sequence is selected from the group consisting of: SEQ ID NO: 13, SEQ ID NO: 23, SEQ ID NO: 33, SEQ ID NO: 43, SEQ ID NO: 53, SEQ ID NO: 63, SEQ ID NO: 73, SEQ ID NO: 83, SEQ ID NO: 93, SEQ ID NO: 103, SEQ ID NO: 113, SEQ ID NO: 123 and SEQ ID NO: 133.

[0024] In some embodiments, the DR6 antibody is an isolated antibody or fragment thereof that specifically binds to DR6, wherein the VL of said antibody or fragment thereof comprises a Kabat light chain complementarity determining region-2 (VL-CDR2) amino acid sequence identical, except for two or fewer amino acid substitutions, to a reference VL-CDR2 amino acid sequence selected from the group consisting of: SEQ ID NO: 14, SEQ ID NO: 24, SEQ ID NO: 34, SEQ ID NO: 44, SEQ ID NO: 54, SEQ ID NO: 64, SEQ ID NO: 74, SEQ ID NO: 84, SEQ ID NO: 94, SEQ ID NO: 104, SEQ ID NO: 114, SEQ ID NO: 124, and SEQ ID NO: 134. In one embodiment, the VL-CDR2 amino acid sequence is selected from the group consisting of: SEQ ID NO: 14, SEQ ID NO: 24, SEQ ID NO: 34, SEQ ID NO: 44, SEQ ID NO: 54, SEQ ID NO: 64, SEQ ID NO: 74, SEQ ID NO: 84, SEQ ID NO: 94, SEQ ID NO: 104, SEQ ID NO: 114, SEQ ID NO: 124, and SEQ ID NO: 134.

[0025] In some embodiments, the DR6 antibody is an isolated antibody or fragment thereof that specifically binds to DR6, wherein the VL of said antibody or fragment thereof comprises a Kabat light chain complementarity determining region-3 (VL-CDR3) amino acid sequence identical, except for four or fewer amino acid substitutions, to a reference VL-CDR3 amino acid sequence selected from the group consisting of: SEQ ID NO: 15, SEQ ID NO: 25, SEQ ID NO: 35, SEQ ID NO: 45, SEQ ID NO: 55, SEQ ID NO: 65, SEQ ID NO: 75, SEQ ID NO: 85, SEQ ID NO: 95, SEQ ID NO: 105, SEQ ID NO: 115, SEQ ID NO: 125, and SEQ ID NO: 135. In one embodiment, the VL-CDR3 amino acid sequence is selected from the group consisting of: SEQ ID NO: 15, SEQ ID NO: 25, SEQ ID NO: 35, SEQ ID NO: 45, SEQ ID NO: 55, SEQ ID NO: 65, SEQ ID NO: 75, SEQ ID NO: 85, SEQ ID NO: 95, SEQ ID NO: 105, SEQ ID NO: 115, SEQ ID NO: 125, and SEQ ID NO: 135.

[0026] In some embodiments, the DR6 antibody is an isolated antibody or fragment thereof that specifically binds to DR6, wherein the VH of said antibody or fragment thereof comprises VH-CDR1, VH-CDR2, and VH-CDR3 amino acid sequences selected from the group consisting of: SEQ ID NOs: 8, 9, and 10; SEQ ID NOs: 18, 19, and 20; SEQ ID NOs: 28, 29, and 30; SEQ ID NOs: 38, 39, and 40; SEQ ID NOs: 48, 49, and 50; SEQ ID NOs: 58, 59, and 60; SEQ ID NOs: 68, 69, and 70; SEQ ID NOs: 78, 79, and 80; SEQ ID NOs: 88, 89, and 90; SEQ

ID NOs: 98, 99, and 100; SEQ ID NOs: 108, 109, and 110; SEQ ID NOs: 118, 119, and 120; and SEQ ID NOs: 128, 129, and 130, except for one, two, three, or four amino acid substitutions in at least one of said VH-CDRs.

[0027] In some embodiments, the DR6 antibody is an isolated antibody or fragment thereof that specifically binds to DR6, wherein the VH of said antibody or fragment thereof comprises VH-CDR1, VH-CDR2, and VH-CDR3 amino acid sequences selected from the group consisting of: SEQ ID NOs: 8, 9, and 10; SEQ ID NOs: 18, 19, and 20; SEQ ID NOs: 28, 29, and 30; SEQ ID NOs: 38, 39, and 40; SEQ ID NOs: 48, 49, and 50; SEQ ID NOs: 58, 59, and 60; SEQ ID NOs: 68, 69, and 70; SEQ ID NOs: 78, 79, and 80; SEQ ID NOs: 88, 89, and 90; SEQ ID NOs: 98, 99, and 100; SEQ ID NOs: 108, 109, and 110; SEQ ID NOs: 118, 119, and 120; and SEQ ID NOs: 128, 129, and 130.

[0028] In some embodiments, the DR6 antibody is an isolated antibody or fragment thereof that specifically binds to DR6, wherein the VL of said antibody or fragment thereof comprises VL-CDR1, VL-CDR2, and VL-CDR3 amino acid sequences selected from the group consisting of: SEQ ID NOs: 13, 14, and 15; SEQ ID NOs: 23, 24, and 25; SEQ ID NOs: 33, 34, and 35; SEQ ID NOs: 43, 44, and 45; SEQ ID NOs: 53, 54, and 55; SEQ ID NOs: 63, 64, and 65; SEQ ID NOs: 73, 74, and 75; SEQ ID NOs: 83, 84, and 85; SEQ ID NOs: 93, 94, and 95; SEQ ID NOs: 103, 104, and 105; SEQ ID NOs: 113, 114, and 115; SEQ ID NOs: 123, 124, and 125; and SEQ ID NOs: 133, 134, and 135, except for one, two, three, or four amino acid substitutions in at least one of said VL-CDRs.

[0029] In some embodiments, the DR6 antibody is an isolated antibody or fragment thereof that specifically binds to DR6, wherein the VL of said antibody or fragment thereof comprises VL-CDR1, VL-CDR2, and VL-CDR3 amino acid sequences selected from the group consisting of: SEQ ID NOs: 13, 14, and 15; SEQ ID NOs: 23, 24, and 25; SEQ ID NOs: 33, 34, and 35; SEQ ID NOs: 43, 44, and 45; SEQ ID NOs: 53, 54, and 55; SEQ ID NOs: 63, 64, and 65; SEQ ID NOs: 73, 74, and 75; SEQ ID NOs: 83, 84, and 85; SEQ ID NOs: 93, 94, and 95; SEQ ID NOs: 103, 104, and 105; SEQ ID NOs: 113, 114, and 115; SEQ ID NOs: 123, 124, and 125; and SEQ ID NOs: 133, 134, and 135.

[0030] In various embodiments of the above-described antibodies or fragments thereof, the VH framework regions and/or VL framework regions are human, except for five or fewer amino acid substitutions.

[0031] In some embodiments, the above-described antibodies or fragments thereof bind to a linear epitope or a non-linear conformation epitope

[0032] In some embodiments, the above-described antibodies or fragments thereof are multivalent, and comprise at least two heavy chains and at least two light chains.

[0033] In some embodiments, the above-described antibodies or fragments thereof are multispecific. In further embodiments, the above-described antibodies or fragments thereof are bispecific.

[0034] In various embodiments of the above-described antibodies or fragments thereof, the heavy and light chain variable domains are murine. In further embodiments, the heavy and light chain variable domains are from a monoclonal antibody selected from the group consisting of 1P1D6.3, 1P2F2.1, and 1P5D10.2.

[0035] In various embodiments of the above-described antibodies or fragments thereof, the heavy and light chain variable domains are fully human. In further embodiments, the heavy and light chain variable domains are from a monoclonal Fab antibody fragment selected from the group consisting of M50-H01, M51-H09, M53-E04, M53-F04, M62-B02, M63-E10, M66-B03, M67-G02, M72-F03, and M73-C04.

[0036] In various embodiments, the above-described antibodies or fragments thereof are humanized.

[0037] In various embodiments, the above-described antibodies or fragments thereof are chimeric.

[0038] In various embodiments, the above-described antibodies or fragments thereof are primatized.

[0039] In various embodiments, the above-described antibodies or fragments thereof are fully human.

[0040] In certain embodiments, the above-described antibodies or fragments thereof are Fab fragments, Fab' fragments, $F(ab)_2$ fragments, or Fv fragments. In certain embodiments, the above-described antibodies are single chain antibodies. In certain embodiments, the antibodies or fragments thereof are conjugated to a polymer. In certain embodiments, the polymer is a polyalkylene glycol. In further embodiments, the polyalkylene gycol is polyethylene glycol (PEG).

[0041] In certain embodiments, the above-described antibodies or fragments thereof comprise light chain constant regions selected from the group consisting of a human kappa constant region and a human lambda constant region.

[0042] In certain embodiments, the above-described antibodies or fragments thereof comprise a heavy chain constant region or fragment thereof. In further embodiments, the heavy chain constant region or fragment thereof is selected from the group consisting of human IgG4, IgG4 agly, IgG1, and IgG1agly.

[0043] In some embodiments, the above-described antibodies or fragments thereof specifically bind to a DR6 polypeptide or fragment thereof, or a DR6 variant polypeptide, with an affinity characterized by a dissociation constant (K_D) which is less than the K_D for said reference monoclonal antibody. In further embodiments, the dissociation constant (K_D) is no greater than 5×10^{-2} M, 10^{-2} M, 5×10^{-3} M, 10^{-3} M, 5×10^{-4} M, 10^{-4} M, 5×10^{-5} M, 10^{-5} M, 5×10^{-6} M, 10^{-6} M, 5×10^{-7} M, 10^{-7} M, 5×10^{-8} M, 10^{-8} M, 5×10^{-9} M, 10^{-9} M, 5×10^{-10} M, 10^{-10} M, 5×10^{-11} M, 10^{-11} M, 5×10^{-12} M, 10^{-12} M, 5×10^{-13} M, 10^{-13} M, 5×10^{-14} M, 10^{-14} M, 5×10^{-15} M, or 10^{-15} M.

[0044] In some embodiments, the above-described antibodies or fragments thereof preferentially bind to a human DR6 polypeptide or fragment thereof, relative to a murine DR6 polypeptide or fragment thereof .

[0045] In addition, the methods described herein relate generally to methods of promoting survival and preventing apoptosis of cells of the nervous system. In certain embodiments, the methods include a method of promoting survival of cells of the nervous system comprising contacting said cells with a DR6 antagonist. In some particular embodiments, the cells of the nervous system cells are cells of the central nervous system, such as brain cells, spinal cord cells, or cell lines derived from such cells. In some embodiments, the cells of the central nervous system are cortical neurons, oligodendrocytes, microglia or astrocytes, or cell lines derived from such cells. In some embodiments the cells of the nervous system are neurons, for example cortical neurons, motor neurons and dorsal root ganglion (DRG) neurons or cell lines derived from such cells. In some embodiments the cells are glial cells including microglia and macroglia or cell lines derived from such cells. Examples of macroglial cells include astrocytes, oligodendrocytes, ependymocytes and radial glial cells. In some embodiments, the cells are precursors of these cells such as oligodendrocyte precursor cells or cell lines derived from such cells. In some embodiments, the cells of the nervous system are peripheral nervous

system cells. In some embodiments, the peripheral nervous system cells are dorsal root ganglion neurons, schwann cells, or cell lines derived from such cells.

[0046] The methods described herein also provide a method of promoting oligodendrocyte proliferation, differentiation or survival comprising contacting oligodendrocyte cells or oligodendrocyte precursor cells with a DR6 antagonist. In some embodiments, the method is a method of treating a condition associated with oligodendrocyte death or lack of differentiation, comprising administering a therapeutically effective amount of a DR6 antagonist.

[0047] The methods described herein also provide a method of promoting myelination comprising contacting a mixture of neuronal cells and oligodendrocyte cells or oligodendrocyte precursor cells with a DR6 antagonist. In some embodiments, the method is a method of of treating a condition associated with dysmyelination or demyelination comprising administering a therapeutically effective amount of a DR6 antagonist.

[0048] The method also relates generally to methods of treating conditions associated with death of cells of the nervous system. In another embodiment, the method is a method of treating a condition associated with death of cells of the nervous system comprising administering an effective amount of a DR6 antagonist to a mammal in need thereof. In some particular embodiments the condition associated with death of cells of the nervous system can be Alzheimer's disease, Parkinson's disease, Huntington's disease, motor neuron disease (*e.g.* amyotrophic lateral sclerosis, which is also called ALS or Lou Gehrig's disease), multiple sclerosis, neuronal trauma or cerebral ischemia (*e.g.* stroke). In some particular embodiments, the condition is neuropathic pain.

[0049] The methods described herein also include methods of inhibiting the binding of DR6 to p75 comprising contacting a DR6 polypeptide and/or p75 polypeptide with a DR6 antagonist under conditions wherein binding of DR6 to p75 is inhibited.

[0050] In various embodiments of the above methods, the DR6 antagonist can be any molecule which interferes with the ability of DR6 to negatively regulate survival of cells of the nervous system. In certain embodiments, the DR6 antagonist is selected from the group consisting of a soluble DR6 polypeptide, a DR6 antagonist compound, a DR6 antagonist antibody or fragment thereof, a DR6 antagonist polynucleotide (*e.g.* RNA interference), a DR6 aptamer, or a combination of two or more DR6 antagonists.

[0051] In certain embodiments, the DR6 antagonist polypeptide is a soluble DR6 polypeptide. Certain soluble DR6 polypeptides as described herein include, but are not limited to

soluble DR6 polypeptides which comprise the DR6 extracellular domain or one or more of the DR6 TNFR-like cysteine-rich motifs . In some embodiments, the soluble DR6 polypeptide lacks one or more of a DR6 TNFR-like cysteine-rich motif, a transmembrane domain, a death domain or a cytoplasmic domain. In some embodiments, the DR6 antagonist polypeptide comprises amino acids 1 to 349 of SEQ ID NO:2 (DR6); 40 to 349 of SEQ ID NO:2; or 41 to 349 of SEQ ID NO:2.

[0052] In some embodiments, the soluble DR6 antagonist is a fusion polypeptide comprising a non-DR6-heterologous polypeptide. In some embodiments, the non-DR6 heterologous polypeptide is selected from the group consisting of an immunoglobulin polypeptide or fragment thereof, a serum albumin polypeptide, a targeting polypeptide, a reporter polypeptide, and a purification-facilitating polypeptide. In some embodiments, the antibody Ig polypeptide is a hinge and an Fc polypeptide.

[0053] In alternative embodiments the DR6 antagonist is an antibody or fragment thereof as described above. In other embodiments, the DR6 antagonist is an an antibody or fragment thereof which binds to a DR6 polypeptide comprising one or more of the following domains (i) a DR6 extracellular domain, and (ii) a DR6 TNFR-like cysteine-rich motif. Additionally, the DR6 antagonist antibody or fragment thereof can specifically bind to an epitope within a polypeptide comprising a DR6 polypeptide as described herein. The DR6 antagonist can also be an antigen-binding fragment of such antibodies or a combination of two or more antibodies or fragments thereof.

[0054] In other embodiments, the DR6 antagonist is a DR6 antagonist polynucleotide such as an antisense polynucleotide, an aptamer, a ribozyme, a small interfering RNA (siRNA), or a small-hairpin RNA (shRNA).

[0055] In additional embodiments, the DR6 antagonist is a DR6 aptamer. A DR6 aptamer is a small polypeptide or a polynucleotide which binds DR6 and promotes nervous system cell survival or prevents cell apoptosis.

[0056] In some embodiments of the above methods, the DR6 antagonist is administered to a subject by a method comprising (a) introducing into a nervous system cell a polynucleotide that encodes the DR6 antagonist through operable association with an expression control sequence; and (b) allowing expression of said DR6 antagonist. In some embodiments the nervous system cells are in a mammal and said introducing comprises (a) administering to said mammal a polynucleotide which encodes a DR6 antagonist through operable association with an

expression control sequence. In some embodiments, the cultured host cell is derived from the mammal to be treated. In certain embodiments, the polynucleotide is introduced into the host cell or nervous system cell via transfection, electroporation, viral transduction or direct microinjection.

[0057] In certain embodiments the DR6 antagonist is a polynucleotide that can be administered to a mammal, at or near the site of the disease, disorder or injury. In some embodiments, the polynucleotide is administered as an expression vector. In certain embodiments, the vector is a viral vector which is selected from the group consisting of an adenoviral vector, an alphavirus vector, an enterovirus vector, a pestivirus vector, a lentiviral vector, a baculoviral vector, a herpesvirus vector (*e.g.* an Epstein Barr viral vector, or a herpes simplex viral vector) a papovaviral vector, a poxvirus vector (*e.g.* a vaccinia viral vector) and a parvovirus. In some embodiments, the vector is administered by a route selected from the group consisting of topical administration, intraocular administration, and parenteral administration (*e.g.* intravenous, intraarterial, intramuscular, intracardiac, subcutaneous, intradermal, intrathecal, intraperitoneal).

[0058] According to the methods described herein, the DR6 antagonist can be used in combination with a p75 antagonist. The p75 antagonist can be used simultaneously or sequentially.

[0059] The methods described herein also include methods of inhibiting the binding of DR6 to p75 comprising contacting a p75 polypeptide and/or DR6 polypeptide with a p75 antagonist under conditions wherein binding of DR6 to p75 is inhibited. The p75 antagonist can be (i) a p75 antagonist compound; (ii) p75 antagonist polypeptide; (iii) a p75 antagonist antibody or fragment thereof; (iv) a p75 antagonist polynucleotide; (v) a p75 aptamer; or (vi) a combination of two or more of said p75 antagonists.

BRIEF DESCRIPTION OF THE DRAWINGS/FIGURES

[0060] Figures 1A-D - DR6 is regulated during development and expressed in the CNS. Graphs displaying results of DR6 quantitative PCR. DR6 mRNA levels from E18, P1, P7, P14, P21 and adult rat brain (Fig. 1A) and spinal cord (Fig. 1B) are expressed as a ratio of the DR6 mRNA level/ DR6 mRNA level at E18. Protein expression in the cortex (CX), hippocampus (HP), striatum (ST), mid brain (MB), cerebellum (CB) and spinal cord (SC) based on Western

blot using anti-DR6 antibody (Fig. 1C). RT-PCR of DR6 and GAPDH from lysates of oligodendrocyte precursor cells (OPCs), cortical neurons, microglias and astrocytes (Fig. 1D).

[0061] Figures 2A-B - DR6 is expressed in oligodendrocytes. RT-PCR of DR6 and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) from lysates of A2B5, O4 and MBP positive oligodendrocytes (Fig. 2A). The DR6 RT-PCR was performed without RT enzyme as a negative control (-RT DR6). Western blot showing expression of DR6 in A2B5, O4, and MBP-positive oligodendrocytes (Fig. 2B).

[0062] Figure 3 - DR6 is regulated in Alzheimer's disease. Graph displaying quantitative PCR of DR6 mRNA levels in Alzheimer's disease brains and control brains. Levels are expressed as a ratio of Alzheimer's Disease brain mRNA level / average control brain mRNA level.

[0063] Figure 4 - DR6 is regulated in response to neuronal injury. Graph displaying quantitative PCR of DR6 mRNA levels in control neurons and neurons subjected to axotomy. DR6 mRNA levels are expressed as a ratio of the mRNA level in axotomy sample / mRNA level in control sample.

[0064] Figures 5A-D – Overexpression of DR6 induces neuronal death. Images of cortical neurons infected with lentivirus expressing FL-DR6 and control cortical neurons (Fig. 5A). Graph displaying absorbance measured in an XTT assay for cell viability performed on untreated cells, cells infected with full-length DR6 (DR6-FL) lentivirus, dominant negative DR6 (DR6-DN) lentivirus and GFP lentivirus (Fig. 5B). Graph displaying free rhodamine measured in an assay for caspase-3 activity in untreated cells, DR6-FL, DR6-DN and GFP lentivirus infected cells (Fig. 5C). Western blots of DR6-FL, DR6-DN and GFP lentivirus infected cells probed with anti-activated caspase-3, anti-βIII-tubulin and anti-GFP antibodies (Fig. 5D).

[0065] Figures 6A-B – Overexpression of DR6 induces death of OPCs. Graph depicting percent cytotoxicity on cells infected with DR6-FL, DR6-DN or GFP lentivirus (Fig. 6A). Graph depicting absorbance as measured in an XTT assay on cells treated with rotenone and cells infected with DR6-FL, DR6-DN or GFP lentivirus (Fig. 6B). Graph depicting absorbance measured in an LDH assay for cell viability in cells infected with DR6-FL, DR6-DN or GFP lentivirus (Fig. 6C).

[0066] Figures 7A-B – Blocking DR6 signaling pathway promotes oligodendrocyte survival and differentiation. Western blots of cell lysates from DR6 FL and DR6 DN infected oligodendrocytes probed with anti-MBP, anti-MOG, anti-GFP and anti- β-actin antibodies (Fig.

7A). An ELISA measuring the level of CNPase in DR6 FL and DR6 DN infected oligodendrocytes (Fig. 7B).

[0067] Figures 8A-B – Soluble DR6 blocks full-length DR6 from inducing neuronal cell death. Time lapse images of cultured E18 cerebral cortical neurons that were treated with control human Fc (top panels), infected with FL-DR6 and treated with control human-Fc (middle panels) and infected with FL-DR6 and treated with DR6-Fc (bottom panels) (Fig. 8A). Graph depicting number of surviving cells after treatment with DR6-Fc or control Fc (Fig. 8B).

[0068] Figures 9A-B – Soluble DR6 promotes aged DRG neuron survival and neurite outgrowth in neurons expressing DR6-FL. Graph depicting neurons bearing processes after treatment with DR6-Fc, control Fc, or no treatment (Fig. 9A). Graph depicting neurons bearing large and complex processes after treatment with DR6-Fc, control Fc, or no treatment (Fig. 9B).

[0069] Figures 10A-B – DR6-induced neuronal death is inhibited by DR6-Fc. Western blots of untreated and DR6-FL lentivirus infected cells incubated in media containing of 0, 1, 3, 10 or 30 μg/ml of recombinant soluble DR6 (DR6 Fc) probed with anti-activated caspase-3, anti-GFP and anti-β-actin antibodies (FIG. 10A). Graph depicting level of activated caspase-3 in cultures treated with DR6-Fc (Fig. 10B).

[0070] Figures 11A-D – DR6 RNAi promotes neuron survival. Graphs depicting percent cytotoxicity as measured in an LDH assay on cells treated with DR6 or control siRNAs (Fig. 11A). Graphs depicting percent cytotoxicity as measured in an XTT assay on cells exposed to DR6 or control siRNAs and treated with increasing concentrations of Abeta42 (Fig. 11B), glutamate (Fig. 11C) or TNF alpha (Fig. 11D).

[0071] Figures 12 A-B – DR6 siRNA promotes oligodendrocyte differentiation. RT-PCR of DR6 and GAPDH from lysates of oligodendrocytes treated with control siRNA or DR6 siRNA (Fig. 12A). The DR6 RT-PCR reaction was also performed wihout the RT enzyme as a negative control (DR6-RT). Western blots of DR6 siRNA or control siRNA treated cells probed with anti-MBP, anti-MOG and anti-β-actin antibodies (Fig. 12B).

[0072] Figures 13 A-C – Anti-DR6 antibodies bind to rat, mouse and human DR6. Graphs depict FACS analysis performed to assess the ability of anti-DR6 antibodies (1D10, 2F2 and 5D10) to bind to DR6 produced from 293T cells transfected with rat (Fig. 13A), human (Fig. 13B) or mouse DR6 (Fig. 13C).

[0073] Figures 14 A-C – Blocking DR6 by anti-DR6 antibodies promotes oligodendrocyte differentiation and inhibits apoptosis. Graph depicting percent caspase 3

activity in oligodendrocyte cultures treated with anti-DR6 antibodies. (Fig. 14A). Graph depicting percent MBP^+ cells in oligodendrocyte cultures treated with anti-DR6 antibodies. (Fig. 14B). Western blot of cell cultures treated with anti-DR6 antibody and probed with rabbit anti-cleaved caspase-3 (1:1000, Cell Signaling), mouse anti-MBP antibody (SMI 94 and SMI 99, 1:4000, Convance) and rabbit anti-β-actin antibodies (1:2000, Sigma) (Fig. 14C).

[0074] Figure 15 – Blocking DR6 by anti-DR6 antibodies promotes oligodendrocyte/DRG myelination in co-culture. Western blot of co-cultures of oligodendrocytes and DRG neurons treated with anti-DR6 antibody and probed with mouse anti-MBP antibody (SMI 94 and SMI 99, 1:4000, Convance), mouse anti-MOG antibody (1:500) and rabbit anti-β-actin antibodies (1:2000, Sigma).

[0075] Figure 16A-B – Blocking DR6 by anti-DR6 antibodies promote remyelination in rat brain slice culture. Images of p17 brain slices no treatment and after treatment with bioactive lipid lysophosphatidylcholine (LPC) and anti-DR6 antibody (Fig. 16A). Graph depicting black gold stainging intensity after no treatment and treatment with bioactive lipid lysophosphatidylcholine (LPC) and anti-DR6 antibody (Fig. 16B).

[0076] Figures 17A-B – Anti-DR6 antibodies promote functional recovery in rat EAE model. Graph depicting EAE scores (measured as a functional recovery of the EAE disease) in MOG induced EAE rats treated with anti-DR6 antibody beginning at day 14 of MOG injection and occuring twice a week for 2 weeks. EAE score was measured as functional recovery of the EAE disease. Graphs depicting nerve conduction velocities in rat EAE model treated with control or anti-DR6 antibody.

[0077] Figures 18A-B – Lymphocyte and whole blood cell numbers are not affected by anti-DR6 antibody treatment in EAE rats. Graphs depicting total lymphocyte number (Fig. 18A) and whole blood cell number (Fig. 18B) determined at the end of EAE study (day 32).

[0078] Figure 19 – DR6 antibodies inhibit T-cell infiltration into spinal cord in EAE rats. Graph depicting results of IHC staining of spinal cord tissue using an anti-CD4 antibody to visualize T cell infiltration in the EAE mice. Stainings were performed at the end of the EAE study.

[0079] Figure 20A-C – TNFα promotes neuron death and upregulates DR6. Graph depicting percent apoptotic cells after treatment with TNFα (Fig 20A). Graph depicting % apoptotic cells versus total number of cells ($DAPI^+$) after treatment with TNFα (Fig 20B). Graph

depicting % DR6+ cells versus total number of cells ($DAPI^+$) after treatment with TNFα (Fig 20C).

[0080] Figures 21A-D – TNFα upregulates DR6 and induces neuron death through NFkB signaling. Western blot of cortical neurons treated with TNFα for 18 and 24 hours and probed with anti-DR6, anti-NFκB, anti-IκBα, and anti-β-actin antibodies (Fig. 21A). Graph depicting quantitative amount of DR6 after treatment with TNFα (Fig. 21B). Graph depicting quantitative amount of NFκB after treatment with TNFα (Fig. 21C). Graph depicting quantitative amount of IκBα after treatment with TNFα (Fig. 21D).

[0081] Figure 22A-D – DR6 RNAi decreases NFκB expression in neurons. Graph depicting DR6 mRNA level after no treatment and treatment with DR6 RNAi and control RNAi (Fig. 22A). Western blot of cortical neurons treated with TNFα, and further treated with DR6 RNAi and probed with anti-NFκB, anti-IκBα, and anti-β-actin antibodies (Fig. 22B). Graph depicting quantitative amount of NFκB after no treatment and treatment with DR6 RNAi (Fig. 22C). Graph depicting quantitative amount of IκBα after no treatment and treatment with DR6 RNAi (Fig. 22D).

[0082] Figure 23A-B - Anti-DR6 antibodies promote schwann cell myelination. Western blot showing MBP and beta-actin levels in schwann cell and DRG neuron co-cultures after treatment with anti-DR6 or control antibodies (Fig. 23A). Bar graph depicting quantitation of MBP levels as compared to beta-actin levels in the co-cultures (Fig. 23B).

[0083] Figure 24 - Levels of DR6 and phosphorylated-AKT are inversely related. Western blot showing DR6, p-AKT, AKT and β-actin levels in cultured rat cortical neurons.

[0084] Figure 25 - DR6 and p75 interact. Western blots showing DR6 and p75 protein levels that result from immunopreciptation of DR6 from cells recombinantly expressing combinations of DR6, p75, and TrkA (negative control) proteins (Fig. 25A). Bar graph depicting quantification of binding of p75 to the surface of cells recombinantly expressing DR6 or pV90 (negative control) (Fig. 25C). Western blots showing DR6 and p75 protein levels the result from immunopreciptation of DR6 from human fetal spinal cord isolates (Fig. 25B).

[0085] Figure 26 - DR6 and p75 are co-expressed in mouse brain. Bar graph showing quantification of DR6 and p75 mRNA levels in various regions of the mouse brain.

[0086] Figure 27 - DR6 antibody 5D10 blocks the interaction of DR6 and p75. Western blot showing p75 and DR6 proteins that immunoprecipitate with anti-DR6 antibodies 2A9 and 5D10 (Fig. 27A). Image showing CHO cells transfected with a control vector or a vector

encoding p75 and exposed to either alkaline phosphatase-DR6 alone, or alkaline phosphatase-DR6 in combination with an anti-DR6 antibody (Figure 27B).

[0087] Figure 28 - Antibodies that block the DR6-p75 interaction bind to the Cys3/Cys4 domain of DR6. Bar graph showing level of expression of recombinant DR6 deletion mutants (Fig. 28A). Western blot showing amounts of myc-DR6 fusion proteins that immunoprecipitate using an anti-myc antibody (positive control), anti-DR6 antibody 5D10, and anti-DR6 antibody 2A9 (Fig. 28B). Bar graph showing quantitation of interaction of DR6 deletion proteins with anti-DR6 antibodies 5D10, 2A9, 1D6, 2F2, and A4A (Fig. 28C). MOPC-21 indicates a mouse monoclonal control antibody.

[0088] Figure 29 - The Cys3/Cys4 region of DR6 is important for binding to p75. Western blot showing the amounts of DR6 deletion protein and p75 protein that immunprepitate with DR6.

[0089] Figure 30 - Anti-DR6 antibodies 5D10 and M53E04 bind to human DR6. Graphs depict FACS analysis performed to assess the ability of anti-DR6 antibodies to bind to human and rat DR6. Anti-myc antibody 9E10 staining results show human and rat DR6 protein expression.

[0090] Figure 31 - Blocking DR6 by anti-DR6 antibodies promotes oligodendrocyte/DRG myelination in co-culture. Western blot of co-cultures of oligodendrocytes and DRG neurons treated with anti-DR6 antibodies (M53E04 and 5D10), an anti-LINGO-1 antibody (Li81), and a control antibody and probed with anti-MBP antibody, anti-MOG antibody, and anti-β-actin antibody.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

[0091] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. In case of conflict, the present application including the definitions will control. Unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. All publications, patents and other references mentioned herein are incorporated by reference in their entireties for all purposes as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference.

[0092] Although methods and materials similar or equivalent to those described herein can be used in practice or testing of the methods described herein, suitable methods and materials are described below. The materials, methods and examples are illustrative only and are not intended to be limiting. Other features and advantages of the antibodies and methods described herein will be apparent from the detailed description and from the claims.

[0093] In order to further define this invention, the following terms and definitions are provided.

[0094] It is to be noted that the term "a" or "an" entity, refers to one or more of that entity; for example, "an immunoglobulin molecule," is understood to represent one or more immunoglobulin molecules. As such, the terms "a" (or "an"), "one or more," and "at least one" can be used interchangeably herein.

[0095] Throughout this specification and claims, the word "comprise," or variations such as "comprises" or "comprising," indicate the inclusion of any recited integer or group of integers but not the exclusion of any other integer or group of integers.

[0096] As used herein, the term "consists of," or variations such as "consist of" or "consisting of," as used throughout the specification and claims, indicate the inclusion of any recited integer or group of integers, but that no additional integer or group of integers can be added to the specified method, structure or composition.

[0097] As used herein, the term "consists essentially of," or variations such as "consist essentially of" or "consisting essentially of," as used throughout the specification and claims, indicate the inclusion of any recited integer or group of integers, and the optional inclusion of any recited integer or group of integers that do not materially change the basic or novel properties of the specified method, structure or composition.

[0098] As used herein, a "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve a desired therapeutic result. A therapeutic result can be, *e.g.*, lessening of symptoms, prolonged survival, improved mobility, or the like. A "therapeutically effective amount" can achieve any one of the desired therapeutic results or any combination of multiple desired therapeutic results A therapeutic result need not be a "cure".

[0099] As used herein, a "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result.

Typically, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount will be less than the therapeutically effective amount.

[00100] As used herein, a "polynucleotide" can contain the nucleotide sequence of the full length cDNA sequence, including the untranslated 5' and 3' sequences, the coding sequences, as well as fragments, epitopes, domains, and variants of the nucleic acid sequence. The polynucleotide can be composed of any polyribonucleotide or polydeoxyribonucleotide, which can be unmodified RNA or DNA or modified RNA or DNA. For example, polynucleotides can be composed of single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that can be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, the polynucleotides can be composed of triple-stranded regions comprising RNA or DNA or both RNA and DNA. polynucleotides can also contain one or more modified bases or DNA or RNA backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications can be made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically, or metabolically modified forms.

[00101] A polypeptide can be composed of amino acids joined to each other by peptide bonds or modified peptide bonds, *i.e.*, peptide isosteres, and can contain amino acids other than the 20 gene-encoded amino acids (*e.g.* non-naturally occurring amino acids). The polypeptides described herein can be modified by either natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in the polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification can be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide can contain many types of modifications. Polypeptides can be branched, for example, as a result of ubiquitination, and they can be cyclic, with or without branching. Cyclic, branched, and branched cyclic polypeptides can result from posttranslation natural processes or can be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or

nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, pegylation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. (*See*, for instance, Proteins - Structure And Molecular Properties, 2nd Ed., T.E. Creighton, W.H. Freeman and Company, New York (1993); Posttranslational Covalent Modification of Proteins, B.C. Johnson, Ed., Academic Press, New York, pgs. 1-12 (1983); Seifter *et al., Meth Enzymol 182*:626-646 (1990); Rattan *et al., Ann NY Acad Sci 663*:48-62 (1992).)

[0100] The terms "fragment," "variant," "derivative" and "analog" when referring to a Death Receptor-6 (DR6) antagonist include any antagonist molecules which promote nervous system cell survival. Soluble DR6 polypeptides can include DR6 proteolytic fragments, deletion fragments and in particular, fragments which more easily reach the site of action when delivered to an animal. Polypeptide fragments further include any portion of the polypeptide which comprises an antigenic or immunogenic epitope of the native polypeptide, including linear as well as three-dimensional epitopes. Soluble DR6 polypeptides can comprise variant DR6 regions, including fragments as described above, and also polypeptides with altered amino acid sequences due to amino acid substitutions, deletions, or insertions. Variants can occur naturally, such as an allelic variant. By an "allelic variant" is intended alternate forms of a gene occupying a given locus on a chromosome of an organism. Genes II, Lewin, B., ed., John Wiley & Sons, New York (1985). Non-naturally occurring variants can be produced using art-known mutagenesis techniques. Soluble DR6 polypeptides can comprise conservative or non-conservative amino acid substitutions, deletions or additions. DR6 antagonists can also include derivative molecules. For example, soluble DR6 polypeptides can include DR6 regions which have been altered so as to exhibit additional features not found on the native polypeptide. Examples include fusion proteins and protein conjugates.

[0101] A "polypeptide fragment" refers to a short amino acid sequence of a DR6 polypeptide. Protein fragments can be "free-standing," or comprised within a larger polypeptide of which the fragment forms a part of region. Representative examples of polypeptide fragments, include, for example, fragments comprising about 5 amino acids, about 10 amino acids, about 15

amino acids, about 20 amino acids, about 30 amino acids, about 40 amino acids, about 50 amino acids, about 60 amino acids, about 70 amino acids, about 80 amino acids, about 90 amino acids, and about 100 amino acids in length.

[0102] As used herein, the term "antigen binding molecule" ("ABM") refers in its broadest sense to a molecule that specifically binds an antigenic determinant. It is understood by those of skill in the art that fragments of mature antibodies can bind specifically to an antigen. Accordingly, an antigen binding molecule, as the term is used herein, includes, but is not limited to, fragments of mature antibodies that bind specifically to a target antigen. An ABM need not contain a constant region. If one or more constant region(s) is present, in particular embodiments, the constant region is substantially identical to human immunoglobulin constant regions, *e.g.*, at least about 85-90%, or about 95% or more identical. The ABMs can be glycoengineered to enhance antibody dependent cellular cytotoxicity.

[0103] *Antibody or Immunoglobulin.* In one embodiment, the DR6 antagonists for use in the treatment methods disclosed herein are "antibody" or "immunoglobulin" molecules, or immunospecific fragments thereof, *e.g.*, naturally occurring antibody or immunoglobulin molecules or engineered antibody molecules or fragments that bind antigen in a manner similar to antibody molecules. The terms "antibody" and "immunoglobulin" are used interchangeably herein. As used herein, the term antibody or immunoglobulin is intended to include whole antibody molecules, including monoclonal, polyclonal and multispecific (*e.g.*, bispecific) antibodies as well as antibody fragments having the Fc region and retaining binding specificity, and fusion proteins that include a region equivalent to the Fc region of an immunoglobulin and that retain binding specificity. Also encompassed are antibody fragments that retain binding specificity including, but not limited to, VH fragments, VL fragments, Fab fragments, $F(ab')_2$ fragments, scFv fragments, Fv fragments, minibodies, diabodies, triabodies, and tetrabodies (*see, e.g.*, Hudson and Souriau, *Nature Med. 9*: 129-134 (2003)). Also encompassed are humanized, primatized and chimeric antibodies.

[0104] As will be discussed in more detail below, the term "immunoglobulin" comprises five broad classes of polypeptides that can be distinguished biochemically. Although all five classes are clearly useful in the methods described, the following discussion will generally be directed to the IgG class of immunoglobulin molecules. With regard to IgG, a standard immunoglobulin molecule comprises two identical light chain polypeptides of molecular weight approximately 23,000 Daltons, and two identical heavy chain polypeptides of molecular weight

53,000-70,000. The four chains are typically joined by disulfide bonds in a "Y" configuration wherein the light chains bracket the heavy chains starting at the mouth of the "Y" and continuing through the variable region.

[0105] Both the light and heavy chains are divided into regions of structural and functional homology. The terms "constant" and "variable" are used functionally. In this regard, it will be appreciated that the variable domains of both the light (V_L) and heavy (V_H) chain portions determine antigen recognition and specificity. Conversely, the constant domains of the light chain (C_L) and the heavy chain (C_H1, C_H2 or C_H3) confer important biological properties such as secretion, transplacental mobility, Fc receptor binding, complement binding, and the like. By convention the numbering of the constant region domains increases as they become more distal from the antigen binding site or amino-terminus of the antibody. The N-terminal portion is a variable region and at the C-terminal portion is a constant region; the C_H3 and C_L domains actually comprise the carboxy-terminus of the heavy and light chain, respectively.

[0106] Light chains are classified as either kappa or lambda (κ, λ). Each heavy chain class can be bound with either a kappa or lambda light chain. In general, the light and heavy chains are covalently bonded to each other, and the "tail" portions of the two heavy chains are bonded to each other by covalent disulfide linkages or non-covalent linkages when the immunoglobulins are generated either by hybridomas, B cells or genetically engineered host cells. In the heavy chain, the amino acid sequences run from an N-terminus at the forked ends of the Y configuration to the C-terminus at the bottom of each chain. Those skilled in the art will appreciate that heavy chains are classified as gamma, mu, alpha, delta, or epsilon, (γ, μ, α, δ, ε)) with some subclasses among them (*e.g.*, $\gamma1$-$\gamma4$). It is the nature of this chain that determines the "class" of the antibody as IgG, IgM, IgA IgG, or IgE, respectively. The immunoglobulin subclasses (isotypes) *e.g.*, IgG_1, IgG_2, IgG_3, IgG_4, IgA_1, etc. are well characterized and are known to confer functional specialization. Modified versions of each of these classes and isotypes are readily discernable to the skilled artisan in view of the instant disclosure and, accordingly, are within the scope of the instant invention.

[0107] As indicated above, the variable region allows the antibody to selectively recognize and specifically bind epitopes on antigens. That is, the V_L domain and V_H domain of an antibody combine to form the variable region that defines a three dimensional antigen binding site. This quaternary antibody structure forms the antigen binding site present at the end of each arm of the Y. More specifically, the antigen binding site is defined by three complementary

determining regions (CDRs) on each of the V_H and V_L chains. In some instances, *e.g.*, certain immunoglobulin molecules derived from camelid species or engineered based on camelid immunoglobulins, a complete immunoglobulin molecule can consist of heavy chains only, with no light chains. *See, e.g.*, Hamers Casterman *et al., Nature 363*:446 448 (1993).

[0108] In naturally occurring antibodies, the six "complementarity determining regions" or "CDRs" present in each antigen binding domain are short, non-contiguous sequences of amino acids that are specifically positioned to form the antigen binding domain as the antibody assumes its three dimensional configuration in an aqueous environment. The remainder of the amino acids in the antigen binding domains, referred to as "framework" regions, show less inter-molecular variability. The framework regions largely adopt a β-sheet conformation and the CDRs form loops which connect, and in some cases form part of, the β-sheet structure. Thus, framework regions act to form a scaffold that provides for positioning the CDRs in correct orientation by inter-chain, non-covalent interactions. The antigen binding domain formed by the positioned CDRs defines a surface complementary to the epitope on the immunoreactive antigen. This complementary surface promotes the non-covalent binding of the antibody to its cognate epitope. The amino acids comprising the CDRs and the framework regions, respectively, can be readily identified for any given heavy or light chain variable region by one of ordinary skill in the art, since they have been precisely defined (*see*, "Sequences of Proteins of Immunological Interest," Kabat, E., *et al.*, U.S. Department of Health and Human Services, (1983); and Chothia and Lesk, *J. Mol. Biol., 196*:901-917 (1987), which are incorporated herein by reference in their entireties).

[0109] In the case where there are two or more definitions of a term which is used and/or accepted within the art, the definition of the term as used herein is intended to include all such meanings unless explicitly stated to the contrary. A specific example is the use of the term "complementarity determining region" ("CDR") to describe the non-contiguous antigen combining sites found within the variable region of both heavy and light chain polypeptides. This particular region has been described by Kabat *et al.*, U.S. Dept. of Health and Human Services, "Sequences of Proteins of Immunological Interest" (1983) and by Chothia *et al.*, *J. Mol. Biol.* 196:901-917 (1987), which are incorporated herein by reference, where the definitions include overlapping or subsets of amino acid residues when compared against each other. Nevertheless, application of either definition to refer to a CDR of an antibody or variants thereof is intended to be within the scope of the term as defined and used herein. The appropriate amino acid residues

which encompass the CDRs as defined by each of the above cited references are set forth below in Table 1 as a comparison. The exact residue numbers which encompass a particular CDR will vary depending on the sequence and size of the CDR. Those skilled in the art can routinely determine which residues comprise a particular CDR given the variable region amino acid sequence of the antibody.

TABLE 1. CDR DEFINITIONS[1]

	Kabat	Chothia
V_H CDR1	31-35	26-32
V_H CDR2	50-65	52-58
V_H CDR3	95-102	95-102
V_L CDR1	24-34	26-32
V_L CDR2	50-56	50-52
V_L CDR3	89-97	91-96

[1]Numbering of all CDR definitions in Table 1 is according to the numbering conventions set forth by Kabat *et al.* (see below).

[0110] Kabat *et al.* also defined a numbering system for variable domain sequences that is applicable to any antibody. One of ordinary skill in the art can unambiguously assign this system of "Kabat numbering" to any variable domain sequence, without reliance on any experimental data beyond the sequence itself. As used herein, "Kabat numbering" refers to the numbering system set forth by Kabat *et al.*, U.S. Dept. of Health and Human Services, "Sequence of Proteins of Immunological Interest" (1983). Unless otherwise specified, references to the numbering of specific amino acid residue positions in a C35 antibody or antigen-binding fragment, variant, or derivative thereof of are according to the Kabat numbering system.

[0111] In camelid species, however, the heavy chain variable region, referred to as V_HH, forms the entire CDR. The main differences between camelid V_HH variable regions and those derived from conventional antibodies (V_H) include (a) more hydrophobic amino acids in the light chain contact surface of V_H as compared to the corresponding region in V_HH, (b) a longer CDR3 in V_HH, and (c) the frequent occurrence of a disulfide bond between CDR1 and CDR3 in V_HH.

[0112] In one embodiment, an antigen binding molecule comprises at least one heavy or light chain CDR of an antibody molecule. In another embodiment, an antigen binding molecule comprises at least two CDRs from one or more antibody molecules. In another embodiment, an antigen binding molecule comprises at least three CDRs from one or more antibody molecules. In another embodiment, an antigen binding molecule comprises at least four CDRs from one or

more antibody molecules. In another embodiment, an antigen binding molecule comprises at least five CDRs from one or more antibody molecules. In another embodiment, an antigen binding molecule comprises at least six CDRs from one or more antibody molecules. Exemplary antibody molecules comprising at least one CDR that can be included in the subject antigen binding molecules are known in the art and exemplary molecules are described herein.

[0113] Antibodies or immunospecific fragments thereof for use in the methods described herein include, but are not limited to, polyclonal, monoclonal, multispecific, human, humanized, primatized, or chimeric antibodies, single chain antibodies, epitope-binding fragments, *e.g.*, Fab, Fab' and F(ab')2, Fd, Fvs, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv), fragments comprising either a V_L or V_H domain, fragments produced by a Fab expression library, and anti-idiotypic (anti-Id) antibodies (including, *e.g.*, anti-Id antibodies to binding molecules disclosed herein). ScFv molecules are known in the art and are described, *e.g.*, in US patent 5,892,019. Immunoglobulin or antibody molecules can be of any type (*e.g.*, IgG, IgE, IgM, IgD, IgA, and IgY), class (*e.g.*, IgG_1, IgG_2, IgG_3, IgG_4, IgA_1 and IgA_2) or subclass of immunoglobulin molecule.

[0114] Antibody fragments, including single-chain antibodies, can comprise the variable region(s) alone or in combination with the entirety or a portion of the following: hinge region, C_H1, C_H2, and C_H3 domains. Antigen-binding fragments can also comprise any combination of variable region(s) with a hinge region, C_H1, C_H2, and C_H3 domains. Antibodies or immunospecific fragments thereof for use in the diagnostic and therapeutic methods disclosed herein can be from any animal origin including birds and mammals. In certain embodiments, the antibodies are human, murine, donkey, rabbit, goat, guinea pig, camel, llama, horse, or chicken antibodies. In another embodiment, the variable region can be condricthoid in origin (*e.g.*, from sharks). As used herein, "human" antibodies include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from human immunoglobulin libraries or from animals transgenic for one or more human immunoglobulins and that do not express endogenous immunoglobulins, as described infra and, for example in, U.S. Pat. No. 5,939,598 by Kucherlapati *et al.*

[0115] As used herein, the term "heavy chain portion" includes amino acid sequences derived from an immunoglobulin heavy chain. A polypeptide comprising a heavy chain portion comprises at least one of: a C_H1 domain, a hinge (*e.g.*, upper, middle, and/or lower hinge region) domain, a C_H2 domain, a C_H3 domain, or a variant or fragment thereof. For example, a binding

polypeptide can comprise a polypeptide chain comprising a C_H1 domain; a polypeptide chain comprising a C_H1 domain, at least a portion of a hinge domain, and a C_H2 domain; a polypeptide chain comprising a C_H1 domain and a C_H3 domain; a polypeptide chain comprising a C_H1 domain, at least a portion of a hinge domain, and a C_H3 domain, or a polypeptide chain comprising a C_H1 domain, at least a portion of a hinge domain, a C_H2 domain, and a C_H3 domain. In another embodiment, a polypeptide comprises a polypeptide chain comprising a C_H3 domain. Further, a binding polypeptide can lack at least a portion of a C_H2 domain (*e.g.*, all or part of a C_H2 domain). As set forth above, it will be understood by one of ordinary skill in the art that these domains (*e.g.*, the heavy chain portions) can be modified such that they vary in amino acid sequence from the naturally occurring immunoglobulin molecule.

[0116] In certain embodiments, DR6 antagonist antibodies or immunospecific fragments thereof for use in the treatment methods disclosed herein, the heavy chain portions of one polypeptide chain of a multimer are identical to those on a second polypeptide chain of the multimer. Alternatively, heavy chain portion-containing monomers for use in the methods described herein are not identical. For example, each monomer can comprise a different target binding site, forming, for example, a bispecific antibody.

[0117] The heavy chain portions of a binding polypeptide for use in the diagnostic and treatment methods disclosed herein can be derived from different immunoglobulin molecules. For example, a heavy chain portion of a polypeptide can comprise a C_H1 domain derived from an IgG_1 molecule and a hinge region derived from an IgG_3 molecule. In another example, a heavy chain portion can comprise a hinge region derived, in part, from an IgG_1 molecule and, in part, from an IgG_3 molecule. In another example, a heavy chain portion can comprise a chimeric hinge derived, in part, from an IgG_1 molecule and, in part, from an IgG_4 molecule.

[0118] As used herein, the term "light chain portion" includes amino acid sequences derived from an immunoglobulin light chain. Typically, the light chain portion comprises at least one of a V_L or C_L domain.

[0119] An isolated nucleic acid molecule encoding a non-natural variant of a polypeptide derived from an immunoglobulin (*e.g.*, an immunoglobulin heavy chain portion or light chain portion) can be created by introducing one or more nucleotide substitutions, additions or deletions into the nucleotide sequence of the immunoglobulin such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein. Mutations can be introduced by standard techniques, such as site-directed mutagenesis and PCR-mediated

mutagenesis. For example, conservative amino acid substitutions are made at one or more non-essential amino acid residues.

[0120] Antibodies or immunospecific fragments thereof for use in the treatment methods disclosed herein can also be described or specified in terms of their binding affinity to a polypeptide. For example, binding affinities include those with a dissociation constant or Kd less than 5×10^{-2} M, 10^{-2} M, 5×10^{-3} M, 10^{-3} M, 5×10^{-4} M, 10^{-4} M, 5×10^{-5} M, 10^{-5} M, 5×10^{-6} M, 10^{-6} M, 5×10^{-7} M, 10^{-7} M, 5×10^{-8} M, 10^{-8} M, 5×10^{-9} M, 10^{-9} M, 5×10^{-10} M, 10^{-10} M, 5×10^{-11} M, 10^{-11} M, 5×10^{-12} M, 10^{-12} M, 5×10^{-13} M, 10^{-13} M, 5×10^{-14} M, 10^{-14} M, 5×10^{-15} M, or 10^{-15} M.

[0121] Antibodies or immunospecific fragments thereof for use in the treatment methods disclosed herein act as antagonists of DR6 as described herein. For example, an antibody for use in the methods described herein can function as an antagonist, blocking or inhibiting the suppressive activity of the DR6 polypeptide.

[0122] As used herein, the term "chimeric antibody" will be held to mean any antibody wherein the immunoreactive region or site is obtained or derived from a first species and the constant region (which can be intact, partial or modified) is obtained from a second species. In certain embodiments the target binding region or site will be from a non-human source (*e.g.* mouse or primate) and the constant region is human.

[0123] As used herein, the term "engineered antibody" refers to an antibody in which the variable domain in either the heavy and light chain or both is altered by at least partial replacement of one or more CDRs from an antibody of known specificity and, if necessary, by partial framework region replacement and sequence changing. Although the CDRs can be derived from an antibody of the same class or even subclass as the antibody from which the framework regions are derived, it is envisaged that the CDRs will be derived from an antibody of different class and/or an antibody from a different species. An engineered antibody in which one or more "donor" CDRs from a non-human antibody of known specificity is grafted into a human heavy or light chain framework region is referred to herein as a "humanized antibody." In some cases it is not necessary to replace all of the CDRs with the complete CDRs from the donor variable region to transfer the antigen binding capacity of one variable domain to another. Rather, in some cases, it is only be necessary to transfer those residues that are necessary to maintain the activity of the target binding site. Given the explanations set forth in, *e.g.*, U. S. Pat. Nos. 5,585,089, 5,693,761, 5,693,762, and 6,180,370, it will be well within the competence of

those skilled in the art, either by carrying out routine experimentation or by trial and error testing to obtain a functional engineered or humanized antibody.

[0124] As used herein, the term humanized is used to refer to an antigen–binding molecule derived from a non-human antigen-binding molecule, for example, a murine antibody, that retains or substantially retains the antigen-binding properties of the parent molecule but which is less immunogenic in humans. This can be achieved by various methods including (a) grafting the entire non-human variable domains onto human constant regions to generate chimeric antibodies, (b) grafting only the non-human CDRs onto human framework and constant regions with or without retention of critical framework residues (*e.g.*, those that are important for retaining good antigen binding affinity or antibody functions), or (c) transplanting the entire non-human variable domains, but "cloaking" them with a human-like section by replacement of surface residues. Such methods are disclosed in Jones *et al.*, Morrison *et al., Proc. Natl. Acad. Sci., 81*:6851-6855 (1984); Morrison and Oi, *Adv. Immunol., 44*:65-92 (1988); Verhoeyen et al., *Science, 239*:1534-1536 (1988); Padlan, *Molec. Immun., 28*:489-498 (1991); Padlan, *Molec. Immun., 31*(3):169-217 (1994), all of which are incorporated by reference in their entirety herein. There are generally 3 complementarity determining regions, or CDRs, (CDR1, CDR2 and CDR3) in each of the heavy and light chain variable domains of an antibody, which are flanked by four framework subregions (*i.e.*, FR1, FR2, FR3, and FR4) in each of the heavy and light chain variable domains of an antibody: FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4. A discussion of humanized antibodies can be found, inter alia, in U.S. Patent No. 6,632,927, and in published U.S. Application No. 2003/0175269, both of which are incorporated herein by reference in their entirety.

[0125] As used herein, the terms "linked," "fused" or "fusion" are used interchangeably. These terms refer to the joining together of two more elements or components, by whatever means including chemical conjugation or recombinant means. An "in-frame fusion" refers to the joining of two or more open reading frames (ORFs) to form a continuous longer ORF, in a manner that maintains the correct reading frame of the original ORFs. Thus, the resulting recombinant fusion protein is a single protein containing two ore more segments that correspond to polypeptides encoded by the original ORFs (which segments are not normally so joined in nature.) Although the reading frame is thus made continuous throughout the fused segments, the segments can be physically or spatially separated by, for example, in-frame linker sequence.

[0126] In the context of polypeptides, a "linear sequence" or a "sequence" is an order of amino acids in a polypeptide in an amino to carboxyl terminal direction in which residues that neighbor each other in the sequence are contiguous in the primary structure of the polypeptide.

[0127] The term “expression” as used herein refers to a process by which a gene produces a biochemical, for example, an RNA or polypeptide. The process includes any manifestation of the functional presence of the gene within the cell including, without limitation, gene knockdown as well as both transient expression and stable expression. It includes without limitation transcription of the gene into messenger RNA (mRNA), transfer RNA (tRNA), small hairpin RNA (shRNA), small interfering RNA (siRNA) or any other RNA product and the translation of such mRNA into polypeptide(s). If the final desired product is biochemical, expression includes the creation of that biochemical and any precursors.

[0128] By "subject" or "individual" or "animal" or "patient" or “mammal,” is meant any subject, particularly a mammalian subject, for whom diagnosis, prognosis, or therapy is desired. Mammalian subjects include, but are not limited to, humans, domestic animals, farm animals, zoo animals, sport animals, pet animals such as dogs, cats, guinea pigs, rabbits, rats, mice, horses, cattle, cows; primates such as apes, monkeys, orangutans, and chimpanzees; canids such as dogs and wolves; felids such as cats, lions, and tigers; equids such as horses, donkeys, and zebras; food animals such as cows, pigs, and sheep; ungulates such as deer and giraffes; bears; and so on. In certain embodiments, the mammal is a human subject.

[0129] The term "RNA interference" or "RNAi" refers to the silencing or decreasing of gene expression by siRNAs. It is the process of sequence-specific, post-transcriptional gene silencing in animals and plants, initiated by siRNA that is homologous in its duplex region to the sequence of the silenced gene. The gene can be endogenous or exogenous to the organism, present integrated into a chromosome or present in a transfection vector that is not integrated into the genome. The expression of the gene is either completely or partially inhibited. RNAi can also be considered to inhibit the function of a target RNA; the function of the target RNA can be complete or partial.

Death Receptor-6 (DR6/ TNFRSF21)

[0130] It has been discovered that DR6 is expressed in cells of the nervous system including neurons and oligodendrocyte precursor cells and that DR6 can induce cell death in these cells.

[0131] DR6 is a polypeptide consisting of 655 amino acids. In certain embodiments, the human polypeptide is encoded by an mRNA comprising the nucleotides of SEQ ID NO:1 (Accession Number: NM_ 014452). In certain embodiments, the human DR6 polypeptide sequence comprises the amino acids of SEQ ID NO:2 (Accession Number: O75509). Mouse DR6 is also a 655 amino acid polypeptide. In certain embodiments, mouse DR6 is encoded by an mRNA comprising the nucleotides of SEQ ID NO:3 (Accession Number: NM_178589). In certain embodiments, the mouse DR6 polypeptide sequence comprises the amino acid sequence of SEQ ID NO:4 (Accession Number: NP_848704).

[0132] Table 2 lists DR6 domains and other regions according to the amino acid residue number based on the sequence of SEQ ID NO:2. As one of skill in the art will appreciate, the beginning and ending residues of the domains listed below can vary depending upon the computer modeling program used, the method used for determining the domain, minor sequence variations etc.

Table 2

Domain or Region	Beginning Residue	Ending Residue
Signal Sequence	1	40 or 41
Extracellular Domain	41 or 42	349 or 350
TNFR-like Cysteine-Rich Motif-1	50	88
TNFR-like Cysteine-Rich Motif-2	90	131
TNFR-like Cysteine-Rich Motif-3	133	167
TNFR-like Cysteine-Rich Motif-4	170	211
Transmembrane	350 or 351	367-370
Cytoplasmic	368-371	655
Death Domain	415	498
Leucine Zipper Motif	497	526

P75/TNR16

[0133] It has also been discovered that p75 neurotrophin receptor is a ligand for DR6. P75, also known as tumor necrosis factor receptor superfamily member 16 (TNR16 or TNFRSF16) or nerve growth factor receptor (NGFR), is a polypeptide consisting of 427 amino acids. The human polypeptide sequence is Accession Number NP_002498 (SEQ ID NO: 165) and the nucleic acid sequence is Accession Number NM_002507 (SEQ ID NO: 166). The p75

protein, like the DR6 protein, includes an extracellular region containing four TNFR Cysteine-Rich motifs, a transmembrane region, and an intracellular region containing a death domain. It has previously been shown that p75 is a low affinity receptor which can bind to NGF, BDNF, NT-3, and NT-4. Mi *et al. Nat. Neuroscience* 7:221-228 (2004). In addition, p75 is a component of the LINGO-1/Nogo-66 receptor signaling pathway and can mediate survival and death of neuronal cells. *Id.*

Methods of Using Antagonists of DR6 and p75

[0134] In one embodiment, the method is a method for promoting survival of cells of the nervous system comprising contacting said cells with a DR6 antagonist. Another embodiment provides methods for promoting oligodendrocyte proliferation, differentiation or survival comprising contacting oligodendrocyte cells or oligodendrocyte precursor cells with a DR6 antagonist. Another embodiment provides methods for promoting myelination comprising contacting a mixture of neuronal cells and oligodendrocytes or oligodendrocyte precursor cells with a DR6 antagonist. Yet another embodiment provides methods of inhibiting the binding of DR6 and p75 comprising contacing a DR6 polypeptide and/or a p75 polypeptide with a DR6 antagonist under conditions wherein binding of DR6 to p75 is inhibited. Similarly, the methods described herein also include methods of inhibiting the binding of DR6 to p75 comprising contacting a DR6 polypeptide and/or a p75 polypeptide with a p75 antagonist.

[0135] A DR6 antagonist can be a DR6 antagonist polypeptide, a DR6 antagonist compound, a DR6 antibody, a DR6 antagonist polynucleotide, a DR6 aptamer or a combination of two or more DR6 antagonists. Additional embodiments include methods for treating a condition associated with death of cells of the nervous system comprising administering a therapeutically effective amount of a DR6 antagonist.

[0136] A p75 antagonist can be a p75 antagonist polypeptide, a p75 antagonist compound, a p75 antibody, a p75 antagonist polynucleotide, a p75 aptamer, or a combination of two or more p75 antagonists. Additional embodiments include methods for treating a condition associated with death of cells of nervous system comprising administering a therapeutically effective amount of a DR6 antagonist in combination with a p75 antagonist.

[0137] In some particular embodiments the condition associated with death of nervous system cells can be Alzheimer's disease, Parkinson's disease, Huntington's disease, motor neuron disease (*e.g.* amyotrophic lateral sclerosis, which is also called ALS or Lou Gehrig's disease), multiple sclerosis, neuronal trauma or cerebral ischemia (*e.g.* stroke). Another embodiment

provides methods for treating a disease of neuronal degeneration comprising administering a therapeutically effective amount of a DR6 antagonist.

[0138] Cells of the nervous system include both cells of the central nervous system (or "CNS cells" and cells of the peripheral nervous system (or "PNS cells). CNS cells include cells in the brain and cells in the spinal cord, as well as cell lines derived from such cells. PNS cells include, for example, dorsal root ganglion neurons, schwann cells, motor neurons and cell lines derived from such cells. Cells of the nervous system include neurons such as cortical neurons, motor neurons, dorsal root ganglion (DRG) neurons and cell lines derived from such cells. Cells of the nervous also include neuronal support cells or glial cells including microglia and macroglia as well as cell lines derived from such cells. Examples of macroglial cells include astrocytes, oligodendrocytes, ependymocytes and radial glial cells. Cells of the nervous system also include precursors of these cells such as oligodendrocyte precursor cells and cell lines derived from such cells.

DR6 Antagonist Polypeptides

[0139] DR6 antagonists to be used herein include those polypeptides which block, inhibit or interfere with the biological function of naturally occurring DR6. Specifically, soluble DR6 polypeptides include fragments, variants, or derivative thereof of a soluble DR6 polypeptide. Table 1 above describes the various domains of a human DR6 polypeptide. Similar domain structures can be deduced for DR6 polypeptides of other species, *e.g.*, mouse or rat DR6. Soluble DR6 polypeptides typically lack the transmembrane domain of the DR6 polypeptide, and optionally lack the cytoplasmic domain of the DR6 polypeptide. For example, certain soluble human DR6 polypeptides lack amino acids 351-367 of SEQ ID NO:2, which comprises the transmembrane domain of human DR6. Another soluble human DR6 polypeptide lacks both the transmembrane domain and the intracellular domain (amino acids 350-655 of SEQ ID NO:2). Additionally, certain soluble DR6 polypeptides comprise one or more of the TNFR-like cysteine rich motifs and/or the entire extracellular domain (corresponding to amino acids 40 to 349 of SEQ ID NO:2, 40 to 350 of SEQ ID NO:2, 41 to 349 of SEQ ID NO:2 or 41 to 350 of SEQ ID NO:2) of the DR6 polypeptide. As one of skill in the art would appreciate, the entire extracellular domain of DR6 can comprise additional or fewer amino acids on either the C-terminal or N-terminal end of the extracellular domain polypeptide. The soluble antagonist DR6 polypeptide can or can not include the signal sequence.

[0140] Additional soluble DR6 polypeptides for use in the methods described herein include, but are not limited to, a soluble DR6 polypeptide comprising, consisting essentially of, or consisting of amino acids 1 to 40 of SEQ ID NO:2; 1 to 41 of SEQ ID NO:2; 65 to 105 of SEQ ID NO:2; 106 to 145 of SEQ ID NO:2; 146 to 185 of SEQ ID NO:2; and 186 to 212 of SEQ ID NO:2; or fragments, variants, or derivatives of such polypeptides.

[0141] Further soluble DR6 polypeptides for use in the methods described herein include, but are not limited to, a soluble DR6 polypeptide comprising, consisting essentially of, or consisting of amino acids 1 to 40 of SEQ ID NO:2; 1 to 41 of SEQ ID NO:2; 1 to 64 of SEQ ID NO:2; 1 to 105 of SEQ ID NO:2; 1 to 145 of SEQ ID NO:2; 1 to 185 of SEQ ID NO:2; 1 to 212 of SEQ ID NO:2; 1 to 349 of SEQ ID NO:2; or fragments, variants, or derivatives of such polypeptides.

[0142] Still further soluble DR6 polypeptides for use in the methods described herein include, but are not limited to, a DR6 polypeptide comprising, consisting essentially of, or consisting of amino acids 41 to 64 of SEQ ID NO:2; 41 to 105 of SEQ ID NO:2; 41 to 145 of SEQ ID NO:2; 41 to 185 of SEQ ID NO:2; 41 to 212 of SEQ ID NO:2; 41 to 349 of SEQ ID NO:2; 41 to 350 of SEQ ID NO:2; 42 to 64 of SEQ ID NO:2; 42 to 105 of SEQ ID NO:2; 42 to 145 of SEQ ID NO:2; 42 to 185 of SEQ ID NO:2; 42 to 212 of SEQ ID NO:2; 42 to 349 of SEQ ID NO:2; and 42 to 350 of SEQ ID NO:2; or fragments, variants, or derivatives of such polypeptides.

[0143] Additional soluble DR6 polypeptide for us in the methods described herein include, but are not limited to, a soluble DR6 polypeptide comprising, consisting essentially of, or consisting of amino acids 65 to 105 of SEQ ID NO:2; 65 to 212 of SEQ ID NO:2; 65 to 349 of SEQ ID NO:2; 106 to 145 of SEQ ID NO:2; 106 to 212 of SEQ ID NO:2; 106 to 349 of SEQ ID NO:2; 146 to 185 of SEQ ID NO:2; 146 to 212 of SEQ ID NO:2; 146 to 349 of SEQ ID NO:2; 186 to 212 of SEQ ID NO:2; 186 to 349 of SEQ ID NO:2; and 213 to 349 of SEQ ID NO:2; or fragments, variants, or derivatives of such polypeptides.

[0144] A variant DR6 polypeptide can also vary in sequence from the corresponding wild-type polypeptide. In particular, certain amino acid substitutions can be introduced into the DR6 sequence without appreciable loss of a DR6 biological activity. In exemplary embodiments, a variant DR6 polypeptide contains one or more amino acid substitutions, and/or comprises an amino acid sequence which is at least 70%, 80%, 85%, 90%, 95%, 98% or 99% identical to a reference amino acid sequence selected from the group consisting of: amino acids

41 to 349 of SEQ ID NO:2 or equivalent fragments of SEQ ID NO:4. A variant DR6 polypeptide differing in sequence from any given fragment of SEQ ID NO:2 or SEQ ID NO:4 can include one or more amino acid substitutions (conservative or non-conservative), one or more deletions, and/or one or more insertions. In certain embodiments, the soluble DR6 polypeptide promotes survival of cells of the neuronal system such as neurons and OPCs, *e.g.*, in a mammal.

[0145] A soluble DR6 polypeptide can comprise a fragment of at least six, *e.g.*, ten, fifteen, twenty, twenty-five, thirty, forty, fifty, sixty, seventy, one hundred, or more amino acids of SEQ ID NO:2 or SEQ ID NO:4. In addition, a soluble or dominant negative DR6 polypeptide can comprise at least one, *e.g.*, five, ten, fifteen or twenty conservative amino acid substitutions. Corresponding fragments of soluble DR6 polypeptides at least 70%, 75%, 80%, 85%, 90%, or 95% identical to a reference DR6 polypeptide of SEQ ID NO:2 or SEQ ID NO:4 are also contemplated.

[0146] By "a DR6 reference amino acid sequence," or "reference amino acid sequence" is meant the specified sequence without the introduction of any amino acid substitutions. As one of ordinary skill in the art would understand, if there are no substitutions, the "isolated polypeptide" comprises an amino acid sequence which is identical to the reference amino acid sequence.

[0147] Conservative substitutions include substitutions within the following groups: valine, alanine and glycine; leucine, valine, and isoleucine; aspartic acid and glutamic acid; asparagine and glutamine; serine, cysteine, and threonine; lysine and arginine; and phenylalanine and tyrosine. The non-polar hydrophobic amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine and glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid. Any substitution of one member of the above-mentioned polar, basic or acidic groups by another member of the same group can be deemed a conservative substitution.

[0148] Non-conservative substitutions include those in which (i) a residue having an electropositive side chain (*e.g.*, Arg, His or Lys) is substituted for, or by, an electDR6egative residue (*e.g.*, Glu or Asp), (ii) a hydrophilic residue (*e.g.*, Ser or Thr) is substituted for, or by, a hydrophobic residue (*e.g.*, Ala, Leu, Ile, Phe or Val), (iii) a cysteine or proline is substituted for, or by, any other residue, or (iv) a residue having a bulky hydrophobic or aromatic side chain

(*e.g.*, Val, Ile, Phe or Trp) is substituted for, or by, one having a smaller side chain (*e.g.*, Ala, Ser) or no side chain (*e.g.*, Gly).

[0149] As known in the art, "sequence identity" between two polypeptides is determined by comparing the amino acid sequence of one polypeptide to the sequence of a second polypeptide. When discussed herein, whether any particular polypeptide is at least about 70%, 75%, 80%, 85%, 90% or 95% identical to another polypeptide can be determined using methods and computer programs/software known in the art such as, but not limited to, the BESTFIT program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711). BESTFIT uses the local homology algorithm of Smith and Waterman, Advances in Applied Mathematics 2:482-489 (1981), to find the best segment of homology between two sequences. When using BESTFIT or any other sequence alignment program to determine whether a particular sequence is, for example, 95% identical to a reference sequence described herein, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference polypeptide sequence and that gaps in homology of up to 5% of the total number of amino acids in the reference sequence are allowed.

[0150] As would be well understood by a person of ordinary skill in the art, the DR6 fragments such as those listed above can vary in length, for example, by 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids at either end (either longer or shorter) based, for example, on alternate predictions of the DR6 domain regions. In addition, any of the fragments listed above can further include a secretory signal peptide at the N-terminus, *e.g.*, amino acids 1 to 40 of SEQ ID NO:2 or amino acids 1 to 41 of SEQ ID NO:2. Other secretory signal peptides, such as those described elsewhere herein, can also be used. Corresponding fragments of soluble DR6 polypeptides at least 70%, 75%, 80%, 85%, 90%, or 95% identical to SEQ ID NO:2, SEQ ID NO:4, or fragments thereof described herein are also contemplated.

[0151] Soluble DR6 polypeptides for use in the methods described herein can include any combination of two or more soluble DR6 polypeptides. Accordingly, soluble DR6 polypeptide dimers, either homodimers or heterodimers, are contemplated. Two or more soluble DR6 polypeptides as described herein can be directly connected, or can be connected via a suitable peptide linker. Such peptide linkers are described elsewhere herein.

[0152] Soluble DR6 polypeptides for use in the methods described herein can be cyclic. Cyclization of the soluble DR6 polypetides reduces the conformational freedom of linear

peptides and results in a more structurally constrained molecule. Many methods of peptide cyclization are known in the art. For example, "backbone to backbone" cyclization by the formation of an amide bond between the N-terminal and the C-terminal amino acid residues of the peptide. The "backbone to backbone" cyclization method includes the formation of disulfide bridges between two ω-thio amino acid residues (*e.g.* cysteine, homocysteine). Certain soluble DR6 peptides described herein include modifications on the N- and C- terminus of the peptide to form a cyclic DR6 polypeptide. Such modifications include, but are not limited, to cysteine residues, acetylated cysteine residues, cysteine residues with a NH2 moiety and biotin. Other methods of peptide cyclization are described in Li & Roller. Curr. Top. Med. Chem. 3:325-341 (2002), which is incorporated by reference herein in its entirety.

[0153] Cyclic DR6 polypeptides for use in the methods described herein include, but are not limited to, $C_1LSPX_9X_{10}X_{11}C_2$ (SEQ ID NO:5) where X_1 is lysine, arginine, histidine, glutamine, or asparagine, X_2 is lysine, arginine, histidine, glutamine, or asparagine, X_3 is lysine, arginine, histidine, glutamine, or asparagine, C_1 optionally has a moiety to promote cyclization (*e.g.* an acetyl group or biotin) attached and C_2 optionally has a moiety to promote cyclization (*e.g.* an NH_2 moiety) attached.

[0154] In some embodiments, the DR6 antagonist polypeptide inhibits binding of DR6 to p75. In some emeodiments, the DR6 antagonist polypeptide inhibis binding of DR6 to p75, but does not prevent DR6 binding to APP.

DR6 Antagonist Compounds

[0155] DR6 antagonists in the methods described herein include any chemical or synthetic compound which inhibits or decreases the activity of DR6 compared to the activity of DR6 in the absence of the antagonist compound. The DR6 antagonist compound can be one that inhibits binding of DR6 to p75. The DR6 antagonist compound can also be one that inhibits binding of DR6 to p75 but does not prevent binding of DR6 to APP.

[0156] One of ordinary skill in the art would know how to screen and test for DR6 antagonist compounds which would be useful in the methods described herein, for example by screening for compounds that modify nervous system cell survival using assays described elsewhere herein.

DR6 Antibodies or Immunospecific Fragments Thereof

[0157] DR6 antagonists for use in the methods described herein also include DR6-antigen binding molecules, DR-specific antibodies or antigen-binding fragments, variants, or derivatives which are antagonists of DR6 activity. For example, binding of certain DR6 antigen binding molecules or DR6 antibodies to DR6, as expressed in neurons inhibit apoptosis or promote cell survival.

[0158] In certain embodiments, the antibody is an antibody or antigen-binding fragment, variant or derivative of that specifically binds to DR6, wherein the antibody promotes survival of cells of the nervous system. In certain embodiments, the antibody is an antibody or antigen-binding fragment, variant or derivative of that specifically binds to DR6, wherein the antibody promotes proliferation, differentiation or survival of oligodendrocytes. In certain embodiments, the DR6 antibody is an antibody or antigen-binding fragment, variant or derivative thereof that specifically binds to DR6, wherein the antibody promotes myelination. In other embodiments, the DR6 antibody is an antibody or antigen-binding fragment, variant or derivative thereof that inhibits binding of DR6 to p75. In other embodiments, the DR6 antibody is an antibody or antigen-binding fragment, variant or derivative thereof that inhibits inding of DR6 to p75 but does not prevent binding of DR6 to APP.

[0159] DR6 antibodies include, but are not limited to, polyclonal, monoclonal, multispecific, human, humanized or chimeric antibodies, single chain antibodies, scFvs, diabodies, triabodies, tetrabodies, minibodies, domain-deleted antibodies, Fab fragments, F(ab')2 fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies (including, *e.g.*, anti-Id antibodies to antibodies described herein) and epitope-binding fragments of any of the above. The term "antibody," as used herein, refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, *i.e.*, molecules that contain an antigen binding site that immunospecifically binds an antigen. The immunoglobulin molecules can be of any type (*e.g.*, IgG, IgE, IgM, IgD, IgA and IgY), class (*e.g.*, IgGl, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule.

[0160] Certain DR6 antagonist antibodies for use in the methods described herein specifically or preferentially binds to a particular DR6 polypeptide fragment or domain, for example, a DR6 polypeptide, fragment, variant, or derivative as described herein. Such DR6 polypeptide fragments include, but are not limited to, a DR6 polypeptide comprising, consisting essentially of, or consisting of one or more TNFR-like cysteine-rich motifs of DR6. Such

fragments include for example, fragments comprising, consisting essentially of or consisting of amino acids 65 to 105 of SEQ ID NO:2; 106 to 145 of SEQ ID NO:2; 146 to 185 of SEQ ID NO:2; 186 to 212 of SEQ ID NO:2; 65 to 145 of SEQ ID NO:2; 65 to 185 of SEQ ID NO:2; 65 to 212 of SEQ ID NO:2; 106 to 185 of SEQ ID NO:2; 106 to 212 of SEQ ID NO:2; and 146 to 212 of SEQ ID NO:2. Such fragments also include amino acids 134-189 of SEQ ID NO:2; 168-189 of SEQ ID NO:2; and 134-168 of SEQ ID NO:2. Corresponding fragments of a variant DR6 polypeptide at least 70%, 75%, 80%, 85%, 90% or 95% identical to amino acids 65 to 105 of SEQ ID NO:2; 106 to 145 of SEQ ID NO:2; 146 to 185 of SEQ ID NO:2; 186 to 212 of SEQ ID NO:2; 65 to 145 of SEQ ID NO:2; 65 to 185 of SEQ ID NO:2; 65 to 212 of SEQ ID NO:2; 106 to 185 of SEQ ID NO:2; 106 to 212 of SEQ ID NO:2; 146 to 212 of SEQ ID NO:2; 134-189 of SEQ ID NO:2; 168-189 of SEQ ID NO:2; and 134-168 of SEQ ID NO:2 are also contemplated. In some embodiments, the DR6 antibody, antigen-binding fragment, variant, or derivative thereof requires both the Cys3 and Cys4 regions of DR6 to interact with DR6.

[0161] In other embodiments, the antibody is an antibody, or antigen-binding fragment, variant, or derivative thereof which specifically or preferentially binds to at least one epitope of DR6, where the epitope comprises, consists essentially of, or consists of at least about four to five amino acids of SEQ ID NO:2 or SEQ ID NO:4, at least seven, at least nine, or between at least about 15 to about 30 amino acids of SEQ ID NO:2 or SEQ ID NO:4. The amino acids of a given epitope of SEQ ID NO:2 or SEQ ID NO:4 as described can be, but need not be contiguous or linear. In certain embodiments, the at least one epitope of DR6 comprises, consists essentially of, or consists of a non-linear epitope formed by the extracellular domain of DR6 as expressed on the surface of a cell or as a soluble fragment, *e.g.*, fused to an IgG Fc region. Thus, in certain embodiments the at least one epitope of DR6 comprises, consists essentially of, or consists of at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 15, at least 20, at least 25, between about 15 to about 30, or at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 contiguous or non-contiguous amino acids of SEQ ID NO:2, or SEQ ID NO:4. where non-contiguous amino acids form an epitope through protein folding.

[0162] In other embodiments, the antibody is an antibody, or antigen-binding fragment, variant, or derivative thereof which specifically or preferentially binds to at least one epitope of DR6, where the epitope comprises, consists essentially of, or consists of, in addition to one, two, three, four, five, six or more contiguous or non-contiguous amino acids of SEQ ID NO:2 or SEQ ID NO:4 as described above, and an additional moiety which modifies the protein, *e.g.*, a

carbohydrate moiety can be included such that the DR6 antibody binds with higher affinity to modified target protein than it does to an unmodified version of the protein. Alternatively, the DR6 antibody does not bind the unmodified version of the target protein at all.

[0163] In certain aspects, the antibody is an antibody, or antigen-binding fragment, variant, or derivative thereof which specifically binds to a DR6 polypeptide or fragment thereof, or a DR6 variant polypeptide, with an affinity characterized by a dissociation constant (K_D) which is less than the K_D for a given reference monoclonal antibody.

[0164] In certain embodiments, an antibody, or antigen-binding fragment, variant, or derivative thereof binds specifically to at least one epitope of DR6 or fragment or variant described above, *i.e.*, binds to such an epitope more readily than it would bind to an unrelated, or random epitope; binds preferentially to at least one epitope of DR6 or fragment or variant described above, *i.e.*, binds to such an epitope more readily than it would bind to a related, similar, homologous, or analogous epitope; competitively inhibits binding of a reference antibody which itself binds specifically or preferentially to a certain epitope of DR6 or fragment or variant described above; or binds to at least one epitope of DR6 or fragment or variant described above with an affinity characterized by a dissociation constant K_D of less than about 5 x 10^{-2} M, about 10^{-2} M, about 5 x 10^{-3} M, about 10^{-3} M, about 5 x 10^{-4} M, about 10^{-4} M, about 5 x 10^{-5} M, about 10^{-5} M, about 5 x 10^{-6} M, about 10^{-6} M, about 5 x 10^{-7} M, about 10^{-7} M, about 5 x 10^{-8} M, about 10^{-8} M, about 5 x 10^{-9} M, about 10^{-9} M, about 5 x 10^{-10} M, about 10^{-10} M, about 5 x 10^{-11} M, about 10^{-11} M, about 5 x 10^{-12} M, about 10^{-12} M, about 5 x 10^{-13} M, about 10^{-13} M, about 5 x 10^{-14} M, about 10^{-14} M, about 5 x 10^{-15} M, or about 10^{-15} M. In a particular aspect, the antibody or fragment thereof preferentially binds to a human DR6 polypeptide or fragment thereof, relative to a murine DR6 polypeptide or fragment thereof. In another particular aspect, the antibody or fragment thereof preferentially binds to one or more DR6 polypeptides or fragments thereof, *e.g.*, one or more mammalian DR6 polypeptides.

[0165] As used in the context of antibody binding dissociation constants, the term "about" allows for the degree of variation inherent in the methods utilized for measuring antibody affinity. For example, depending on the level of precision of the instrumentation used, standard error based on the number of samples measured, and rounding error, the term "about 10^{-2} M" might include, for example, from 0.05 M to 0.005 M.

[0166] In specific embodiments, an antibody, or antigen-binding fragment, variant, or derivative thereof binds DR6 polypeptides or fragments or variants thereof with an off rate

(k(off)) of less than or equal to 5 X 10^{-2} sec^{-1}, 10^{-2} sec^{-1}, 5 X 10^{-3} sec^{-1} or 10^{-3} sec^{-1}. Alternatively, an antibody, or antigen-binding fragment, variant, or derivative thereof binds DR6 polypeptides or fragments or variants thereof with an off rate (k(off)) of less than or equal to 5 X 10^{-4} sec^{-1}, 10^{-4} sec^{-1}, 5 X 10^{-5} sec^{-1}, or 10^{-5} sec^{-1} 5 X 10^{-6} sec^{-1}, 10^{-6} sec^{-1}, 5 X 10^{-7} sec^{-1} or 10^{-7} sec^{-1}.

[0167] In other embodiments, an antibody, or antigen-binding fragment, variant, or derivative thereof binds DR6 polypeptides or fragments or variants thereof with an on rate (k(on)) of greater than or equal to 10^{3} M^{-1} sec^{-1}, 5 X 10^{3} M^{-1} sec^{-1}, 10^{4} M^{-1} sec^{-1} or 5 X 10^{4} M^{-1} sec^{-1}. Alternatively, an antibody, or antigen-binding fragment, variant, or derivative thereof binds DR6 polypeptides or fragments or variants thereof with an on rate (k(on)) greater than or equal to 10^{5} M^{-1} sec^{-1}, 5 X 10^{5} M^{-1} sec^{-1}, 10^{6} M^{-1} sec^{-1}, or 5 X 10^{6} M^{-1} sec^{-1} or 10^{7} M^{-1} sec^{-1}.

[0168] In one embodiment, the DR6 antibody includes DR6 antibodies, or antigen-binding fragments, variants, or derivatives thereof which at least the antigen-binding domains of certain monoclonal antibodies, and fragments, variants, and derivatives thereof shown in Tables 3 and 4. Table 3 lists human anti-DR6 Fab regions identified from a phage display library. Table 4 lists mouse anti-DR6 antibodies derived from hybridomas.

Table 3: DR6-specific human Fabs.

	Fab
1	M50-H01
2	M51-H09
3	M53-E04
4.	M53-F04
5	M62-B02
6.	M63-E10
7.	M66-B03
8.	M67-G02
9.	M72-F03
10.	M73-C04

Table 4: DR6-specific Murine Monoclonal Antibodies.

	Murine Antibody
1	1P1D6.3
2	1P2F2.1
3	1P5D10.2

[0169] As used herein, the term "antigen binding domain" includes a site that specifically binds an epitope on an antigen (*e.g.*, an epitope of DR6). The antigen binding domain of an antibody typically includes at least a portion of an immunoglobulin heavy chain variable region

and at least a portion of an immunoglobulin light chain variable region. The binding site formed by these variable regions determines the specificity of the antibody.

[0170] In some embodiments, the DR6 antibody is a DR6 antibody, or antigen-binding fragment, variant or derivatives thereof, where the DR6 antibody specifically binds to the same DR6 epitope as a reference monoclonal Fab antibody fragment selected from the group consisting of M50-H01, M51-H09, M53-E04, M53-F04, M62-B02, M63-E10, M66-B03, M67-G02, M72-F03, and M73-C04 or a reference monoclonal antibody selected from the group consisting of 1P1D6.3, 1P2F2.1, and 1P5D10.2.

[0171] In some embodiments, the DR6 antibody is a DR6 antibody, or antigen-binding fragment, variant or derivatives thereof, where the DR6 antibody competitively inhibits a reference monoclonal Fab antibody fragment selected from the group consisting of M50-H01, M51-H09, M53-E04, M53-F04, M62-B02, M63-E10, M66-B03, M67-G02, M72-F03, and M73-C04 or a reference monoclonal antibody selected from the group consisting of 1P1D6.3, 1P2F2.1, and 1P5D10.2 from binding to DR6.

[0172] In some embodiments, the DR6 antibody is a DR6 antibody, or antigen-binding fragment, variant or derivatives thereof, where the DR6 antibody comprises an antigen binding domain identical to that of a monoclonal Fab antibody fragment selected from the group consisting of M50-H01, M51-H09, M53-E04, M53-F04, M62-B02, M63-E10, M66-B03, M67-G02, M72-F03, and M73-C04 or a reference monoclonal antibody selected from the group consisting of 1P1D6.3, 1P2F2.1, and 1P5D10.2.

[0173] In some embodiments, the DR6 antibody is not an antibody selected from the group consisting of 3F4.48, 4B6.9.7 or 1E5.57 as described in International Publication No. WO2008/080045, filed December 21, 2007. In some embodiments, the DR6 antibody is not antibody selected from the group consisting of antibodies that competitively inhibit binding of 3F4.48, 4B6.9.7 or 1E5.57 to DR6.

[0174] In some embodiments, the DR6 antibody is an antagonist antibody.

[0175] Methods of making antibodies are well known in the art and described herein. Once antibodies to various fragments of, or to the full-length DR6 without the signal sequence, have been produced, determining which amino acids, or epitope, of DR6 to which the antibody or antigen binding fragment binds can be determined by epitope mapping protocols as described herein as well as methods known in the art (*e.g.* double antibody-sandwich ELISA as described in "Chapter 11 - Immunology," *Current Protocols in Molecular Biology*, Ed. Ausubel *et al.*, v.2,

John Wiley & Sons, Inc. (1996)). Additional epitope mapping protocols can be found in Morris, G. *Epitope Mapping Protocols,* New Jersey: Humana Press (1996), which are both incorporated herein by reference in their entireties. Epitope mapping can also be performed by commercially available means (*i.e.* ProtoPROBE, Inc. (Milwaukee, Wisconsin)).

[0176] Additionally, antibodies produced which bind to any portion of DR6 can then be screened for their ability to act as an antagonist of DR6 for example, promoting survival of cells of the nervous system, treating a condition associated with death of cells of the nervous and preventing apoptosis of cells of the nervous system Antibodies can be screened for these and other properties according to methods described in detail in the Examples. Other functions of antibodies described herein can be tested using other assays as described in the Examples herein.

[0177] In one embodiment, a DR6 antagonist for use in the methods described herein is an antibody molecule, or immunospecific fragment thereof. Unless it is specifically noted, as used herein a "fragment thereof" in reference to an antibody refers to an immunospecific fragment, *i.e.*, an antigen-specific fragment.

[0178] In one embodiment, a binding molecule or antigen binding molecule for use in the methods described herein comprises a synthetic constant region wherein one or more domains are partially or entirely deleted ("domain-deleted antibodies"). Certain methods described herein comprise administration of a DR6 antagonist antibody, or immunospecific fragment thereof, in which at least a fraction of one or more of the constant region domains has been deleted or otherwise altered so as to provide desired biochemical characteristics such as reduced effector functions, the ability to non-covalently dimerize, increased ability to localize at the site of action, reduced serum half-life, or increased serum half-life when compared with a whole, unaltered antibody of approximately the same immunogenicity. For example, certain antibodies for use in the treatment methods described herein are domain deleted antibodies which comprise a polypeptide chain similar to an immunoglobulin heavy chain, but which lack at least a portion of one or more heavy chain domains.

[0179] In certain embodiments compatible modified antibodies will comprise domain deleted constructs or variants wherein the entire C_H2 domain has been removed (ΔC_H2 constructs). For other embodiments a short connecting peptide can be substituted for the deleted domain to provide flexibility and freedom of movement for the variable region. Those skilled in the art will appreciate that such constructs can be desirable under certain circumstances due to the regulatory properties of the C_H2 domain on the catabolic rate of the antibody. Domain

deleted constructs can be derived using a vector (*e.g.*, from Biogen IDEC Incorporated) encoding an IgG_1 human constant domain (see, *e.g.*, WO 02/060955A2 and WO02/096948A2). This exemplary vector was engineered to delete the C_H2 domain and provide a synthetic vector expressing a domain deleted IgG_1 constant region.

[0180] In certain embodiments, modified antibodies for use in the methods disclosed herein are minibodies. Minibodies can be made using methods described in the art (see, *e.g.*, see *e.g.*, US patent 5,837,821 or WO 94/09817A1).

[0181] In one embodiment, a DR6 antagonist antibody or fragment thereof for use in the treatment methods disclosed herein comprises an immunoglobulin heavy chain having deletion or substitution of a few or even a single amino acid as long as it permits association between the monomeric subunits. For example, the mutation of a single amino acid in selected areas of the C_H2 domain can be enough to substantially reduce Fc binding and thereby increase localization to the intended site of action. Similarly, it can be desirable to simply delete that part of one or more constant region domains that control the effector function (*e.g.* complement binding) to be modulated. Such partial deletions of the constant regions can improve selected characteristics of the antibody (serum half-life) while leaving other desirable functions associated with the subject constant region domain intact. Moreover, as alluded to above, the constant regions of the disclosed antibodies can be synthetic through the mutation or substitution of one or more amino acids that enhances the profile of the resulting construct. In this respect it can be possible to disrupt the activity provided by a conserved binding site (*e.g.* Fc binding) while substantially maintaining the configuration and immunogenic profile of the modified antibody. Yet other embodiments comprise the addition of one or more amino acids to the constant region to enhance desirable characteristics such as effector function or provide for more cytotoxin or carbohydrate attachment. In such embodiments it can be desirable to insert or replicate specific sequences derived from selected constant region domains.

[0182] In certain DR6 antagonist antibodies or immunospecific fragments thereof for use in the therapeutic methods described herein, the Fc portion can be mutated to decrease effector function using techniques known in the art. For example, modifications of the constant region can be used to modify disulfide linkages or oligosaccharide moieties that allow for enhanced localization due to increased antigen specificity or antibody flexibility. The resulting physiological profile, bioavailability and other biochemical effects of the modifications can

easily be measured and quantified using well know immunological techniques without undue experimentation.

[0183] The methods described herein also provide the use of antibodies that comprise, consist essentially of, or consist of, variants (including derivatives) of antibody molecules (*e.g.*, the V_H regions and/or V_L regions) described herein, which antibodies or fragments thereof immunospecifically bind to a DR6 polypeptide. Standard techniques known to those of skill in the art can be used to introduce mutations in the nucleotide sequence encoding a binding molecule, including, but not limited to, site-directed mutagenesis and PCR-mediated mutagenesis which result in amino acid substitutions. In various embodiments, the variants (including derivatives) encode less than 50 amino acid substitutions, less than 40 amino acid substitutions, less than 30 amino acid substitutions, less than 25 amino acid substitutions, less than 20 amino acid substitutions, less than 15 amino acid substitutions, less than 10 amino acid substitutions, less than 5 amino acid substitutions, less than 4 amino acid substitutions, less than 3 amino acid substitutions, or less than 2 amino acid substitutions relative to the reference V_H region, V_HCDR1, V_HCDR2, V_HCDR3, V_L region, V_LCDR1, V_LCDR2, or V_LCDR3. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a side chain with a similar charge. Families of amino acid residues having side chains with similar charges have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Alternatively, mutations can be introduced randomly along all or part of the coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for biological activity to identify mutants that retain activity.

[0184] For example, it is possible to introduce mutations only in framework regions or only in CDR regions of an antibody molecule. Introduced mutations can be silent or neutral missense mutations, *i.e.*, have no, or little, effect on an antibody's ability to bind antigen. These types of mutations can be useful to optimize codon usage, or improve a hybridoma's antibody production. Alternatively, non-neutral missense mutations can alter an antibody's ability to bind antigen. The location of most silent and neutral missense mutations is likely to be in the

framework regions, while the location of most non-neutral missense mutations is likely to be in CDR, though this is not an absolute requirement. One of skill in the art would be able to design and test mutant molecules with desired properties such as no alteration in antigen binding activity or alteration in binding activity (*e.g.*, improvements in antigen binding activity or change in antibody specificity). Following mutagenesis, the encoded protein can routinely be expressed and the functional and/or biological activity of the encoded protein can be determined using techniques described herein or by routinely modifying techniques known in the art.

[0185] In one embodiment, an antibody is a bispecific binding molecule, binding polypeptide, or antibody, *e.g.*, a bispecific antibody, minibody, domain deleted antibody, or fusion protein having binding specificity for more than one epitope, *e.g.*, more than one antigen or more than one epitope on the same antigen. In one embodiment, a bispecific antibody has at least one binding domain specific for at least one epitope on DR6. A bispecific antibody can be a tetravalent antibody that has two target binding domains specific for an epitope of DR6 and two target binding domains specific for a second target. Thus, a tetravalent bispecific antibody can be bivalent for each specificity.

[0186] Modified forms of antibodies or immunospecific fragments thereof for use in the diagnostic and therapeutic methods disclosed herein can be made from whole precursor or parent antibodies using techniques known in the art. Exemplary techniques are discussed in more detail herein.

[0187] DR6 antagonist antibodies or immunospecific fragments thereof for use in the diagnostic and treatment methods disclosed herein can be made or manufactured using techniques that are known in the art. In certain embodiments, antibody molecules or fragments thereof are "recombinantly produced," *i.e.*, are produced using recombinant DNA technology. Exemplary techniques for making antibody molecules or fragments thereof are discussed in more detail elsewhere herein.

[0188] DR6 antagonist antibodies or fragments thereof for use in the methods described herein can be generated by any suitable method known in the art.

[0189] Polyclonal antibodies can be produced by various procedures well known in the art. For example, a DR6 immunospecific fragment can be administered to *various* host animals including, but not limited to, rabbits, mice, rats, etc. to induce the production of sera containing polyclonal antibodies specific for the antigen. Various adjuvants can be used to increase the immunological response, depending on the host species, and include but are not limited to,

Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and *Corynebacterium parvum*. Such adjuvants are also well known in the art.

[0190] Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. For example, monoclonal antibodies can be produced using hybridoma techniques including those known in the art and taught, for example, in Harlow *et al., Antibodies: A Laboratory Manual,* Cold Spring Harbor Laboratory Press, 2nd ed. (1988); Hammerling *et al.*, in: *Monoclonal Antibodies and T-Cell Hybridomas* Elsevier, N.Y., 563-681 (1981) (said references incorporated by reference in their entireties). The term "monoclonal antibody" as used herein is not limited to antibodies produced through hybridoma technology. The term "monoclonal antibody" refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced. Thus, the term "monoclonal antibody" is not limited to antibodies produced through hybridoma technology. Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma and recombinant and phage display technology.

[0191] Using art recognized protocols, in one example, antibodies are raised in mammals by multiple subcutaneous or intraperitoneal injections of the relevant antigen (*e.g.*, purified DR6 antigens or cells or cellular extracts comprising such antigens) and an adjuvant. This immunization typically elicits an immune *response* that comprises production of antigen-reactive antibodies from activated splenocytes or lymphocytes. While the resulting antibodies can be harvested from the serum of the animal to provide polyclonal preparations, it is often desirable to isolate individual lymphocytes from the spleen, lymph nodes or peripheral blood to provide homogenous preparations of monoclonal antibodies (MAbs). In certain specific embodiments, the lymphocytes are obtained from the spleen.

[0192] In this well known process (Kohler *et al., Nature 256*:495 (1975)) the relatively short-lived, or mortal, lymphocytes from a mammal which has been injected with antigen are fused with an immortal tumor cell line (*e.g.* a myeloma cell line), thus, producing hybrid cells or "hybridomas" which are both immortal and capable of producing the genetically coded antibody of the B cell. The resulting hybrids are segregated into single genetic strains by selection, dilution, and regrowth with each individual strain comprising specific genes for the formation of

a single antibody. They produce antibodies which are homogeneous against a desired antigen and, in reference to their pure genetic parentage, are termed "monoclonal."

[0193] Typically, hybridoma cells thus prepared are seeded and grown in a suitable culture medium that contains one or more substances that inhibit the growth or survival of the unfused, parental myeloma cells. Those skilled in the art will appreciate that reagents, cell lines and media for the formation, selection and growth of hybridomas are commercially available from a number of sources and standardized protocols are well established. Generally, culture medium in which the hybridoma cells are growing is assayed for production of monoclonal antibodies against the desired antigen. In certain embodiments, the binding specificity of the monoclonal antibodies produced by hybridoma cells is determined by *in vitro* assays such as immunoprecipitation, radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA). After hybridoma cells are identified that produce antibodies of the desired specificity, affinity and/or activity, the clones can be subcloned by limiting dilution procedures and grown by standard methods (*Goding, Monoclonal Antibodies: Principles and Practice*, Academic Press, pp. 59-103 (1986)). It will further be appreciated that the monoclonal antibodies secreted by the subclones can be separated from culture medium, ascites fluid or serum by conventional purification procedures such as, for example, protein-A, hydroxylapatite chromatography, gel electrophoresis, dialysis or affinity chromatography.

[0194] Antibody fragments that recognize specific epitopes can be generated by known techniques. For example, Fab and $F(ab')_2$ fragments can be produced by proteolytic cleavage of immunoglobulin molecules, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce $F(ab')_2$ fragments). $F(ab')_2$ fragments contain the variable region, the light chain constant region and the C_H1 domain of the heavy chain.

[0195] Those skilled in the art will also appreciate that DNA encoding antibodies or antibody fragments (*e.g.*, antigen binding sites) can also be derived from antibody phage libraries. In a particular, such phage can be utilized to display antigen-binding domains expressed from a repertoire or combinatorial antibody library (*e.g.*, human or murine). Phage expressing an antigen binding domain that binds the antigen of interest can be selected or identified with antigen, *e.g.*, using labeled antigen or antigen bound or captured to a solid surface or bead. Phage used in these methods are typically filamentous phage including fd and M13 binding domains expressed from phage with Fab, Fv or disulfide stabilized Fv antibody domains recombinantly fused to either the phage gene III or gene VIII protein. Exemplary methods are set forth, for

example, in EP 368 684 B1; U.S. patent. 5,969,108, Hoogenboom, H.R. and Chames, *Immunol. Today 21*:371 (2000); Nagy *et al. Nat. Med. 8*:801 (2002); Huie *et al., Proc. Natl. Acad. Sci. USA 98*:2682 (2001); Lui *et al., J. Mol. Biol. 315*:1063 (2002), each of which is incorporated herein by reference. Several publications (*e.g.*, Marks *et al., Bio/Technology 10*:779-783 (1992)) have described the production of high affinity human antibodies by chain shuffling, as well as combinatorial infection and *in vivo* recombination as a strategy for constructing large phage libraries. In another embodiment, Ribosomal display can be used to replace bacteriophage as the display platform (*see, e.g.*, Hanes *et al., Nat. Biotechnol. 18*:1287 (2000); Wilson *et al., Proc. Natl. Acad. Sci. USA 98*:3750 (2001); or Irving *et al., J. Immunol. Methods 248*:31 (2001)). In yet another embodiment, cell surface libraries can be screened for antibodies (Boder *et al., Proc. Natl. Acad. Sci. USA 97*:10701 (2000); Daugherty *et al., J. Immunol. Methods 243*:211 (2000)). Such procedures provide alternatives to traditional hybridoma techniques for the isolation and subsequent cloning of monoclonal antibodies.

[0196] In phage display methods, functional antibody domains are displayed on the surface of phage particles which carry the polynucleotide sequences encoding them. In particular, DNA sequences encoding V_H and V_L regions are amplified from animal cDNA libraries (*e.g.*, human or murine cDNA libraries of lymphoid tissues) or synthetic cDNA libraries. In certain embodiments, the DNA encoding the V_H and V_L regions are joined together by an scFv linker by PCR and cloned into a phagemid vector (*e.g.*, p CANTAB 6 or pComb 3 HSS). The vector is electroporated in *E. coli* and the *E. coli* is infected with *helper* phage. Phage used in these methods are typically filamentous phage including fd and M13 and the V_H or V_L regions are usually recombinantly fused to either the phage gene III or gene VIII. Phage expressing an antigen binding domain that binds to an antigen of interest (*i.e.*, a DR6 polypeptide or a fragment thereof) can be selected or identified with antigen, *e.g.*, using labeled antigen or antigen bound or captured to a solid surface or bead.

[0197] Additional examples of phage display methods that can be used to make the antibodies include those disclosed in Brinkman *et al., J. Immunol. Methods 182*:41-50 (1995); Ames *et al., J. Immunol. Methods 184*:177-186 (1995); Kettleborough *et al., Eur. J. Immunol. 24*:952-958 (1994); Persic *et al., Gene 187*:9-18 (1997); Burton *et al., Advances in Immunology 57*:191-280 (1994); PCT Application No. PCT/GB91/01134; PCT publications WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and U.S. Pat. Nos. 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047;

5,571,698; 5,427,908; 5,516,637; 5,780,225; 5,658,727; 5,733,743 and 5,969,108; each of which is incorporated herein by reference in its entirety.

[0198] As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies, or any other desired antigen binding fragment, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria. For example, techniques to recombinantly produce Fab, Fab' and F(ab')2 fragments can also be employed using methods known in the art such as those disclosed in PCT publication WO 92/22324; Mullinax *et al.*, *BioTechniques 12(6)*:864-869 (1992); and Sawai *et al.*, *AJRI 34*:26-34 (1995); and Better *et al.*, *Science 240*:1041-1043 (1988) (said references incorporated by reference in their entireties).

[0199] In another embodiment, DNA encoding desired monoclonal antibodies can be readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). In certain embodiments, isolated and subcloned hybridoma cells serve as a source of such DNA. Once isolated, the DNA can be placed into expression vectors, which are then transfected into prokaryotic or eukaryotic host cells such as *E. coli* cells, simian COS cells, Chinese Hamster Ovary (CHO) cells or myeloma cells that do not otherwise produce immunoglobulins. More particularly, the isolated DNA (which can be synthetic as described herein) can be used to clone constant and variable region sequences for the manufacture antibodies as described in Newman *et al.*, U.S. Pat. No. 5,658,570, filed January 25, 1995, which is incorporated by reference herein. Essentially, this entails extraction of RNA from the selected cells, conversion to cDNA, and amplification by PCR using Ig specific primers. Suitable primers for this purpose are also described in U.S. Pat. No. 5,658,570. As will be discussed in more detail below, transformed cells expressing the desired antibody can be grown up in relatively large quantities to provide clinical and commercial supplies of the immunoglobulin.

[0200] In a specific embodiment, the amino acid sequence of the heavy and/or light chain variable domains can be inspected to identify the sequences of the complementarity determining regions (CDRs) by methods that are well know in the art, *e.g.*, by comparison to known amino acid sequences of other heavy and light chain variable regions to determine the regions of sequence hypervariability. Using routine recombinant DNA techniques, one or more of the CDRs can be inserted within framework regions, *e.g.*, into human framework regions to humanize a non-human antibody. The framework regions can be naturally occurring or consensus framework

regions, *e.g.*, human framework regions (see, *e.g.*, Chothia *et al.*, *J. Mol. Biol. 278*:457-479 (1998) for a listing of human framework regions). In certain embodiments, the polynucleotide generated by the combination of the framework regions and CDRs encodes an antibody that specifically binds to at least one epitope of a desired polypeptide, *e.g.*, DR6. In further embodiments, one or more amino acid substitutions can be made within the framework regions, for example, to improve binding of the antibody to its antigen. Additionally, such methods can be used to make amino acid substitutions or deletions of one or more variable region cysteine residues participating in an intrachain disulfide bond to generate antibody molecules lacking one or more intrachain disulfide bonds. Other alterations to the polynucleotide are contemplated and within the skill of the art.

[0201] In certain embodiments, a DR6 antagonist antibody or immunospecific fragment thereof for use in the treatment methods disclosed herein will not elicit a deleterious immune response in the animal to be treated, *e.g.*, in a human. In one embodiment, DR6 antagonist antibodies or immunospecific fragments thereof for use in the treatment methods disclosed herein be modified to reduce their immunogenicity using art-recognized techniques. For example, antibodies can be humanized, primatized, deimmunized, or chimeric antibodies can be made. These types of antibodies are derived from a non-human antibody, typically a murine or primate antibody, that retains or substantially retains the antigen-binding properties of the parent antibody, but which is less immunogenic in humans. This can be achieved by various methods, including (a) grafting the entire non-human variable domains onto human constant regions to generate chimeric antibodies; (b) grafting at least a part of one or more of the non-human complementarity determining regions (CDRs) into a human framework and constant regions with or without retention of critical framework residues; or (c) transplanting the entire non-human variable domains, but "cloaking" them with a human-like section by replacement of surface residues. Such methods are disclosed in Morrison *et al.*, *Proc. Natl. Acad. Sci. 81*:6851-6855 (1984); Morrison *et al.*, *Adv. Immunol. 44*:65-92 (1988); Verhoeyen *et al.*, *Science 239*:1534-1536 (1988); Padlan, *Molec. Immun. 28*:489-498 (1991); Padlan, *Molec. Immun. 31*:169-217 (1994), and U.S. Pat. Nos. 5,585,089, 5,693,761, 5,693,762, and 6,190,370, all of which are hereby incorporated by reference in their entirety.

[0202] De-immunization can also be used to decrease the immunogenicity of an antibody. As used herein, the term "de-immunization" includes alteration of an antibody to modify T cell epitopes (see, *e.g.*, WO9852976A1, WO0034317A2). For example, V_H and V_L sequences from

the starting antibody are analyzed and a human T cell epitope "map" from each V region showing the location of epitopes in relation to complementarity-determining regions (CDRs) and other key residues within the sequence. Individual T cell epitopes from the T cell epitope map are analyzed in order to identify alternative amino acid substitutions with a low risk of altering activity of the final antibody. A range of alternative V_H and V_L sequences are designed comprising combinations of amino acid substitutions and these sequences are subsequently incorporated into a range of binding polypeptides, *e.g.*, DR6 antagonist antibodies or immunospecific fragments thereof for use in the diagnostic and treatment methods disclosed herein, which are then tested for function. Typically, between 12 and 24 variant antibodies are generated and tested. Complete heavy and light chain genes comprising modified V and human C regions are then cloned into expression vectors and the subsequent plasmids introduced into cell lines for the production of whole antibody. The antibodies are then compared in appropriate biochemical and biological assays, and the optimal variant is identified.

[0203] A chimeric antibody is a molecule in which different portions of the antibody are derived from different animal species, such as antibodies having a variable region derived from a murine monoclonal antibody and a human immunoglobulin constant region. Methods for producing chimeric antibodies are known in the art. *See, e.g.*, Morrison, *Science 229*:1202 (1985); Oi *et al.*, *BioTechniques 4*:214 (1986); Gillies *et al.*, *J. Immunol. Methods 125*:191-202 (1989); Takeda *et al.*, *Nature 314*:452-454 (1985), Neuberger *et al., Nature 312*:604-608 (1984); U.S. Pat. Nos. 5,807,715; 4,816,567; and 4,816397, which are incorporated herein by reference in their entireties. Humanized antibodies are antibody molecules from non-human species antibody that binds the desired antigen having one or more complementarity determining regions (CDRs) from the non-human species and framework regions from a human immunoglobulin molecule. Often, framework residues in the human framework regions will be substituted with the corresponding residue from the CDR donor antibody to alter, *e.g.*, improve, antigen binding. These framework substitutions are identified by methods well known in the art, *e.g.*, by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. (See, *e.g.*, Queen *et al.*, U.S. Pat. No. 5,585,089; Riechmann *et al.*, *Nature 332*:323 (1988), which are incorporated herein by reference in their entireties.) Antibodies can be humanized using a variety of techniques known in the art including, for example, CDR-grafting (EP 239,400; PCT publication WO 91/09967; U.S. Pat. Nos. 5,225,539; 5,530,101; and

5,585,089), veneering or resurfacing (EP 592,106; EP 519,596; Padlan, *Molecular Immunology 28(4/5)*:489-498 (1991); Studnicka *et al.*, *Protein Engineering 7(6)*:805-814 (1994); Roguska. *et al.*, *PNAS 91*:969-973 (1994)), and chain shuffling (U.S. Pat. No. 5,565,332).

[0204] Yet another highly efficient means for generating recombinant antibodies is disclosed by Newman, *Biotechnology 10*: 1455-1460 (1992). Specifically, this technique results in the generation of primatized antibodies that contain monkey variable domains and human constant sequences. This reference is incorporated by reference in its entirety herein. Moreover, this technique is also described in commonly assigned U.S. Pat. Nos. 5,658,570, 5,693,780 and 5,756,096 each of which is incorporated herein by reference.

[0205] Completely human antibodies are particularly desirable for therapeutic treatment of human patients. Human antibodies can be made by a variety of methods known in the art including phage display methods described above using antibody libraries derived from human immunoglobulin sequences. See also, U.S. Pat. Nos. 4,444,887 and 4,716,111; and PCT publications WO 98/46645, WO 98/50433, WO 98/24893, WO 98/16654, WO 96/34096, WO 96/33735, and WO 91/10741; each of which is incorporated herein by reference in its entirety.

[0206] Human antibodies can also be produced using transgenic mice which are incapable of expressing functional endogenous immunoglobulins, but which can express human immunoglobulin genes. that are incapable of endogenous immunoglobulin production (see *e.g.*, U.S. Pat. Nos. 6,075,181, 5,939,598, 5,591,669 and 5,589,369 each of which is incorporated herein by reference). For example, it has been described that the homozygous deletion of the antibody heavy-chain joining region in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. The human heavy and light chain immunoglobulin gene complexes can be introduced randomly or by homologous recombination into mouse embryonic stem cells. Alternatively, the human variable region, constant region, and diversity region can be introduced into mouse embryonic stem cells in addition to the human heavy and light chain genes. The mouse heavy and light chain immunoglobulin genes can be rendered non-functional separately or simultaneously with the introduction of human immunoglobulin loci by homologous recombination. In particular, homozygous deletion of the JH region prevents endogenous antibody production. The modified embryonic stem cells are expanded and microinjected into blastocysts to produce chimeric mice. The chimeric mice are then bred to produce homozygous offspring that express human antibodies. The transgenic mice are immunized in the normal fashion with a selected antigen, *e.g.*, all or a portion of a desired

target polypeptide. Monoclonal antibodies directed against the antigen can be obtained from the immunized, transgenic mice using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B-cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA, IgM and IgE antibodies. For an overview of this technology for producing human antibodies, see Lonberg and Huszar *Int. Rev. Immunol. 13*:65-93 (1995). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, see, *e.g.*, PCT publications WO 98/24893; WO 96/34096; WO 96/33735; U.S. Pat. Nos. 5,413,923; 5,625,126; 5,633,425; 5,569,825; 5,661,016; 5,545,806; 5,814,318; and 5,939,598, which are incorporated by reference herein in their entirety. In addition, companies such as Abgenix, Inc. (Freemont, Calif.) and GenPharm (San Jose, Calif.) can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above.

[0207] Another means of generating human antibodies using SCID mice is disclosed in U.S. Pat. No. 5,811,524 which is incorporated herein by reference. It will be appreciated that the genetic material associated with these human antibodies can also be isolated and manipulated as described herein.

[0208] Completely human antibodies which recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach a selected non-human monoclonal antibody, *e.g.*, a mouse antibody, is used to guide the selection of a completely human antibody recognizing the same epitope. (Jespers *et al.*, *Bio/Technology 12*:899-903 (1988)). *See also*, U.S. Patent No. 5,565,332, which discloses, for example, selection from random libraries where only V_HCDR3 is unvaried.

[0209] Alternatively, techniques described for the production of single chain antibodies (U.S. Pat. No. 4,694,778; Bird, *Science 242*:423-442 (1988); Huston *et al.*, *Proc. Natl. Acad. Sci. USA 85*:5879-5883 (1988); and Ward *et al.*, *Nature 334*:544-554 (1989)) can be used. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain antibody. Techniques for the assembly of functional Fv fragments in *E. coli* can also be used (Skerra *et al.*, *Science 242*:1038-1041 (1988)). Examples of techniques which can be used to produce single-chain Fvs and antibodies

include those described in U.S. Pat. Nos. 4,946,778 and 5,258,498; Huston *et al.*, *Methods in Enzymology 203*:46-88 (1991); and Shu *et al.*, *PNAS 90*:7995-7999 (1993).

[0210] In another embodiment, lymphocytes can be selected by micromanipulation and the variable genes isolated. For example, peripheral blood mononuclear cells can be isolated from an immunized mammal and cultured for about 7 days *in vitro*. The cultures can be screened for specific IgGs that meet the screening criteria. Cells from positive wells can be isolated. Individual Ig-producing B cells can be isolated by FACS or by identifying them in a complement-mediated hemolytic plaque assay. Ig-producing B cells can be micromanipulated into a tube and the V_H and V_L genes can be amplified using, *e.g.*, RT-PCR. The V_H and V_L genes can be cloned into an antibody expression vector and transfected into cells (*e.g.*, eukaryotic or prokaryotic cells) for expression.

[0211] Alternatively, antibody-producing cell lines can be selected and cultured using techniques well known to the skilled artisan. Such techniques are described in a variety of laboratory manuals and primary publications. In this respect, techniques suitable for use in the methods as described below are described in *Current Protocols in Immunology*, Coligan *et al.*, Eds., Green Publishing Associates and Wiley-Interscience, John Wiley and Sons, New York (1991) which is herein incorporated by reference in its entirety, including supplements.

[0212] Antibodies for use in the therapeutic methods disclosed herein can be produced by any method known in the art for the synthesis of antibodies, in particular, by chemical synthesis or by recombinant expression techniques as described herein.

[0213] It will further be appreciated that the alleles, variants and mutations of antigen binding DNA sequences can be used in the methods described herein.

[0214] In one embodiment, cDNAs that encode the light and the heavy chains of the antibody can be made, either simultaneously or separately, using reverse transcriptase and DNA polymerase in accordance with well known methods. PCR can be initiated by consensus constant region primers or by more specific primers based on the published heavy and light chain DNA and amino acid sequences. As discussed above, PCR also can be used to isolate DNA clones encoding the antibody light and heavy chains. In this case the libraries can be screened by consensus primers or larger homologous probes, such as mouse constant region probes.

[0215] DNA, typically plasmid DNA, can be isolated from the cells using techniques known in the art, restriction mapped and sequenced in accordance with standard, well known techniques set forth in detail, *e.g.*, in the foregoing references relating to recombinant DNA

techniques. Of course, the DNA can be synthetic at any point during the isolation process or subsequent analysis.

[0216] Recombinant expression of an antibody, or fragment, derivative or analog thereof, *e.g.*, a heavy or light chain of an antibody which is a DR6 antagonist, requires construction of an expression vector containing a polynucleotide that encodes the antibody. Once a polynucleotide encoding an antibody molecule or a heavy or light chain of an antibody, or portion thereof (*e.g.*, containing the heavy or light chain variable domain), has been obtained, the vector for the production of the antibody molecule can be produced by recombinant DNA technology using techniques well known in the art. Thus, methods for preparing a protein by expressing a polynucleotide containing an antibody encoding nucleotide sequence are described herein. Methods which are well known to those skilled in the art can be used to construct expression vectors containing antibody coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. Also considered herein are replicable vectors comprising a nucleotide sequence encoding an antibody molecule, or a heavy or light chain thereof, or a heavy or light chain variable domain, operably linked to a promoter. Such vectors can include the nucleotide sequence encoding the constant region of the antibody molecule (see, *e.g.*, PCT Publication WO 86/05807; PCT Publication WO 89/01036; and U.S. Pat. No. 5,122,464) and the variable domain of the antibody can be cloned into such a vector for expression of the entire heavy or light chain.

[0217] The expression vector is transferred to a host cell by conventional techniques and the transfected cells are then cultured by conventional techniques to produce an antibody for use in the methods described herein. Thus, host cells containing a polynucleotide encoding an antibody, or a heavy or light chain thereof, operably linked to a heterologous promoter are also described herein. In certain embodiments for the expression of double-chained antibodies, vectors encoding both the heavy and light chains can be co-expressed in the host cell for expression of the entire immunoglobulin molecule, as detailed below.

[0218] A variety of host-expression vector systems can be utilized to express antibody molecules for use in the methods described elsewhere herein.

[0219] The host cell can be co-transfected with two expression vectors, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. The two vectors can contain identical selectable markers which enable equal

expression of heavy and light chain polypeptides. Alternatively, a single vector can be used which encodes both heavy and light chain polypeptides. In such situations, the light chain is advantageously placed before the heavy chain to avoid an excess of toxic free heavy chain (Proudfoot, *Nature 322*:52 (1986); Kohler, *Proc. Natl. Acad. Sci. USA 77*:2197 (1980)). The coding sequences for the heavy and light chains can comprise cDNA or genomic DNA.

[0220] Once an antibody molecule has been recombinantly expressed, it can be purified by any method known in the art for purification of an immunoglobulin molecule, for example, by chromatography (*e.g.*, ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. Alternatively, a method for increasing the affinity of antibodies is disclosed in US 2002 0123057 A1.

[0221] Furthermore, as described in more detail below, any of the DR6 antibodies or antibody fragments as described herein can be conjugated (covalently linked) to one or more polymers. In one particular embodiment, an antibody fragment that recognizes a specific epitope, for example, a Fab, $F(ab')_2$, Fv fragment or single chain antibody can be conjugated to a polymer. Examples of polymers suitable for such conjugation include polypeptides, sugar polymers and polyalkylene glycol chains (as described in more detail below). The class of polymer generally used is a polyalkylene glycol. Polyethylene glycol (PEG) is most frequently used. PEG moieties, *e.g.*, 1, 2, 3, 4 or 5 PEG polymers, can be conjugated to DR6 antibodies or fragments thereof to increase serum half life. PEG moieties are non-antigenic and essentially biologically inert. PEG moieties used can be branched or unbranched.

Polynucleotides Enocding DR6 Antibodies

[0222] The polynucleotides described herein include nucleic acid molecules encoding DR6 antibodies, or antigen-binding fragments, variants, or derivatives thereof.

[0223] In one embodiment, the polynucleotide an isolated polynucleotide comprising, consisting essentially of, or consisting of a nucleic acid encoding an immunoglobulin heavy chain variable region (VH), where at least one of the CDRs of the heavy chain variable region or at least two of the VH-CDRs of the heavy chain variable region are at least 80%, 85%, 90% or 95% identical to reference heavy chain VH-CDR1, VH-CDR2, or VH-CDR3 amino acid sequences from monoclonal DR6 antibodies disclosed herein. Alternatively, the VH-CDR1, VH-CDR2, and VH-CDR3 regions of the VH are at least 80%, 85%, 90% or 95% identical to reference heavy chain VH-CDR1, VH-CDR2, and VH-CDR3 amino acid sequences from

monoclonal DR6 antibodies disclosed herein. Thus, according to this embodiment a heavy chain variable region has VH-CDR1, VH-CDR2, or VH-CDR3 polypeptide sequences related to the polypeptide sequences shown in Table 5.

[0224] In another embodiment, the polynucleotide is an isolated polynucleotide comprising, consisting essentially of, or consisting of a nucleic acid encoding an immunoglobulin light chain variable region (VL), where at least one of the VL-CDRs of the light chain variable region or at least two of the VL-CDRs of the light chain variable region are at least 80%, 85%, 90% or 95% identical to reference light chain VL-CDR1, VL-CDR2, or VL-CDR3 amino acid sequences from monoclonal DR6 antibodies disclosed herein. Alternatively, the VL-CDR1, VL-CDR2, and VL-CDR3 regions of the VL are at least 80%, 85%, 90% or 95% identical to reference light chain VL-CDR1, VL-CDR2, and VL-CDR3 amino acid sequences from monoclonal DR6 antibodies disclosed herein. Thus, according to this embodiment a light chain variable region has VL-CDR1, VL-CDR2, or VL-CDR3 polypeptide sequences related to the polypeptide sequences shown in Table 5.

Table 5: DR6 Antibody Sequence SEQ ID NOs

Antibody	VH PN	VH PP	VH CDR1	VH CDR2	VH CDR3	VL PN	VL PP	VL CDR1	VL CDR2	VL CDR2
M50-H01	6	7	8	9	10	11	12	13	14	15
M51-H09	16	17	18	19	20	21	22	23	24	25
M53-E04	26	27	28	29	30	31	32	33	34	35
M53-F04	36	37	38	39	40	41	42	43	44	45
M62-B02	46	47	48	49	50	51	52	53	54	55
M63-E10	56	57	58	59	60	61	62	63	64	65
M66-B03	66	67	68	69	70	71	72	73	74	75
M67-G02	76	77	78	79	80	81	82	83	84	85
M72-F03	86	87	88	89	90	91	92	93	94	95
M73-C04	96	97	98	99	100	101	102	103	104	105
1P1D6.3	106	107	108	109	110	111	112	113	114	115
1P2F2.1	116	117	118	129	130	131	132	133	134	135
1P5D10.2	126	127	128	139	140	141	142	143	144	145

[0225] As known in the art, "sequence identity" between two polypeptides or two polynucleotides is determined by comparing the amino acid or nucleic acid sequence of one polypeptide or polynucleotide to the sequence of a second polypeptide or polynucleotide. When discussed herein, whether any particular polypeptide is at least about 40%, 45%, 50%, 55%,

60%, 65%, 70%, 75%, 80%, 85%, 90% or 95% identical to another polypeptide can be determined using methods and computer programs/software known in the art such as, but not limited to, the BESTFIT program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711). BESTFIT uses the local homology algorithm of Smith and Waterman, Advances in Applied Mathematics 2:482-489 (1981), to find the best segment of homology between two sequences. When using BESTFIT or any other sequence alignment program to determine whether a particular sequence is, for example, 95% identical to a reference sequence, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference polypeptide sequence and that gaps in homology of up to 5% of the total number of amino acids in the reference sequence are allowed.

[0226] In certain embodiments, an antibody or antigen-binding fragment comprising the VH encoded by the polynucleotide specifically or preferentially binds to DR6. In certain embodiments the nucleotide sequence encoding the VH polypeptide is altered without altering the amino acid sequence encoded thereby. For instance, the sequence can be altered for improved codon usage in a given species, to remove splice sites, or the remove restriction enzyme sites. Sequence optimizations such as these are described in the examples and are well known and routinely carried out by those of ordinary skill in the art.

[0227] In another embodiment, the polynucleotide is isolated polynucleotide comprising, consisting essentially of, or consisting of a nucleic acid encoding an immunoglobulin heavy chain variable region (VH) in which the VH-CDR1, VH-CDR2, and VH-CDR3 regions have polypeptide sequences which are identical to the VH-CDR1, VH-CDR2, and VH-CDR3 groups shown in Table 5. In certain embodiments, an antibody or antigen-binding fragment comprising the VH encoded by the polynucleotide specifically or preferentially binds to DR6.

[0228] In some embodiments, the polynucleotide is an isolated polynucleotide comprising a nucleic acid which encodes an antibody VH polypeptide, where the VH polypeptide comprises VH-CDR1, VH-CDR2, and VH-CDR3 amino acid sequences selected from the group consisting of: SEQ ID NOs: 8, 9, and 10; SEQ ID NOs: 18, 19, and 20; SEQ ID NOs: 28, 29, and 30; SEQ ID NOs: 38, 39, and 40; SEQ ID NOs: 48, 49, and 50; SEQ ID NOs: 58, 59, and 60; SEQ ID NOs: 68, 69, and 70; SEQ ID NOs: 78, 79, and 80; SEQ ID NOs: 88, 89, and 90; SEQ ID NOs: 98, 99, and 100; SEQ ID NOs: 108, 109, and 110; SEQ ID NOs: 118, 119, and 120; and

SEQ ID NOs: 128, 129, and 130; and where an antibody or antigen binding fragment thereof comprising the VH-CDR3 specifically binds to DR6.

[0229] In certain embodiments, an antibody or antigen-binding fragment thereof comprising, consisting essentially of, or consisting of a VH encoded by one or more of the polynucleotides described above specifically or preferentially binds to the same DR6 epitope as a reference monoclonal Fab antibody fragment selected from the group consisting of M50-H01, M51-H09, M53-E04, M53-F04, M62-B02, M63-E10, M66-B03, M67-G02, M72-F03, and M73-C04 or a reference monoclonal antibody selected from the group consisting of 1P1D6.3, 1P2F2.1, and 1P5D10.2, or will competitively inhibit such a monoclonal antibody or fragment from binding to DR6.

[0230] In certain embodiments, an antibody or antigen-binding fragment thereof comprising, consisting essentially of, or consisting of a VH encoded by one or more of the polynucleotides described above specifically or preferentially binds to a DR6 polypeptide or fragment thereof, or a DR6 variant polypeptide, with an affinity characterized by a dissociation constant (KD) no greater than 5×10^{-2} M, 10^{-2} M, 5×10^{-3} M, 10^{-3} M, 5×10^{-4} M, 10^{-4} M, 5×10^{-5} M, 10^{-5} M, 5×10^{-6} M, 10^{-6} M, 5×10^{-7} M, 10^{-7} M, 5×10^{-8} M, 10^{-8} M, 5×10^{-9} M, 10^{-9} M, 5×10^{-10} M, 10^{-10} M, 5×10^{-11} M, 10^{-11} M, 5×10^{-12} M, 10^{-12} M, 5×10^{-13} M, 10^{-13} M, 5×10^{-14} M, 10^{-14} M, 5×10^{-15} M, or 10^{-15} M.

[0231] In certain embodiments, an antibody or antigen-binding fragment comprising the VL encoded by the polynucleotide specifically or preferentially binds to DR6.

[0232] In another embodiment, the polynucleotide is an isolated polynucleotide comprising, consisting essentially of, or consisting of a nucleic acid encoding an immunoglobulin light chain variable region (VL) in which the VL-CDR1, VL-CDR2, and VL-CDR3 regions have polypeptide sequences which are identical to the VL-CDR1, VL-CDR2, and VL-CDR3 groups shown in Table 5. In certain embodiments, an antibody or antigen-binding fragment comprising the VL encoded by the polynucleotide specifically or preferentially binds to DR6.

[0233] In a further aspect, the polynucleotide is an isolated polynucleotide comprising, consisting essentially of, or consisting of a nucleic acid encoding an immunoglobulin light chain variable region (VL) in which the VL-CDR1, VL-CDR2, and VL-CDR3 regions are encoded by nucleotide sequences which are identical to the nucleotide sequences which encode the VL-CDR1, VL-CDR2, and VL-CDR3 groups shown in Table 5. In certain embodiments, an

antibody or antigen-binding fragment comprising the VL encoded by the polynucleotide specifically or preferentially binds to DR6.

[0234] In some embodiments, the polynucleotide is an isolated polynucleotide comprising a nucleic acid which encodes an antibody VL polypeptide, wherein said VL polypeptide comprises VL-CDR1, VL-CDR2, and VL-CDR3 amino acid sequences selected from the group consisting of: SEQ ID NOs: 13, 14, and 15; SEQ ID NOs: 23, 24, and 25; SEQ ID NOs: 33, 34, and 35; SEQ ID NOs: 43, 44, and 45; SEQ ID NOs: 53, 54, and 55; SEQ ID NOs: 63, 64, and 65; SEQ ID NOs: 73, 74, and 75; SEQ ID NOs: 83, 84, and 85; SEQ ID NOs: 93, 94, and 95; SEQ ID NOs: 103, 104, and 105; SEQ ID NOs: 113, 114, and 115; SEQ ID NOs: 123, 124, and 125; and SEQ ID NOs: 133, 134, and 135; and wherein an antibody or antigen binding fragment thereof comprising said VL-CDR3 specifically binds to DR6.

[0235] In certain embodiments, an antibody or antigen-binding fragment thereof comprising, consisting essentially of, or consisting of a VL encoded by one or more of the polynucleotides described above specifically or preferentially binds to the same DR6 epitope as a reference monoclonal Fab antibody fragment selected from the group consisting of M50-H01, M51-H09, M53-E04, M53-F04, M62-B02, M63-E10, M66-B03, M67-G02, M72-F03, and M73-C04 or a reference monoclonal antibody selected from the group consisting of 1P1D6.3, 1P2F2.1, and 1P5D10.2 or will competitively inhibit such a monoclonal antibody or fragment from binding to DR6.

[0236] In certain embodiments, an antibody or antigen-binding fragment thereof comprising, consisting essentially of, or consisting of a VL encoded by one or more of the polynucleotides described above specifically or preferentially binds to a DR6 polypeptide or fragment thereof, or a DR6 variant polypeptide, with an affinity characterized by a dissociation constant (K_D) no greater than 5×10^{-2} M, 10^{-2} M, 5×10^{-3} M, 10^{-3} M, 5×10^{-4} M, 10^{-4} M, 5×10^{-5} M, 10^{-5} M, 5×10^{-6} M, 10^{-6} M, 5×10^{-7} M, 10^{-7} M, 5×10^{-8} M, 10^{-8} M, 5×10^{-9} M, 10^{-9} M, 5×10^{-10} M, 10^{-10} M, 5×10^{-11} M, 10^{-11} M, 5×10^{-12} M, 10^{-12} M, 5×10^{-13} M, 10^{-13} M, 5×10^{-14} M, 10^{-14} M, 5×10^{-15} M, or 10^{-15} M.

[0237] In a further embodiment, the polynucleotide can be an isolated polynucleotide comprising, consisting essentially of, or consisting of a nucleic acid encoding a VH at least 80%, 85%, 90% 95% or 100% identical to a reference VH polypeptide sequence selected from the group consisting of SEQ ID NOs: 7, 17, 27, 37, 47, 57, 67, 77, 87, 97, 107, 117 and 127. In

certain embodiments, an antibody or antigen-binding fragment comprising the VH encoded by the polynucleotide specifically or preferentially binds to DR6.

[0238] In another aspect, the polynucleotide can be an isolated polynucleotide comprising, consisting essentially of, or consisting of a nucleic acid sequence encoding a VH having a polypeptide sequence selected from the group consisting of SEQ ID NOs: 7, 17, 27, 37, 47, 57, 67, 77, 87, 97, 107, 117 and 127. In certain embodiments, an antibody or antigen-binding fragment comprising the VH encoded by the polynucleotide specifically or preferentially binds to DR6.

[0239] In a further embodiment, the polynucleotide can be an isolated polynucleotide comprising, consisting essentially of, or consisting of a VH-encoding nucleic acid at least 80%, 85%, 90% 95% or 100% identical to a reference nucleic acid sequence selected from the group consisting of SEQ ID NOs: 6, 16, 26, 36, 46, 56, 66, 76, 86, 96, 106, 116, and 126. In certain embodiments, an antibody or antigen-binding fragment comprising the VH encoded by such polynucleotides specifically or preferentially binds to DR6.

[0240] In another aspect, the polynucleotide can be an isolated polynucleotide comprising, consisting essentially of, or consisting of a nucleic acid sequence encoding a VH, where the amino acid sequence of the VH is selected from the group consisting of SEQ ID NOs: 7, 17, 27, 37, 47, 57, 67, 77, 87, 97, 107, 117 and 127. The polynucleotide can also be an isolated polynucleotide comprising, consisting essentially of, or consisting of a nucleic acid sequence encoding a VH, where the sequence of the nucleic acid is selected from the group consisting of SEQ ID NOs: 6, 16, 26, 36, 46, 56, 66, 76, 86, 96, 106, 116, and 126. In certain embodiments, an antibody or antigen-binding fragment comprising the VH encoded by such polynucleotides specifically or preferentially binds to DR6.

[0241] In certain embodiments, an antibody or antigen-binding fragment thereof comprising, consisting essentially of, or consisting of a VH encoded by one or more of the polynucleotides described above specifically or preferentially binds to the same DR6 epitope as a reference monoclonal Fab antibody fragment selected from the group consisting of M50-H01, M51-H09, M53-E04, M53-F04, M62-B02, M63-E10, M66-B03, M67-G02, M72-F03, and M73-C04 or a reference monoclonal antibody selected from the group consisting of 1P1D6.3, 1P2F2.1, and 1P5D10.2, or will competitively inhibit such a monoclonal antibody or fragment from binding to DR6, or will competitively inhibit such a monoclonal antibody from binding to DR6.

[0242] In certain embodiments, an antibody or antigen-binding fragment thereof comprising, consisting essentially of, or consisting of a VH encoded by one or more of the polynucleotides described above specifically or preferentially binds to a DR6 polypeptide or fragment thereof, or a DR6 variant polypeptide, with an affinity characterized by a dissociation constant (K_D) no greater than 5×10^{-2} M, 10^{-2} M, 5×10^{-3} M, 10^{-3} M, 5×10^{-4} M, 10^{-4} M, 5×10^{-5} M, 10^{-5} M, 5×10^{-6} M, 10^{-6} M, 5×10^{-7} M, 10^{-7} M, 5×10^{-8} M, 10^{-8} M, 5×10^{-9} M, 10^{-9} M, 5×10^{-10} M, 10^{-10} M, 5×10^{-11} M, 10^{-11} M, 5×10^{-12} M, 10^{-12} M, 5×10^{-13} M, 10^{-13} M, 5×10^{-14} M, 10^{-14} M, 5×10^{-15} M, or 10^{-15} M.

[0243] In a further embodiment, the polynucleotide can be an isolated polynucleotide comprising, consisting essentially of, or consisting of a nucleic acid encoding a VL at least 80%, 85%, 90% 95% or 100% identical to a reference VL polypeptide sequence having an amino acid sequence selected from the group consisting of SEQ ID NOs: 12, 22, 32, 42, 52, 62, 72, 82, 92, 102, 112, 122 and 132. In a further embodiment, the polynucleotide can be an isolated polynucleotide comprising, consisting essentially of, or consisting of a VL-encoding nucleic acid at least 80%, 85%, 90% 95% or 100% identical to a reference nucleic acid sequence selected from the group consisting of SEQ ID NOs: 11, 21, 31, 41, 51, 61, 71, 81, 91, 101, 111, 121, and 131. In certain embodiments, an antibody or antigen-binding fragment comprising the VL encoded by such polynucleotides specifically or preferentially binds to DR6.

[0244] In another aspect, the polynucleotide can be an isolated polynucleotide comprising, consisting essentially of, or consisting of a nucleic acid sequence encoding a VL having a polypeptide sequence selected from the group consisting of SEQ ID NOs: 12, 22, 32, 42, 52, 62, 72, 82, 92, 102, 112, 122 and 132. The the polynucleotide can be an isolated polynucleotide comprising, consisting essentially of, or consisting of a nucleic acid sequence encoding a VL, where the sequence of the nucleic acid is selected from the group consisting of SEQ ID NOs: 11, 21, 31, 41, 51, 61, 71, 81, 91, 101, 111, 121, and 131. In certain embodiments, an antibody or antigen-binding fragment comprising the VL encoded by such polynucleotides specifically or preferentially binds to DR6.

[0245] In certain embodiments, an antibody or antigen-binding fragment thereof comprising, consisting essentially of, or consisting of a VL encoded by one or more of the polynucleotides described above specifically or preferentially binds to the same DR6 epitope as a reference monoclonal Fab antibody fragment selected from the group consisting of M50-H01, M51-H09, M53-E04, M53-F04, M62-B02, M63-E10, M66-B03, M67-G02, M72-F03, and M73-

C04 or a reference monoclonal antibody selected from the group consisting of 1P1D6.3, 1P2F2.1, and 1P5D10.2, or will competitively inhibit such a monoclonal antibody or fragment from binding to DR6.

[0246] In certain embodiments, an antibody or antigen-binding fragment thereof comprising, consisting essentially of, or consisting of a VL encoded by one or more of the polynucleotides described above specifically or preferentially binds to a DR6 polypeptide or fragment thereof, or a DR6 variant polypeptide, with an affinity characterized by a dissociation constant (K_D) no greater than 5×10^{-2} M, 10^{-2} M, 5×10^{-3} M, 10^{-3} M, 5×10^{-4} M, 10^{-4} M, 5×10^{-5} M, 10^{-5} M, 5×10^{-6} M, 10^{-6} M, 5×10^{-7} M, 10^{-7} M, 5×10^{-8} M, 10^{-8} M, 5×10^{-9} M, 10^{-9} M, 5×10^{-10} M, 10^{-10} M, 5×10^{-11} M, 10^{-11} M, 5×10^{-12} M, 10^{-12} M, 5×10^{-13} M, 10^{-13} M, 5×10^{-14} M, 10^{-14} M, 5×10^{-15} M, or 10^{-15} M.

[0247] Any of the polynucleotides described above can further include additional nucleic acids, encoding, *e.g.*, a signal peptide to direct secretion of the encoded polypeptide, antibody constant regions as described herein, or other heterologous polypeptides as described herein.

[0248] Also, as described in more detail elsewhere herein, the compositions include compositions comprising the polynucleotides comprising one or more of the polynucleotides described above. In one embodiment, the compositions includes compositions comprising a first polynucleotide and second polynucleotide wherein said first polynucleotide encodes a VH polypeptide as described herein and wherein said second polynucleotide encodes a VL polypeptide as described herein. Specifically a composition which comprises, consists essentially of, or consists of a VH polynucleotide, and a VL polynucleotide, wherein the VH polynucleotide and the VL polynucleotide encode polypeptides, respectively at least 80%, 85%, 90% 95% or 100% identical to reference VH and VL polypeptide amino acid sequences selected from the group consisting of SEQ ID NOs: 7 and 12, 17 and 22, 27 and 32, 37 and 42, 47 and 52, 57 and 62, 67 and 72, 77 and 82, 87 and 92, 97 and 102, 107 and 112, 117 and 122 and 127 and 132. Or alternatively, a composition which comprises, consists essentially of, or consists of a VH polynucleotide, and a VL polynucleotide at least 80%, 85%, 90% 95% or 100% identical, respectively, to reference VL and VL nucleic acid sequences selected from the group consisting of SEQ ID NOs: 6 and 11, 16 and 21, 26 and 31, 36 and 41, 46 and 51, 56 and 61, 66 and 71, 76 and 81, 86 and 91, 96 and 101, 106 and 111, 116 and 121, and 126 and 131. In certain embodiments, an antibody or antigen-binding fragment comprising the VH and VL encoded by the polynucleotides in such compositions specifically or preferentially binds to DR6.

[0249] The polynucleotides described herein also include fragments of the polynucleotides, as described elsewhere. Additionally polynucleotides which encode fusion polynucleotides, Fab fragments, and other derivatives, as described herein, are also contemplated.

[0250] The polynucleotides can be produced or manufactured by any method known in the art. For example, if the nucleotide sequence of the antibody is known, a polynucleotide encoding the antibody can be assembled from chemically synthesized oligonucleotides (*e.g.*, as described in Kutmeier *et al.*, *BioTechniques 17*:242 (1994)), which, briefly, involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding the antibody, annealing and ligating of those oligonucleotides, and then amplification of the ligated oligonucleotides by PCR.

[0251] Alternatively, a polynucleotide encoding a DR6 antibody, or antigen-binding fragment, variant, or derivative thereof can be generated from nucleic acid from a suitable source. If a clone containing a nucleic acid encoding a particular antibody is not available, but the sequence of the antibody molecule is known, a nucleic acid encoding the antibody can be chemically synthesized or obtained from a suitable source (*e.g.*, an antibody cDNA library, or a cDNA library generated from, or nucleic acid, such as poly A+RNA, isolated from, any tissue or cells expressing the antibody or other DR6 antibody, such as hybridoma cells selected to express an antibody) by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence to identify, *e.g.*, a cDNA clone from a cDNA library that encodes the antibody or other DR6 antibody. Amplified nucleic acids generated by PCR can then be cloned into replicable cloning vectors using any method well known in the art.

[0252] Once the nucleotide sequence and corresponding amino acid sequence of the DR6 antibody, or antigen-binding fragment, variant, or derivative thereof is determined, its nucleotide sequence can be manipulated using methods well known in the art for the manipulation of nucleotide sequences, *e.g.*, recombinant DNA techniques, site directed mutagenesis, PCR, etc. (see, for example, the techniques described in Sambrook *et al.*, *Molecular Cloning, A Laboratory Manual*, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. (1990) and Ausubel *et al.*, eds., *Current Protocols in Molecular Biology*, John Wiley & Sons, NY (1998), which are both incorporated by reference herein in their entireties), to generate antibodies having a different amino acid sequence, for example to create amino acid substitutions, deletions, and/or insertions.

[0253] A polynucleotide encoding a DR6 antibody, or antigen-binding fragment, variant, or derivative thereof can be composed of any polyribonucleotide or polydeoxribonucleotide, which can be unmodified RNA or DNA or modified RNA or DNA. For example, a polynucleotide encoding DR6 antibody, or antigen-binding fragment, variant, or derivative thereof can be composed of single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that can be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, a polynucleotide encoding a DR6 antibody, or antigen-binding fragment, variant, or derivative thereof can be composed of triple-stranded regions comprising RNA or DNA or both RNA and DNA. A polynucleotide encoding a DR6 antibody, or antigen-binding fragment, variant, or derivative thereof can also contain one or more modified bases or DNA or RNA backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications can be made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically, or metabolically modified forms.

[0254] An isolated polynucleotide encoding a non-natural variant of a polypeptide derived from an immunoglobulin (*e.g.*, an immunoglobulin heavy chain portion or light chain portion) can be created by introducing one or more nucleotide substitutions, additions or deletions into the nucleotide sequence of the immunoglobulin such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein. Mutations can be introduced by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Conservative amino acid substitutions can be made at one or more non-essential amino acid residues.

DR6 Antibody Polypeptides

[0255] Isolated polypeptides which make up DR6 antibodies, and polynucleotides encoding such polypeptides are also described herein. DR6 antibodies comprise polypeptides, *e.g.*, amino acid sequences encoding DR6-specific antigen binding regions derived from immunoglobulin molecules. A polypeptide or amino acid sequence "derived from" a designated protein refers to the origin of the polypeptide having a certain amino acid sequence. In certain cases, the polypeptide or amino acid sequence which is derived from a particular starting polypeptide or amino acid sequence has an amino acid sequence that is essentially identical to

that of the starting sequence, or a portion thereof, wherein the portion consists of at least 10-20 amino acids, at least 20-30 amino acids, at least 30-50 amino acids, or which is otherwise identifiable to one of ordinary skill in the art as having its origin in the starting sequence.

[0256] In one embodiment, the polypeptide can be an isolated polypeptide comprising, consisting essentially of, or consisting of an immunoglobulin heavy chain variable region (VH), where at least one of VH-CDRs of the heavy chain variable region or at least two of the VH-CDRs of the heavy chain variable region are at least 80%, 85%, 90% or 95% identical to reference heavy chain VH-CDR1, VH-CDR2 or VH-CDR3 amino acid sequences from monoclonal DR6 antibodies disclosed herein. Alternatively, the VH-CDR1, VH-CDR2 and VH-CDR3 regions of the VH are at least 80%, 85%, 90% or 95% identical to reference heavy chain VH-CDR1, VH-CDR2 and VH-CDR3 amino acid sequences from monoclonal DR6 antibodies disclosed herein. Thus, according to this embodiment a heavy chain variable region has VH-CDR1, VH-CDR2 and VH-CDR3 polypeptide sequences related to the groups shown in Table 5, *supra*. While Table 5 shows VH-CDRs defined by the Kabat system, other CDR definitions, *e.g.*, VH-CDRs defined by the Chothia system, are also described. In certain embodiments, an antibody or antigen-binding fragment comprising the VH specifically or preferentially binds to DR6.

[0257] In another embodiment, the polypeptide can be an isolated polypeptide comprising, consisting essentially of, or consisting of an immunoglobulin heavy chain variable region (VH) in which the VH-CDR1, VH-CDR2 and VH-CDR3 regions have polypeptide sequences which are identical to the VH-CDR1, VH-CDR2 and VH-CDR3 groups shown in Table 5. In certain embodiments, an antibody or antigen-binding fragment comprising the VH specifically or preferentially binds to DR6.

[0258] In another embodiment, the polypeptide can be an isolated polypeptide comprising, consisting essentially of, or consisting of an immunoglobulin heavy chain variable region (VH) in which the VH-CDR1, VH-CDR2 and VH-CDR3 regions have polypeptide sequences which are identical to the VH-CDR1, VH-CDR2 and VH-CDR3 groups shown in Table 5, except for one, two, three, four, five, or six amino acid substitutions in any one VH-CDR. In larger CDRs, *e.g.*, VH-CDR-3, additional substitutions can be made in the CDR, as long as the a VH comprising the VH-CDR specifically or preferentially binds to DR6. In certain embodiments the amino acid substitutions are conservative. In certain embodiments, an antibody or antigen-binding fragment comprising the VH specifically or preferentially binds to DR6.

[0259] In some embodiments, the polypeptide can be an isolated polypeptide comprising, consisting essentially of, or consisting of an immunoglobulin heavy chain variable region (VH) in which the VH-CDR1, VH-CDR2 and VH-CDR3 regions have polypeptide sequences selected from the group consisting of: SEQ ID NOs: 8, 9, and 10; SEQ ID NOs: 18, 19, and 20; SEQ ID NOs: 28, 29, and 30; SEQ ID NOs: 38, 39, and 40; SEQ ID NOs: 48, 49, and 50; SEQ ID NOs: 58, 59, and 60; SEQ ID NOs: 68, 69, and 70; SEQ ID NOs: 78, 79, and 80; SEQ ID NOs: 88, 89, and 90; SEQ ID NOs: 98, 99, and 100; SEQ ID NOs: 108, 109, and 110; SEQ ID NOs: 118, 119, and 120; and SEQ ID NOs: 128, 129 and 130, except for one, two, three, four, five or six amino acid substitutions in at least one of said VH-CDRs.

[0260] In some embodiments, the polypeptide can be an isolated polypeptide comprising, consisting essentially of, or consisting of an immunoglobulin heavy chain variable region (VH) in which the VH-CDR1, VH-CDR2 and VH-CDR3 regions have polypeptide sequences selected from the group consisting of: SEQ ID NOs: 8, 9, and 10; SEQ ID NOs: 18, 19, and 20; SEQ ID NOs: 28, 29, and 30; SEQ ID NOs: 38, 39, and 40; SEQ ID NOs: 48, 49, and 50; SEQ ID NOs: 58, 59, and 60; SEQ ID NOs: 68, 69, and 70; SEQ ID NOs: 78, 79, and 80; SEQ ID NOs: 88, 89, and 90; SEQ ID NOs: 98, 99, and 100; SEQ ID NOs: 108, 109, and 110; SEQ ID NOs: 118, 119, and 120; and SEQ ID NOs: 128, 129 and 130.

[0261] In a further embodiment, the polypeptide can be an isolated polypeptide comprising, consisting essentially of, or consisting of a VH polypeptide at least 80%, 85%, 90% 95% or 100% identical to a reference VH polypeptide amino acid sequence selected from the group consisting of SEQ ID NOs: 7, 17, 27, 37, 47, 57, 67, 77, 87, 97, 107, 117, and 127. In certain embodiments, an antibody or antigen-binding fragment comprising the VH polypeptide specifically or preferentially binds to DR6.

[0262] In another aspect, the polypeptide can be an isolated polypeptide comprising, consisting essentially of, or consisting of a VH polypeptide selected from the group consisting of SEQ ID NOs: 7, 17, 27, 37, 47, 57, 67, 77, 87, 97, 107, 117, and 127. In certain embodiments, an antibody or antigen-binding fragment comprising the VH polypeptide specifically or preferentially binds to DR6.

[0263] In certain embodiments, an antibody or antigen-binding fragment thereof comprising, consisting essentially of, or consisting of a one or more of the VH polypeptides described above specifically or preferentially binds to the same DR6 epitope as a reference monoclonal Fab antibody fragment selected from the group consisting of M50-H01, M51-H09,

M53-E04, M53-F04, M62-B02, M63-E10, M66-B03, M67-G02, M72-F03, and M73-C04 or a reference monoclonal antibody selected from the group consisting of 1P1D6.3, 1P2F2.1, and 1P5D10.2, or will competitively inhibit such a monoclonal antibody or fragment from binding to DR6.

[0264] In certain embodiments, an antibody or antigen-binding fragment thereof comprising, consisting essentially of, or consisting of one or more of the VH polypeptides described above specifically or preferentially binds to a DR6 polypeptide or fragment thereof, or a DR6 variant polypeptide, with an affinity characterized by a dissociation constant (K_D) no greater than 5×10^{-2} M, 10^{-2} M, 5×10^{-3} M, 10^{-3} M, 5×10^{-4} M, 10^{-4} M, 5×10^{-5} M, 10^{-5} M, 5×10^{-6} M, 10^{-6} M, 5×10^{-7} M, 10^{-7} M, 5×10^{-8} M, 10^{-8} M, 5×10^{-9} M, 10^{-9} M, 5×10^{-10} M, 10^{-10} M, 5×10^{-11} M, 10^{-11} M, 5×10^{-12} M, 10^{-12} M, 5×10^{-13} M, 10^{-13} M, 5×10^{-14} M, 10^{-14} M, 5×10^{-15} M, or 10^{-15} M.

[0265] In another embodiment, the polypeptide can be an isolated polypeptide comprising, consisting essentially of, or consisting of an immunoglobulin light chain variable region (VL), where at least one of the VL-CDRs of the light chain variable region or at least two of the VL-CDRs of the light chain variable region are at least 80%, 85%, 90% or 95% identical to reference light chain VL-CDR1, VL-CDR2 or VL-CDR3 amino acid sequences from monoclonal DR6 antibodies disclosed herein. Alternatively, the VL-CDR1, VL-CDR2 and VL-CDR3 regions of the VL are at least 80%, 85%, 90% or 95% identical to reference light chain VL-CDR1, VL-CDR2 and VL-CDR3 amino acid sequences from monoclonal DR6 antibodies disclosed herein. Thus, according to this embodiment a light chain variable region has VL-CDR1, VL-CDR2 and VL-CDR3 polypeptide sequences related to the polypeptides shown in Table 5, *supra*. While Table 5 shows VL-CDRs defined by the Kabat system, other CDR definitions, *e.g.*, VL-CDRs defined by the Chothia system, are also described. In certain embodiments, an antibody or antigen-binding fragment comprising the VL polypeptide specifically or preferentially binds to DR6.

[0266] In another embodiment, the polypeptide can be an isolated polypeptide comprising, consisting essentially of, or consisting of an immunoglobulin light chain variable region (VL) in which the VL-CDR1, VL-CDR2 and VL-CDR3 regions have polypeptide sequences which are identical to the VL-CDR1, VL-CDR2 and VL-CDR3 groups shown in Table 5. In certain embodiments, an antibody or antigen-binding fragment comprising the VL polypeptide specifically or preferentially binds to DR6.

[0267] In another embodiment, the polypeptide can be an isolated polypeptide comprising, consisting essentially of, or consisting of an immunoglobulin heavy chain variable region (VL) in which the VL-CDR1, VL-CDR2 and VL-CDR3 regions have polypeptide sequences which are identical to the VL-CDR1, VL-CDR2 and VL-CDR3 groups shown in Table 5, except for one, two, three, four, five, or six amino acid substitutions in any one VL-CDR. In larger CDRs, additional substitutions can be made in the VL-CDR, as long as the a VL comprising the VL-CDR specifically or preferentially binds to DR6. In certain embodiments the amino acid substitutions are conservative. In certain embodiments, an antibody or antigen-binding fragment comprising the VL specifically or preferentially binds to DR6.

[0268] In some embodiments, the polypeptide can be an isolated polypeptide comprising, consisting essentially of, or consisting of an immunoglobulin heavy chain variable region (VL) in which the VL-CDR1, VL-CDR2 and VL-CDR3 regions have polypeptide sequences selected from the group consisting of: SEQ ID NOs: 13, 14, and 15; SEQ ID NOs: 23, 24, and 25; SEQ ID NOs: 33, 34, and 35; SEQ ID NOs: 43, 44, and 45; SEQ ID NOs: 53, 54, and 55; SEQ ID NOs: 63, 64, and 65; SEQ ID NOs: 73, 74, and 75; SEQ ID NOs: 83, 84, and 85; SEQ ID NOs: 93, 94, and 95; SEQ ID NOs: 103, 104, and 105; SEQ ID NOs: 113, 114 and 115; SEQ ID NOs: 123, 124, and 125; and SEQ ID NOs: 133, 134 and 135, except for one, two, three, four, five or six amino acid substitutions in at least one of said VL-CDRs.

[0269] In some embodiments, the polypeptide can be an isolated polypeptide comprising, consisting essentially of, or consisting of an immunoglobulin heavy chain variable region (VL) in which the VL-CDR1, VL-CDR2 and VL-CDR3 regions have polypeptide sequences selected from the group consisting of: SEQ ID NOs: 13, 14, and 15; SEQ ID NOs: 23, 24, and 25; SEQ ID NOs: 33, 34, and 35; SEQ ID NOs: 43, 44, and 45; SEQ ID NOs: 53, 54, and 55; SEQ ID NOs: 63, 64, and 65; SEQ ID NOs: 73, 74, and 75; SEQ ID NOs: 83, 84, and 85; SEQ ID NOs: 93, 94, and 95; SEQ ID NOs: 103, 104, and 105; SEQ ID NOs: 113, 114 and 115; SEQ ID NOs: 123, 124, and 125; and SEQ ID NOs: 133, 134 and 135.

[0270] In a further embodiment, the polypeptide can be an isolated polypeptide comprising, consisting essentially of, or consisting of a VL polypeptide at least 80%, 85%, 90% 95% or 100% identical to a reference VL polypeptide sequence selected from the group consisting of SEQ ID NOs: 12, 22, 32, 42, 52, 62, 72, 82, 92, 102, 112, 122, and 132. In certain embodiments, an antibody or antigen-binding fragment comprising the VL polypeptide specifically or preferentially binds to DR6.

[0271] In another aspect, the polypeptide can be an isolated polypeptide comprising, consisting essentially of, or consisting of a VL polypeptide selected from the group consisting of SEQ ID NOs: 12, 22, 32, 42, 52, 62, 72, 82, 92, 102, 112, 122, and 132. In certain embodiments, an antibody or antigen-binding fragment comprising the VL polypeptide specifically or preferentially binds to DR6.

[0272] In certain embodiments, an antibody or antigen-binding fragment thereof comprising, consisting essentially of, one or more of the VL polypeptides described above specifically or preferentially binds to the same DR6 epitope as a reference monoclonal Fab antibody fragment selected from the group consisting of M50-H01, M51-H09, M53-E04, M53-F04, M62-B02, M63-E10, M66-B03, M67-G02, M72-F03, and M73-C04 or a reference monoclonal antibody selected from the group consisting of 1P1D6.3, 1P2F2.1, and 1P5D10.2, or will competitively inhibit such a monoclonal antibody or fragment from binding to DR6.

[0273] In certain embodiments, an antibody or antigen-binding fragment thereof comprising, consisting essentially of, or consisting of a one or more of the VL polypeptides described above specifically or preferentially binds to a DR6 polypeptide or fragment thereof, or a DR6 variant polypeptide, with an affinity characterized by a dissociation constant (K_D) no greater than 5×10^{-2} M, 10^{-2} M, 5×10^{-3} M, 10^{-3} M, 5×10^{-4} M, 10^{-4} M, 5×10^{-5} M, 10^{-5} M, 5×10^{6} M, 10^{-6} M, 5×10^{-7} M, 10^{-7} M, 5×10^{-8} M, 10^{-8} M, 5×10^{-9} M, 10^{-9} M, 5×10^{-10} M, 10^{-10} M, 5×10^{-11} M, 10^{-11} M, 5×10^{-12} M, 10^{-12} M, 5×10^{-13} M, 10^{-13} M, 5×10^{-14} M, 10^{-14} M, 5×10^{-15} M, or 10^{-15} M.

[0274] In other embodiments, an antibody or antigen-binding fragment thereof comprises, consists essentially of or consists of a VH polypeptide, and a VL polypeptide, where the VH polypeptide and the VL polypeptide, respectively are at least 80%, 85%, 90% 95% or 100% identical to reference VH and VL polypeptide amino acid sequences selected from the group consisting of SEQ ID NOs: 7 and 12, 17 and 22, 27 and 32, 37 and 42, 47 and 52, 57 and 62, 67 and 72, 77 and 82, 87 and 92, 97 and 102, 107 and 112, 117 and 122 and 127 and 132. In certain embodiments, an antibody or antigen-binding fragment comprising these VH and VL polypeptides specifically or preferentially binds to DR6.

[0275] Any of the polypeptides described above can further include additional polypeptides, *e.g.*, a signal peptide to direct secretion of the encoded polypeptide, antibody constant regions as described herein, or other heterologous polypeptides as described herein. Additionally, polypeptides include polypeptide fragments as described elsewhere. Additionally

polypeptides include fusion polypeptide, Fab fragments, and other derivatives, as described herein.

[0276] Also, as described in more detail elsewhere herein, the present compositions include compositions comprising the polypeptides described above.

[0277] It will also be understood by one of ordinary skill in the art that DR6 antibody polypeptides as disclosed herein can be modified such that they vary in amino acid sequence from the naturally occurring binding polypeptide from which they were derived. For example, a polypeptide or amino acid sequence derived from a designated protein can be similar, *e.g.*, have a certain percent identity to the starting sequence, *e.g.*, it can be 60%, 70%, 75%, 80%, 85%, 90%, or 95% identical to the starting sequence.

[0278] Furthermore, nucleotide or amino acid substitutions, deletions, or insertions leading to conservative substitutions or changes at "non-essential" amino acid regions can be made. For example, a polypeptide or amino acid sequence derived from a designated protein can be identical to the starting sequence except for one or more individual amino acid substitutions, insertions, or deletions, *e.g.*, one, two, three, four, five, six, seven, eight, nine, ten, fifteen, twenty or more individual amino acid substitutions, insertions, or deletions. A polypeptide or amino acid sequence derived from a designated protein can be identical to the starting sequence except for one or more individual amino acid substitutions, insertions, or deletions, *e.g.*, one, two, three, four, five, six, seven, eight, nine, ten, fifteen, twenty or more individual amino acid substitutions, insertions, or deletions. In other embodiments, a polypeptide or amino acid sequence derived from a designated protein can be identical to the starting sequence except for two or fewer, three or fewer, four or fewer, five or fewer, six or fewer, seven or fewer, eight or fewer, nine or fewer, ten or fewer, fifteen or fewer, or twenty or fewer individual amino acid substitutions, insertions, or deletions. In certain embodiments, a polypeptide or amino acid sequence derived from a designated protein has one to five, one to ten, one to fifteen, or one to twenty individual amino acid substitutions, insertions, or deletions relative to the starting sequence.

[0279] Certain DR6 antibody polypeptides comprise, consist essentially of, or consist of an amino acid sequence derived from a human amino acid sequence. However, certain DR6 antibody polypeptides comprise one or more contiguous amino acids derived from another mammalian species. For example, a DR6 antibody can include a primate heavy chain portion, hinge portion, or antigen binding region. In another example, one or more murine-derived amino acids can be present in a non-murine antibody polypeptide, *e.g.*, in an antigen binding site of a

DR6 antibody. In another example, the antigen binding site of a DR6 antibody is fully murine. In certain therapeutic applications, DR6-specific antibodies, or antigen-binding fragments, variants, or analogs thereof are designed so as to not be immunogenic in the animal to which the antibody is administered.

[0280] In certain embodiments, a DR6 antibody polypeptide comprises an amino acid sequence or one or more moieties not normally associated with an antibody. Exemplary modifications are described in more detail below. For example, a single-chain fv antibody fragment can comprise a flexible linker sequence, or can be modified to add a functional moiety (*e.g.*, PEG, a drug, a toxin, or a label).

[0281] An DR6 antibody polypeptide can comprise, consist essentially of, or consist of a fusion protein. Fusion proteins are chimeric molecules which comprise, for example, an immunoglobulin antigen-binding domain with at least one target binding site, and at least one heterologous portion, *i.e.*, a portion with which it is not naturally linked in nature. The amino acid sequences can normally exist in separate proteins that are brought together in the fusion polypeptide or they can normally exist in the same protein but are placed in a new arrangement in the fusion polypeptide. Fusion proteins can be created, for example, by chemical synthesis, or by creating and translating a polynucleotide in which the peptide regions are encoded in the desired relationship.

[0282] The term "heterologous" as applied to a polynucleotide or a polypeptide, means that the polynucleotide or polypeptide is derived from a distinct entity from that of the rest of the entity to which it is being compared. For instance, as used herein, a "heterologous polypeptide" to be fused to a DR6 antibody, or an antigen-binding fragment, variant, or analog thereof is derived from a non-immunoglobulin polypeptide of the same species, or an immunoglobulin or non-immunoglobulin polypeptide of a different species.

[0283] A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art, including basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Thus, a nonessential amino acid residue in an

immunoglobulin polypeptide can be replaced with another amino acid residue from the same side chain family. In another embodiment, a string of amino acids can be replaced with a structurally similar string that differs in order and/or composition of side chain family members.

[0284] Alternatively, in another embodiment, mutations can be introduced randomly along all or part of the immunoglobulin coding sequence, such as by saturation mutagenesis, and the resultant mutants can be incorporated into DR6 antibodies for use in the diagnostic and treatment methods disclosed herein and screened for their ability to bind to the desired antigen, *e.g.*, DR6.

Fusion Polypeptides and Antibodies

[0285] DR6 polypeptides and antibodies for use in the treatment methods disclosed herein can further be recombinantly fused to a heterologous polypeptide at the N- or C-terminus. For example, DR6 antagonist polypeptides or antibodies can be recombinantly fused or conjugated to molecules useful as labels in detection assays and effector molecules such as heterologous polypeptides, drugs, radionuclides, or toxins. *See, e.g.*, PCT publications WO 92/08495; WO 91/14438; WO 89/12624; U.S. Patent No. 5,314,995; and EP 396,387.

[0286] DR6 antagonist polypeptides and antibodies for use in the treatment methods disclosed herein can be composed of amino acids joined to each other by peptide bonds or modified peptide bonds, *i.e.*, peptide isosteres, and can contain amino acids other than the 20 gene-encoded amino acids.

[0287] DR6 antagonists include fusion proteins comprising, consisting essentially of, or consisting of a DR6 antagonist polypeptide or antibody fusion that inhibits DR6 function. In certain embodiments, the heterologous polypeptide to which the DR6 antagonist polypeptide or antibody is fused is useful for function or is useful to target the DR6 antagonist polypeptide or antibody. In certain embodiments, a soluble DR6 antagonist polypeptide, *e.g.*, a DR6 polypeptide comprising the extracellular domain (corresponding to amino acids 1 to 349 or 41 to 349 of SEQ ID NO: 2), or any other soluble DR6 polypeptide fragment, variant or derivative described herein, is fused to a heterologous polypeptide moiety to form a DR6 antagonist fusion polypeptide. DR6 antagonist fusion proteins and antibodies can be used to accomplish various objectives, *e.g.*, increased serum half-life, improved bioavailability, *in vivo* targeting to a specific organ or tissue type, improved recombinant expression efficiency, improved host cell secretion, ease of purification, and higher avidity. Depending on the objective(s) to be achieved, the heterologous moiety can be inert or biologically active. Also, it can be chosen to be stably fused

to the DR6 antagonist polypeptide or antibody or to be cleavable, *in vitro* or *in vivo*. Heterologous moieties to accomplish these other objectives are known in the art.

[0288] As an alternative to expression of a DR6 antagonist fusion polypeptide or antibody, a chosen heterologous moiety can be preformed and chemically conjugated to the DR6 antagonist polypeptide or antibody. In most cases, a chosen heterologous moiety will function similarly, whether fused or conjugated to the DR6 antagonist polypeptide or antibody. Therefore, in the following discussion of heterologous amino acid sequences, unless otherwise noted, it is to be understood that the heterologous sequence can be joined to the DR6 antagonist polypeptide or antibody in the form of a fusion protein or as a chemical conjugate.

[0289] Pharmacologically active polypeptides such as DR6 antagonist polypeptides or antibodies often exhibit rapid *in vivo* clearance, necessitating large doses to achieve therapeutically effective concentrations in the body. In addition, polypeptides smaller than about 60 kDa potentially undergo glomerular filtration, which sometimes leads to nephrotoxicity. Fusion or conjugation of relatively small polypeptides such as DR6 antagonist polypeptides or antibodies can be employed to reduce or avoid the risk of such nephrotoxicity. Various heterologous amino acid sequences, *i.e.*, polypeptide moieties or "carriers," for increasing the *in vivo* stability, *i.e.*, serum half-life, of therapeutic polypeptides are known.

[0290] Due to its long half-life, wide *in vivo* distribution, and lack of enzymatic or immunological function, essentially full-length human serum albumin (HSA), or an HSA fragment, is commonly used as a heterologous moiety. Through application of methods and materials such as those taught in Yeh *et al., Proc. Natl. Acad. Sci. USA 89*:1904-08 (1992) and Syed *et al., Blood 89*:3243-52 (1997), HSA can be used to form a DR6 antagonist fusion polypeptide or antibody or polypeptide/antibody conjugate that displays pharmacological activity by virtue of the DR6 moiety while displaying significantly increased *in vivo* stability, *e.g.*, 10-fold to 100-fold higher. The C-terminus of the HSA can be fused to the N-terminus of the DR6 polypeptide. Since HSA is a naturally secreted protein, the HSA signal sequence can be exploited to obtain secretion of a soluble DR6 fusion protein into the cell culture medium when the fusion protein is produced in a eukaryotic, *e.g.*, mammalian, expression system.

[0291] In certain embodiments, DR6 antagonist polypeptides or antibodies for use in the methods described herein further comprise a targeting moiety. Targeting moieties include a protein or a peptide which directs localization to a certain part of the body, for example, to the brain or compartments therein. In certain embodiments, DR6 antagonist polypeptides or

antibody for use in the methods described herein are attached or fused to a brain targeting moiety. The brain targeting moieties are attached covalently (*e.g.*, direct, translational fusion, or by chemical linkage either directly or through a spacer molecule, which can be optionally cleavable) or non-covalently attached (*e.g.*, through reversible interactions such as avidin, biotin, protein A, IgG, etc.). In other embodiments, a DR6 antagonist polypeptide or antibody for use in the methods described herein is attached to one more brain targeting moieties. In additional embodiments, the brain targeting moiety is attached to a plurality of DR6 antagonist polypeptides or antibodies for use in the methods described herein.

[0292] A brain targeting moiety associated with a DR6 antagonist polypeptide or antibody enhances brain delivery of such a DR6 antagonist polypeptide or antibody. A number of polypeptides have been described which, when fused to a protein or therapeutic agent, delivers the protein or therapeutic agent through the blood brain barrier (BBB). Non-limiting examples include the single domain antibody FC5 (Abulrob *et al.* (2005) *J. Neurochem.* 95, 1201-1214); mAB 83-14, a monoclonal antibody to the human insulin receptor (Pardridge *et al.* (1995) *Pharmacol. Res.* 12, 807-816); the B2, B6 and B8 peptides binding to the human transferrin receptor (hTfR) (Xia *et al.* (2000) *J. Virol.* 74, 11359-11366); the OX26 monoclonal antibody to the transferrin receptor (Pardridge *et al.* (1991) *J. Pharmacol. Exp. Ther.* 259, 66-70); and SEQ ID NOs: 1-18 of U.S. Patent No. 6,306,365. The contents of the above references are incorporated herein by reference in their entirety.

[0293] Enhanced brain delivery of a DR6 antagonist composition is determined by a number of means well established in the art. For example, administering to an animal a radioactively, enzymatically or fluorescently labeled DR6 antagonist polypeptide or antibody linked to a brain targeting moiety; determining brain localization; and comparing localization with an equivalent radioactively labeled DR6 antagonist polypeptide o antibody that is not associated with a brain targeting moiety. Other means of determining enhanced targeting are described in the above references.

[0294] The signal sequence is a polynucleotide that encodes an amino acid sequence that initiates transport of a protein across the membrane of the endoplasmic reticulum. Signal sequences useful for constructing an immunofusin include antibody light chain signal sequences, *e.g.*, antibody 14.18 (Gillies *et al., J. Immunol. Meth. 125*:191-202 (1989)), antibody heavy chain signal sequences, *e.g.*, the MOPC141 antibody heavy chain signal sequence (Sakano *et al., Nature 286*:5774 (1980)). Alternatively, other signal sequences can be used. *See, e.g.*, Watson,

Nucl. Acids Res. 12:5145 (1984). The signal peptide is usually cleaved in the lumen of the endoplasmic reticulum by signal peptidases. This results in the secretion of an immunofusin protein containing the Fc region and the DR6 polpeptide.

[0295] In some embodiments, the DNA sequence can encode a proteolytic cleavage site between the secretion cassette and the DR6 polypeptide. Such a cleavage site can provide, *e.g.*, for the proteolytic cleavage of the encoded fusion protein, thus separating the Fc domain from the target protein. Useful proteolytic cleavage sites include amino acid sequences recognized by proteolytic enzymes such as trypsin, plasmin, thrombin, factor Xa, or enterokinase K.

[0296] The secretion cassette can be incorporated into a replicable expression vector. Useful vectors include linear nucleic acids, plasmids, phagemids, cosmids and the like. An exemplary expression vector is pdC, in which the transcription of the immunofusin DNA is placed under the control of the enhancer and promoter of the human cytomegalovirus. *See, e.g.*, Lo *et al., Biochim. Biophys. Acta 1088*:712 (1991); and Lo *et al., Protein Engineering 11*:495-500 (1998). An appropriate host cell can be transformed or transfected with a DNA that encodes a DR6 polypeptide and used for the expression and secretion of the DR6 polypeptide. Host cells that are typically used include immortal hybridoma cells, myeloma cells, 293 cells, Chinese hamster ovary (CHO) cells, Hela cells, and COS cells.

[0297] In one embodiment, a DR6 polypeptide is fused to a hinge and Fc region, *i.e.*, the C-terminal portion of an Ig heavy chain constant region. Potential advantages of a DR6-Fc fusion include solubility, *in vivo* stability, and multivalency, *e.g.*, dimerization. The Fc region used can be an IgA, IgD, or IgG Fc region (hinge- C_H2- C_H3). Alternatively, it can be an IgE or IgM Fc region (hinge- C_H2- C_H3-C_H4). An IgG Fc region is generally used, *e.g.*, an IgG_1 Fc region or IgG_4 Fc region. In one embodiment, a sequence beginning in the hinge region just upstream of the papain cleavage site which defines IgG Fc chemically (*i.e.* residue 216, taking the first residue of heavy chain constant region to be 114 according to the Kabat system), or analogous sites of other immunoglobulins is used in the fusion. The precise site at which the fusion is made is not critical; particular sites are well known and can be selected in order to optimize the biological activity, secretion, or binding characteristics of the molecule. Materials and methods for constructing and expressing DNA encoding Fc fusions are known in the art and can be applied to obtain DR6 fusions without undue experimentation. Some methods described herein employ a DR6 fusion protein such as those described in Capon *et al.*, U.S. Patent Nos. 5,428,130 and 5,565,335.

[0298] In some embodiments, fully intact, wild-type Fc regions display effector functions that can be unnecessary and undesired in an Fc fusion protein used in the methods described herein. Therefore, certain binding sites can be deleted from the Fc region during the construction of the secretion cassette. For example, since coexpression with the light chain is unnecessary, the binding site for the heavy chain binding protein, Bip (Hendershot *et al., Immunol. Today 8*:111-14 (1987)), is deleted from the C_H2 domain of the Fc region of IgE, such that this site does not interfere with the efficient secretion of the immunofusin. Transmembrane domain sequences, such as those present in IgM, also are generally deleted.

[0299] In certain embodiments, the IgG_1 Fc region is used. Alternatively, the Fc region of the other subclasses of immunoglobulin gamma (gamma-2, gamma-3 and gamma-4) can be used in the secretion cassette. The IgG_1 Fc region of immunoglobulin gamma-1 includes at least part of the hinge region, the C_H2 region, and the C_H3 region. In some embodiments, the Fc region of immunoglobulin gamma-1 is a C_H2-deleted-Fc, which includes part of the hinge region and the C_H3 region, but not the C_H2 region. A C_H2-deleted-Fc has been described by Gillies *et al., Hum. Antibod. Hybridomas* 1:47 (1990). In some embodiments, the Fc region of one of IgA, IgD, IgE, or IgM, is used.

[0300] DR6-Fc fusion proteins can be constructed in several different configurations. In one configuration the C-terminus of the DR6 polypeptide is fused directly to the N-terminus of the Fc hinge moiety. In a slightly different configuration, a short polypeptide, *e.g.*, 2-10 amino acids, is incorporated into the fusion between the N-terminus of the DR6 moiety and the C-terminus of the Fc moiety. Such a linker provides conformational flexibility, which can improve biological activity in some circumstances. If a sufficient portion of the hinge region is retained in the Fc moiety, the DR6-Fc fusion will dimerize, thus forming a divalent molecule. A homogeneous population of monomeric Fc fusions will yield monospecific, bivalent dimers. A mixture of two monomeric Fc fusions each having a different specificity will yield bispecific, bivalent dimers.

[0301] Soluble DR6 polypeptides can be fused to heterologous peptides to facilitate purification or identification of the soluble DR6 moiety. For example, a histidine tag can be fused to a soluble DR6 polypeptide to facilitate purification using commercially available chromatography media.

[0302] A "linker" sequence is a series of one or more amino acids separating two polypeptide coding regions in a fusion protein. A typical linker comprises at least 5 amino acids.

Additional linkers comprise at least 10 or at least 15 amino acids. In certain embodiments, the amino acids of a peptide linker are selected so that the linker is hydrophilic. The linker (Gly-Gly-Gly-Gly-Ser)$_3$ (G_4S)$_3$ (SEQ ID NO:136) is a useful linker that is widely applicable to many antibodies as it provides sufficient flexibility. Other linkers include (Gly-Gly-Gly-Gly-Ser)$_2$ (G_4S)$_2$ (SEQ ID NO:137), Glu Ser Gly Arg Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser (SEQ ID NO:138), Glu Gly Lys Ser Ser Gly Ser Gly Ser Glu Ser Lys Ser Thr (SEQ ID NO:139), Glu Gly Lys Ser Ser Gly Ser Gly Ser Glu Ser Lys Ser Thr Gln (SEQ ID NO:140), Glu Gly Lys Ser Ser Gly Ser Gly Ser Glu Ser Lys Val Asp (SEQ ID NO:141), Gly Ser Thr Ser Gly Ser Gly Lys Ser Ser Glu Gly Lys Gly (SEQ ID NO:142), Lys Glu Ser Gly Ser Val Ser Ser Glu Gln Leu Ala Gln Phe Arg Ser Leu Asp (SEQ ID NO:143), and Glu Ser Gly Ser Val Ser Ser Glu Glu Leu Ala Phe Arg Ser Leu Asp (SEQ ID NO:144). Examples of shorter linkers include fragments of the above linkers, and examples of longer linkers include combinations of the linkers above, combinations of fragments of the linkers above, and combinations of the linkers above with fragments of the linkers above.

[0303] DR6 polypeptides can be fused to a polypeptide tag. The term "polypeptide tag," as used herein, is intended to mean any sequence of amino acids that can be attached to, connected to, or linked to a DR6 polypeptide and that can be used to identify, purify, concentrate or isolate the DR6 polypeptide. The attachment of the polypeptide tag to the DR6 polypeptide can occur, *e.g.*, by constructing a nucleic acid molecule that comprises: (a) a nucleic acid sequence that encodes the polypeptide tag, and (b) a nucleic acid sequence that encodes a DR6 polypeptide. Exemplary polypeptide tags include, *e.g.*, amino acid sequences that are capable of being post-translationally modified, *e.g.*, amino acid sequences that are biotinylated. Other exemplary polypeptide tags include, *e.g.*, amino acid sequences that are capable of being recognized and/or bound by an antibody (or fragment thereof) or other specific binding reagent. Polypeptide tags that are capable of being recognized by an antibody (or fragment thereof) or other specific binding reagent include, *e.g.*, those that are known in the art as "epitope tags." An epitope tag can be a natural or an artificial epitope tag. Natural and artificial epitope tags are known in the art, including, *e.g.*, artificial epitopes such as FLAG, Strep, or poly-histidine peptides. FLAG peptides include the sequence Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys (SEQ ID NO:145) or Asp-Tyr-Lys-Asp-Glu-Asp-Asp-Lys (SEQ ID NO:146) (Einhauer, A. and Jungbauer, A., J. Biochem. Biophys. Methods 49:1-3:455-465 (2001)). The Strep epitope has the sequence Ala-Trp-Arg-His-Pro-Gln-Phe-Gly-Gly (SEQ ID NO:147). The VSV-G epitope can

also be used and has the sequence Tyr-Thr-Asp-Ile-Glu-Met-Asn-Arg-Leu-Gly-Lys (SEQ ID NO:148). Another artificial epitope is a poly-His sequence having six histidine residues (His-His-His-His-His-His) (SEQ ID NO:149). Naturally-occurring epitopes include the influenza virus hemagglutinin (HA) sequence Tyr-Pro-Tyr-Asp-Val-Pro-Asp-Tyr-Ala-Ile-Glu-Gly-Arg (SEQ ID NO:150) recognized by the monoclonal antibody 12CA5 (Murray *et al., Anal. Biochem. 229*:170-179 (1995)) and the eleven amino acid sequence from human c-myc (Myc) recognized by the monoclonal antibody 9E10 (Glu-Gln-Lys-Leu-Leu-Ser-Glu-Glu-Asp-Leu-Asn) (SEQ ID NO:151) (Manstein *et al., Gene 162*:129-134 (1995)). Another useful epitope is the tripeptide Glu-Glu-Phe which is recognized by the monoclonal antibody YL 1/2. (Stammers *et al. FEBS Lett. 283*:298-302(1991)).

[0304] In certain embodiments, the DR6 polypeptide and the polypeptide tag can be connected via a linking amino acid sequence. As used herein, a "linking amino acid sequence" can be an amino acid sequence that is capable of being recognized and/or cleaved by one or more proteases. Amino acid sequences that can be recognized and/or cleaved by one or more proteases are known in the art. Exemplary amino acid sequences are those that are recognized by the following proteases: factor VIIa, factor IXa, factor Xa, APC, t-PA, u-PA, trypsin, chymotrypsin, enterokinase, pepsin, cathepsin B,H,L,S,D, cathepsin G, renin, angiotensin converting enzyme, matrix metalloproteases (collagenases, stromelysins, gelatinases), macrophage elastase, Cir, and Cis. The amino acid sequences that are recognized by the aforementioned proteases are known in the art. Exemplary sequences recognized by certain proteases can be found, *e.g.*, in U.S. Patent No. 5,811,252.

[0305] In some methods, a soluble DR6 fusion construct is used to enhance the production of a soluble DR6 moiety in bacteria. In such constructs a bacterial protein normally expressed and/or secreted at a high level is employed as the N-terminal fusion partner of a soluble DR6 polypeptide. *See, e.g.*, Smith *et al.*, Gene *67*:31 (1988); Hopp *et al., Biotechnology 6*:1204 (1988); La Vallie *et al., Biotechnology 11*:187 (1993).

[0306] By fusing a soluble DR6 moiety at the amino and carboxy termini of a suitable fusion partner, bivalent or tetravalent forms of a soluble DR6 polypeptide can be obtained. For example, a soluble DR6 moiety can be fused to the amino and carboxy termini of an Ig moiety to produce a bivalent monomeric polypeptide containing two soluble DR6 moieties. Upon dimerization of two of these monomers, by virtue of the Ig moiety, a tetravalent form of a soluble DR6 protein is obtained. Such multivalent forms can be used to achieve increased binding

affinity for the target. Multivalent forms of soluble DR6 also can be obtained by placing soluble DR6 moieties in tandem to form concatamers, which can be employed alone or fused to a fusion partner such as Ig or HSA.

DR6 Antagonist Conjugates

[0307] DR6 antagonist polypeptides and antibodies for use in the treatment methods disclosed herein include derivatives that are modified, *i.e.*, by the covalent attachment of any type of molecule such that covalent attachment does not prevent the DR6 antagonist polypeptide or antibody from inhibiting the biological function of DR6. For example, but not by way of limitation, the DR6 antagonist polypeptides and antibodies can be modified *e.g.*, by glycosylation, acetylation, pegylation, phosphylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications can be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Additionally, the derivative can contain one or more non-classical amino acids.

[0308] DR6 antagonist polypeptides and antibodies can be modified by natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in the DR6 antagonist polypeptide or antibody, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini, or on moieties such as carbohydrates. It will be appreciated that the same type of modification can be present in the same or varying degrees at several sites in a given DR6 antagonist polypeptide or antibody. Also, a given DR6 antagonist polypeptide or antibody can contain many types of modifications. DR6 antagonist polypeptides or antibodies can be branched, for example, as a result of ubiquitination, and they can be cyclic, with or without branching. Cyclic, branched, and branched cyclic DR6 antagonist polypeptides and antibodies can result from posttranslation natural processes or can be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate,

formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, pegylation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. (*See*, for instance, *Proteins - Structure And Molecular Properties*, T. E. Creighton, W. H. Freeman and Company, New York 2nd Ed., (1993); *Posttranslational Covalent Modification Of Proteins*, B. C. Johnson, Ed., Academic Press, New York, pgs. 1-12 (1983); Seifter *et al.*, *Meth Enzymol 182*:626-646 (1990); Rattan *et al.*, *Ann NY Acad Sci 663*:48-62 (1992)).

[0309] Any of a number of cross-linkers that contain a corresponding amino-reactive group and thiol-reactive group can be used to link DR6 antagonist polypeptides to a heterologous fusion partner. Examples of suitable linkers include amine reactive cross-linkers that insert a thiol-reactive maleimide, *e.g.*, SMCC, AMAS, BMPS, MBS, EMCS, SMPB, SMPH, KMUS, and GMBS. Other suitable linkers insert a thiol-reactive haloacetate group, *e.g.*, SBAP, SIA, SIAB. Linkers that provide a protected or non-protected thiol for reaction with sulfhydryl groups to product a reducible linkage include SPDP, SMPT, SATA, and SATP. Such reagents are commercially available (*e.g.*, Pierce Chemicals).

[0310] Conjugation does not have to involve the N-terminus of a soluble DR6 polypeptide or the thiol moiety on serum albumin. For example, soluble DR6-albumin fusions can be obtained using genetic engineering techniques, wherein the soluble DR6 moiety is fused to the serum albumin gene at its N-terminus, C-terminus, or both.

[0311] Soluble DR6 polypeptides or DR6 antibodies can be polypeptides or antibodies wherein one or more polymers are conjugated (covalently linked) to the DR6 polypeptide or antibody. Examples of polymers suitable for such conjugation include polypeptides (discussed above), sugar polymers and polyalkylene glycol chains. Typically, but not necessarily, a polymer is conjugated to the soluble DR6 polypeptide or DR6 antibody for the purpose of improving one or more of the following: solubility, stability, or bioavailability.

[0312] The class of polymer generally used for conjugation to a DR6 antagonist polypeptide or antibody is a polyalkylene glycol. Polyethylene glycol (PEG) is most frequently used. PEG moieties, *e.g.*, 1, 2, 3, 4 or 5 PEG polymers, can be conjugated to each DR6 antagonist polypeptide or antibody to increase serum half life, as compared to the DR6 antagonist polypeptide or antibody alone. PEG moieties are non-antigenic and essentially biologically inert. PEG moieties can be branched or unbranched.

[0313] The number of PEG moieties attached to the DR6 antagonist polypeptide or antibody and the molecular weight of the individual PEG chains can vary. In general, the higher the molecular weight of the polymer, the fewer polymer chains attached to the polypeptide. Usually, the total polymer mass attached to the DR6 antagonist polypeptide or antibody is from 20 kDa to 40 kDa. Thus, if one polymer chain is attached, the molecular weight of the chain is generally 20-40 kDa. If two chains are attached, the molecular weight of each chain is generally 10-20 kDa. If three chains are attached, the molecular weight is generally 7-14 kDa.

[0314] The polymer, *e.g.*, PEG, can be linked to the DR6 antagonist polypeptide or antibody through any suitable, exposed reactive group on the polypeptide. The exposed reactive group(s) can be, *e.g.*, an N-terminal amino group or the epsilon amino group of an internal lysine residue, or both. An activated polymer can react and covalently link at any free amino group on the DR6 antagonist polypeptide or antibody. Free carboxylic groups, suitably activated carbonyl groups, hydroxyl, guanidyl, imidazole, oxidized carbohydrate moieties and mercapto groups of the DR6 antagonist polypeptide or antibody (if available) also can be used as reactive groups for polymer attachment.

[0315] In a conjugation reaction, from about 1.0 to about 10 moles of activated polymer per mole of polypeptide, depending on polypeptide concentration, is typically employed. Usually, the ratio chosen represents a balance between maximizing the reaction while minimizing side reactions (often non-specific) that can impair the desired pharmacological activity of the DR6 antagonist polypeptide or antibody. In certain embodiments, at least 50% of the biological activity (as demonstrated, *e.g.*, in any of the assays described herein or known in the art) of the DR6 antagonist polypeptide or antibody is retained. In further embodiments, nearly 100% is retained.

[0316] The polymer can be conjugated to the DR6 antagonist polypeptide or antibody using conventional chemistry. For example, a polyalkylene glycol moiety can be coupled to a lysine epsilon amino group of the DR6 antagonist polypeptide or antibody. Linkage to the lysine side chain can be performed with an N-hydroxylsuccinimide (NHS) active ester such as PEG succinimidyl succinate (SS-PEG) and succinimidyl propionate (SPA-PEG). Suitable polyalkylene glycol moieties include, *e.g.*, carboxymethyl-NHS and norleucine-NHS, SC. These reagents are commercially available. Additional amine-reactive PEG linkers can be substituted for the succinimidyl moiety. These include, *e.g.*, isothiocyanates, nitrophenylcarbonates (PNP), epoxides, benzotriazole carbonates, SC-PEG, tresylate, aldehyde, epoxide, carbonylimidazole

and PNP carbonate. Conditions are usually optimized to maximize the selectivity and extent of reaction. Such optimization of reaction conditions is within ordinary skill in the art.

[0317] PEGylation can be carried out by any of the PEGylation reactions known in the art. *See, e.g., Focus on Growth* Factors 3:4-10 (1992), and European patent applications EP0154316 and EP0401384. PEGylation can be carried out using an acylation reaction or an alkylation reaction with a reactive polyethylene glycol molecule (or an analogous reactive water-soluble polymer).

[0318] PEGylation by acylation generally involves reacting an active ester derivative of polyethylene glycol. Any reactive PEG molecule can be employed in the PEGylation. PEG esterified to N-hydroxysuccinimide (NHS) is a frequently used activated PEG ester. As used herein, "acylation" includes without limitation the following types of linkages between the therapeutic protein and a water-soluble polymer such as PEG: amide, carbamate, urethane, and the like. *See, e.g., Bioconjugate Chem.* 5:133-140, 1994. Reaction parameters are generally selected to avoid temperature, solvent, and pH conditions that would damage or inactivate the soluble DR6 polypeptide.

[0319] Generally, the connecting linkage is an amide and typically at least 95% of the resulting product is mono-, di- or tri-PEGylated. However, some species with higher degrees of PEGylation can be formed in amounts depending on the specific reaction conditions used. Optionally, purified PEGylated species are separated from the mixture, particularly unreacted species, by conventional purification methods, including, *e.g.*, dialysis, salting-out, ultrafiltration, ion-exchange chromatography, gel filtration chromatography, hydrophobic exchange chromatography, and electrophoresis.

[0320] PEGylation by alkylation generally involves reacting a terminal aldehyde derivative of PEG with DR6 antagonist polypeptide or antibody in the presence of a reducing agent. In addition, one can manipulate the reaction conditions to favor PEGylation substantially only at the N-terminal amino group of a DR6 antagonist polypeptide or antibody, *i.e.* a mono-PEGylated protein. In either case of mono-PEGylation or poly-PEGylation, the PEG groups are typically attached to the protein via a - C_H2-NH- group. With particular reference to the - C_H2- group, this type of linkage is known as an "alkyl" linkage.

[0321] Derivatization via reductive alkylation to produce an N-terminally targeted mono-PEGylated product exploits differential reactivity of different types of primary amino groups (lysine versus the N-terminal) available for derivatization. The reaction is performed at a pH that

allows one to take advantage of the pKa differences between the epsilon-amino groups of the lysine residues and that of the N-terminal amino group of the protein. By such selective derivatization, attachment of a water-soluble polymer that contains a reactive group, such as an aldehyde, to a protein is controlled: the conjugation with the polymer takes place predominantly at the N-terminus of the protein and no significant modification of other reactive groups, such as the lysine side chain amino groups, occurs.

[0322] The polymer molecules used in both the acylation and alkylation approaches are selected from among water-soluble polymers. The polymer selected is typically modified to have a single reactive group, such as an active ester for acylation or an aldehyde for alkylation, so that the degree of polymerization can be controlled as provided for in the present methods. An exemplary reactive PEG aldehyde is polyethylene glycol propionaldehyde, which is water stable, or mono C1-C10 alkoxy or aryloxy derivatives thereof (*see, e.g.*, Harris *et al.*, U.S. Pat. No. 5,252,714). The polymer can be branched or unbranched. For the acylation reactions, the polymer(s) selected typically have a single reactive ester group. For reductive alkylation, the polymer(s) selected typically have a single reactive aldehyde group. Generally, the water-soluble polymer will not be selected from naturally occurring glycosyl residues, because these are usually made more conveniently by mammalian recombinant expression systems.

[0323] Methods for preparing a PEGylated soluble DR6 polypeptide or antibody generally includes the steps of (a) reacting a DR6 antagonist polypeptide or antibody with polyethylene glycol (such as a reactive ester or aldehyde derivative of PEG) under conditions whereby the molecule becomes attached to one or more PEG groups, and (b) obtaining the reaction product(s). In general, the optimal reaction conditions for the acylation reactions will be determined case-by-case based on known parameters and the desired result. For example, a larger the ratio of PEG to protein, generally leads to a greater the percentage of poly-PEGylated product.

[0324] Reductive alkylation to produce a substantially homogeneous population of mono-polymer/soluble DR6 polypeptide or DR6 antibody generally includes the steps of: (a) reacting a soluble DR6 protein or polypeptide with a reactive PEG molecule under reductive alkylation conditions, at a pH suitable to permit selective modification of the N-terminal amino group of the polypeptide or antibody; and (b) obtaining the reaction product(s).

[0325] For a substantially homogeneous population of mono-polymer/soluble DR6 polypeptide or DR6 antibody, the reductive alkylation reaction conditions are those that permit

the selective attachment of the water-soluble polymer moiety to the N-terminus of the polypeptide or antibody. Such reaction conditions generally provide for pKa differences between the lysine side chain amino groups and the N-terminal amino group. For purposes described herein, the pH is generally in the range of 3-9, typically 3-6.

[0326] Soluble DR6 polypeptides or antibodies can include a tag, *e.g.*, a moiety that can be subsequently released by proteolysis. Thus, the lysine moiety can be selectively modified by first reacting a His-tag modified with a low-molecular-weight linker such as Traut's reagent (Pierce) which will react with both the lysine and N-terminus, and then releasing the His tag. The polypeptide will then contain a free SH group that can be selectively modified with a PEG containing a thiol-reactive head group such as a maleimide group, a vinylsulfone group, a haloacetate group, or a free or protected SH.

[0327] Traut's reagent can be replaced with any linker that will set up a specific site for PEG attachment. For example, Traut's reagent can be replaced with SPDP, SMPT, SATA, or SATP (Pierce). Similarly one could react the protein with an amine-reactive linker that inserts a maleimide (for example SMCC, AMAS, BMPS, MBS, EMCS, SMPB, SMPH, KMUS, or GMBS), a haloacetate group (SBAP, SIA, SIAB), or a vinylsulfone group and react the resulting product with a PEG that contains a free SH.

[0328] In some embodiments, the polyalkylene glycol moiety is coupled to a cysteine group of the DR6 antagonist polypeptide or antibody. Coupling can be effected using, *e.g.*, a maleimide group, a vinylsulfone group, a haloacetate group, or a thiol group.

[0329] Optionally, the soluble DR6 polypeptide or antibody is conjugated to the polyethylene-glycol moiety through a labile bond. The labile bond can be cleaved in, *e.g.*, biochemical hydrolysis, proteolysis, or sulfhydryl cleavage. For example, the bond can be cleaved under *in vivo* (physiological) conditions.

[0330] The reactions can take place by any suitable method used for reacting biologically active materials with inert polymers, generally at about pH 5-8, *e.g.*, pH 5, 6, 7, or 8, if the reactive groups are on the alpha amino group at the N-terminus. Generally the process involves preparing an activated polymer and thereafter reacting the protein with the activated polymer to produce the soluble protein suitable for formulation.

[0331] In some embodiments, the antibodies or polypeptides are fusion proteins comprising a DR6 antibody, or antigen-binding fragment, variant, or derivative thereof, and a heterologous polypeptide. The heterologous polypeptide to which the antibody is fused can be

useful for function or is useful to target the DR6 polypeptide expressing cells. In one embodiment, a fusion protein comprises, consists essentially of, or consists of, a polypeptide having the amino acid sequence of any one or more of the VH regions of an antibody or the amino acid sequence of any one or more of the VL regions of an antibody or fragments or variants thereof, and a heterologous polypeptide sequence. In another embodiment, a fusion protein for use in the diagnostic and treatment methods disclosed herein comprises, consists essentially of, or consists of a polypeptide having the amino acid sequence of any one, two, three of the VH-CDRs of a DR6-specific antibody, or fragments, variants, or derivatives thereof, or the amino acid sequence of any one, two, three of the VL-CDRs of a DR6-specific antibody, or fragments, variants, or derivatives thereof, and a heterologous polypeptide sequence. In one embodiment, the fusion protein comprises a polypeptide having the amino acid sequence of a VH-CDR3 of a DR6-specific antibody, or fragment, derivative, or variant thereof, and a heterologous polypeptide sequence, which fusion protein specifically binds to at least one epitope of DR6. In another embodiment, a fusion protein comprises a polypeptide having the amino acid sequence of at least one VH region of a DR6-specific antibody and the amino acid sequence of at least one VL region of a DR6-specific antibody or fragments, derivatives or variants thereof, and a heterologous polypeptide sequence. In one embodiment, the VH and VL regions of the fusion protein correspond to a single source antibody (or scFv or Fab fragment) which specifically binds at least one epitope of DR6. In yet another embodiment, a fusion protein for use in the diagnostic and treatment methods disclosed herein comprises a polypeptide having the amino acid sequence of any one, two, three or more of the VH CDRs of a DR6-specific antibody and the amino acid sequence of any one, two, three or more of the VL CDRs of a DR6-specific antibody, or fragments or variants thereof, and a heterologous polypeptide sequence. In some embodiments, two, three, four, five, six, or more of the VH-CDR(s) or VL-CDR(s) correspond to single source antibody (or scFv or Fab fragment). Nucleic acid molecules encoding these fusion proteins are also encompassed.

DR6 Polynucleotide Antagonists

[0332] Specific embodiments comprise a method of promoting nervous system cell survival by contacting the cells with a DR6 polynucleotide antagonist. The polynucleotide antagonist can be any polynucleotide that encodes a DR6-antagonist polypeptide. The polynuceotide antagonist can also be a nucleic acid molecule which specifically binds to a polynucleotide which encodes DR6. The DR6 polynucleotide antagonist prevents expression of

DR6 (knockdown). In certain embodiments, the DR6 polynucleotide antagonist promotes nervous system cell survival or inhibits nervous system cell apoptosis. DR6 polynucleotide antagonists include, but are not limited to antisense molecules, ribozymes, siRNA, shRNA and RNAi. Typically, such binding molecules are separately administered to the animal (*see*, for example, O'Connor, J. *Neurochem. 56*:560 (1991), but such binding molecules can also be expressed *in vivo* from polynucleotides taken up by a host cell and expressed *in vivo*. *See also* Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988).

[0333] RNAi refers to the expression of an RNA which interferes with the expression of the targeted mRNA. Specifically, the RNAi silences a targeted gene via interacting with the specific mRNA (*e.g.* DR6) through a siRNA (short interfering RNA). The ds RNA complex is then targeted for degradation by the cell. Additional RNAi molecules include Short hairpin RNA (shRNA); also short interfering hairpin. The shRNA molecule contains sense and antisense sequences from a target gene connected by a loop. The shRNA is transported from the nucleus into the cytoplasm, it is degraded along with the mRNA. Pol III or U6 promoters can be used to express RNAs for RNAi.

[0334] RNAi is mediated by double stranded RNA (dsRNA) molecules that have sequence-specific homology to their "target" mRNAs (Caplen *et al.*, *Proc Natl Acad Sci USA 98*:9742-9747, 2001). Biochemical studies in *Drosophila* cell-free lysates indicates that the mediators of RNA-dependent gene silencing are 21-25 nucleotide "small interfering" RNA duplexes (siRNAs). Accordingly, siRNA molecules are advantageously used in the methods described herein. The siRNAs are derived from the processing of dsRNA by an RNase known as DICER (Bernstein *et al.*, *Nature 409*:363-366, 2001). It appears that siRNA duplex products are recruited into a multi-protein siRNA complex termed RISC (RNA Induced Silencing Complex). Without wishing to be bound by any particular theory, it is believed that a RISC is guided to a target mRNA, where the siRNA duplex interacts sequence-specifically to mediate cleavage in a catalytic fashion (Bernstein *et al.*, *Nature 409*:363-366, 2001; Boutla *et al.*, *Curr Biol 11*:1776-1780, 2001).

[0335] RNAi has been used to analyze gene function and to identify essential genes in mammalian cells (Elbashir *et al.*, *Methods 26*:199-213, 2002; Harborth *et al.*, *J Cell Sci 114*:4557-4565, 2001), including by way of non-limiting example neurons (Krichevsky *et al.*, *Proc Natl Acad Sci USA 99*:11926-11929, 2002). RNAi is also being evaluated for therapeutic

modalities, such as inhibiting or blocking the infection, replication and/or growth of viruses, including without limitation poliovirus (Gitlin *et al.*, *Nature 418*:379-380, 2002) and HIV (Capodici *et al.*, *J Immunol 169*:5196-5201, 2002), and reducing expression of oncogenes (*e.g.*, the bcr-abl gene; Scherr *et al.*, *Blood* Sep 26 epub ahead of print, 2002). RNAi has been used to modulate gene expression in mammalian (mouse) and amphibian (*Xenopus*) embryos (respectively, Calegari *et al.*, *Proc Natl Acad Sci USA 99*:14236-14240, 2002; and Zhou, *et al.*, *Nucleic Acids Res 30*:1664-1669, 2002), and in postnatal mice (Lewis *et al.*, *Nat Genet 32*:107-108, 2002), and to reduce trangsene expression in adult transgenic mice (McCaffrey *et al.*, *Nature 418*:38-39, 2002). Methods have been described for determining the efficacy and specificity of siRNAs in cell culture and *in vivo* (see, *e.g.*, Bertrand *et al.*, *Biochem Biophys Res Commun 296*:1000-1004, 2002; Lassus *et al.*, *Sci STKE 2002(147)*:PL13, 2002; and Leirdal *et al.*, *Biochem Biophys Res Commun 295*:744-748, 2002).

[0336] Molecules that mediate RNAi, including without limitation siRNA, can be produced *in vitro* by chemical synthesis (Hohjoh, *FEBS Lett 521*:195-199, 2002), hydrolysis of dsRNA (Yang *et al.*, *Proc Natl Acad Sci USA 99*:9942-9947, 2002), by *in vitro* transcription with T7 RNA polymerase (Donzeet *et al.*, *Nucleic Acids Res 30*:e46, 2002; Yu *et al.*, *Proc Natl Acad Sci USA 99*:6047-6052, 2002), and by hydrolysis of double-stranded RNA using a nuclease such as *E. coli* RNase III (Yang *et al.*, *Proc Natl Acad Sci USA 99*:9942-9947, 2002).

[0337] siRNA molecules can also be formed by annealing two oligonucleotides to each other, typically have the following general structure, which includes both double-stranded and single-stranded portions:

```
                    |–m–|    (Overhang)
      |———x———|              ("Core")
   5'-XXXXXXXXXXXXXNNNNN-3'  (SEQ ID NO:152)
      ::::::::::::
3'-NNNNNYYYYYYYYYYY-5'  (SEQ ID NO:153)
  |–n–|                     (Overhang)
```

Wherein N, X and Y are nucleotides; X hydrogen bonds to Y; ":" signifies a hydrogen bond between two bases; *x* is a natural integer having a value between 1 and about 100; and *m* and *n* are whole integers having, independently, values between 0 and about 100. In some embodiments, N, X and Y are independently A, G, C and T or U. Non-naturally occurring bases and nucleotides can be present, particularly in the case of synthetic siRNA (*i.e.*, the product of annealing two oligonucleotides). The double-stranded central section is called the "core" and has base pairs (bp) as units of measurement; the single-stranded portions are overhangs, having nucleotides (nt) as units of measurement. The overhangs shown are 3' overhangs, but molecules

with 5' overhangs are also contemplated. Also contemplated are siRNA molecules with no overhangs (*i.e.*, $m = 0$ and $n = 0$), and those having an overhang on one side of the core but not the other (*e.g.*, $m = 0$ and $n \geq 1$, or vice-versa).

[0338] Initially, RNAi technology did not appear to be readily applicable to mammalian systems. This is because, in mammals, dsRNA activates dsRNA-activated protein kinase (PKR) resulting in an apoptotic cascade and cell death (Der *et al, Proc. Natl. Acad. Sci. USA 94*:3279-3283, 1997). In addition, it has long been known that dsRNA activates the interfeDR6 cascade in mammalian cells, which can also lead to altered cell physiology (Colby *et al, Annu. Rev. Microbiol. 25*:333, 1971; Kleinschmidt *et al., Annu. Rev. Biochem. 41*:517, 1972; Lampson *et al., Proc. Natl. Acad. Sci. USA 58*L782, 1967; Lomniczi *et al., J. Gen. Virol. 8*:55, 1970; and Younger *et al., J. Bacteriol. 92*:862, 1966). However, dsRNA-mediated activation of the PKR and interfeDR6 cascades requires dsRNA longer than about 30 base pairs. In contrast, dsRNA less than 30 base pairs in length has been demonstrated to cause RNAi in mammalian cells (Caplen *et al., Proc. Natl. Acad. Sci. USA 98*:9742-9747, 2001). Thus, it is expected that undesirable, non-specific effects associated with longer dsRNA molecules can be avoided by preparing short RNA that is substantially free from longer dsRNAs.

[0339] References regarding siRNA: Bernstein *et al.*, *Nature 409*:363-366, 2001; Boutla *et al.*, *Curr Biol 11*:1776-1780, 2001; Cullen, *Nat Immunol. 3*:597-599, 2002; Caplen *et al.*, *Proc Natl Acad Sci USA 98*:9742-9747, 2001; Hamilton *et al.*, *Science 286*:950-952, 1999; Nagase *et al.*, *DNA Res. 6*:63-70, 1999; Napoli *et al.*, *Plant Cell 2*:279-289, 1990; Nicholson *et al.*, *Mamm. Genome* 13:67-73, 2002; Parrish *et al.*, *Mol Cell 6*:1077-1087, 2000; Romano *et al.*, *Mol Microbiol 6*:3343-3353, 1992; Tabara *et al.*, *Cell 99*:123-132, 1999; and Tuschl, *Chembiochem. 2*:239-245, 2001.

[0340] Paddison *et al.* (*Genes & Dev. 16*:948-958, 2002) have used small RNA molecules folded into hairpins as a means to effect RNAi. Accordingly, such short hairpin RNA (shRNA) molecules are also advantageously used in the methods described herein. The length of the stem and loop of functional shRNAs varies; stem lengths can range anywhere from about 25 to about 30 nt, and loop size can range between 4 to about 25 nt without affecting silencing activity. While not wishing to be bound by any particular theory, it is believed that these shRNAs resemble the dsRNA products of the DICER RNase and, in any event, have the same capacity for inhibiting expression of a specific gene. The shRNA can be expressed from a lentiviral vector (*e.g.*, pLL3.7).

[0341] Antisense technology can be used to control gene expression through antisense DNA or RNA, or through triple-helix formation. Antisense techniques are discussed for example, in Okano, J. *Neurochem. 56*:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Triple helix formation is discussed in, for instance, Lee *et al.*, *Nucleic Acids Research 6*:3073 (1979); Cooney *et al.*, *Science 241*:456 (1988); and Dervan *et al.*, *Science 251*:1300 (1991). The methods are based on binding of a polynucleotide to a complementary DNA or RNA.

[0342] For example, the 5' coding portion of a polynucleotide that encodes DR6 can be used to design an antisense RNA oligonucleotide of from about 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription thereby preventing transcription and the production of the target protein. The antisense RNA oligonucleotide hybridizes to the mRNA *in vivo* and blocks translation of the mRNA molecule into the target polypeptide.

[0343] In one embodiment, antisense nucleic acids specific for the DR6 gene are produced intracellularly by transcription from an exogenous sequence. For example, a vector or a portion thereof, is transcribed, producing an antisense nucleic acid (RNA). Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors can be plasmid, viral, or others known in the art, used for replication and expression in vertebrate cells. Expression of the antisense molecule can be by any promoter known in the art to act in vertebrate, *e.g.*, human cells, such as those described elsewhere herein.

[0344] Absolute complementarity of an antisense molecule is not required. A sequence complementary to at least a portion of an RNA encoding DR6, means a sequence having sufficient complementarity to be able to hybridize with the RNA, forming a stable duplex; or triplex. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid. Generally, the larger the hybridizing nucleic acid, the more base mismatches it can contain and still form a stable duplex (or triplex as the case can be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

[0345] Oligonucleotides that are complementary to the 5' end of a messenger RNA, *e.g.*, the 5' untranslated sequence up to and including the AUG initiation codon, should work most

efficiently at inhibiting translation. However, sequences complementary to the 3' untranslated sequences of mRNAs have been shown to be effective at inhibiting translation of mRNAs as well. See generally, Wagner, R., *Nature 372*:333-335 (1994). Thus, oligonucleotides complementary to either the 5'- or 3'- non- translated, non-coding regions could be used in an antisense approach to inhibit translation of DR6. Oligonucleotides complementary to the 5' untranslated region of the mRNA should include the complement of the AUG start codon. Antisense oligonucleotides complementary to mRNA coding regions are less efficient inhibitors of translation but could be used in accordance with the methods described herein. Antisense nucleic acids are typically at least six nucleotides in length and include, for example, oligonucleotides ranging from 6 to about 50 nucleotides in length. In specific aspects the oligonucleotide is at least 10 nucleotides, at least 17 nucleotides, at least 25 nucleotides or at least 50 nucleotides.

[0346] Polynucleotides for use the therapeutic methods disclosed herein can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone, for example, to improve stability of the molecule, hybridization, etc. The oligonucleotide can include other appended groups such as peptides (*e.g.*, for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (see, *e.g.*, Letsinger *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 86*:6553-6556 (1989); Lemaitre *et al.*, *Proc. Natl. Acad. Sci. 84*:648-652 (1987)); PCT Publication No. WO88/09810, published December 15, 1988) or the blood-brain barrier (see, *e.g.*, PCT Publication No. WO89/10134, published April 25, 1988), hybridization-triggered cleavage agents. (*See*, *e.g.*, Krol *et al.*, *BioTechniques 6*:958-976 (1988)) or intercalating agents. (*See*, *e.g.*, Zon, *Pharm. Res. 5*:539-549(1988)). To this end, the oligonucleotide can be conjugated to another molecule, *e.g.*, a peptide, hybridization triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent, etc.

[0347] An antisense oligonucleotide for use in the therapeutic methods disclosed herein can comprise at least one modified base moiety such as, but not limited to, 5fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N-6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N-6-adenine, 7-methylguanine, 5-

methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N-6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3(3-amino-3-N2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine.

[0348] An antisense oligonucleotide for use in the therapeutic methods disclosed herein can also comprise at least one modified sugar moiety selected from the group including, but not limited to, arabinose, 2-fluoroarabinose, xylulose, and hexose.

[0349] In yet another embodiment, an antisense oligonucleotide for use in the therapeutic methods disclosed herein comprises at least one modified phosphate backbone such as, but not limited to, a phosphorothioate, a phosphorodithioate, a phosphoramidothioate, a phosphoramidate, a phosphordiamidate, a methylphosphonate, an alkyl phosphotriester, and a formacetal or analog thereof.

[0350] In yet another embodiment, an antisense oligonucleotide for use in the therapeutic methods disclosed herein is an α-anomeric oligonucleotide. An α-anomeric oligonucleotide forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual situation, the strands run parallel to each other (Gautier *et al.*, *Nucl.* Acids *Res. 15*:6625-6641(1987)). The oligonucleotide is a 2'-0-methylribonucleotide (Inoue *et al.*, *Nucl. Acids Res. 15*:6131-6148(1987)), or a chimeric RNA-DNA analogue (Inoue *et al.*, *FEBS Lett. 215*:327-330(1987)).

[0351] Polynucleotides can be synthesized by standard methods known in the art, *e.g.* by use of an automated DNA synthesizer (such as are commercially available from Biosearch, Applied Biosystems, etc.). As examples, phosphorothioate oligonucleotides can be synthesized by the method of Stein *et al.*, *Nucl. Acids Res. 16*:3209 (1988), methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports (Sarin *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 85*:7448-7451(1988)), etc.

[0352] Polynucleotide compositions for use in the therapeutic methods disclosed herein further include catalytic RNA, or a ribozyme (*See, e.g.*, PCT International Publication WO 90/11364, published October 4, 1990; Sarver *et al.*, *Science 247*:1222-1225 (1990). Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. The sole requirement is that the target mRNA

have the following sequence of two bases: 5'-UG-3'. The construction and production of hammerhead ribozymes is well known in the art and is described more fully in Haseloff and Gerlach, *Nature 334*:585-591 (1988). In certain embodiments, the ribozyme is engineered so that the cleavage recognition site is located near the 5' end of the target mRNA; *i.e.*, to increase efficiency and minimize the intracellular accumulation of non-functional mRNA transcripts.

[0353] As in the antisense approach, ribozymes for use in the diagnostic and therapeutic methods disclosed herein can be composed of modified oligonucleotides (*e.g.* for improved stability, targeting, etc.) and can be delivered to cells which express DR6 *in vivo*. DNA constructs encoding the ribozyme can be introduced into the cell in the same manner as described above for the introduction of antisense encoding DNA. One method of delivery involves using a DNA construct "encoding" the ribozyme under the control of a stDR6g constitutive or inducible promoter, such as, for example, pol III or pol II promoter, so that transfected cells will produce sufficient quantities of the ribozyme to destroy endogenous DR6 messages and inhibit translation. Since ribozymes unlike antisense molecules, are catalytic, a lower intracellular concentration is required for efficiency.

DR6 Aptamer Antagonists

[0354] In another embodiment, the DR6 antagonist for use in the methods described herein is an aptamer. An aptamer can be a nucleotide or a polypeptide which has a unique sequence, has the property of binding specifically to a desired target (*e.g.*, a polypeptide), and is a specific ligand of a given target. Nucleotide aptamers include double stranded DNA and single stranded RNA molecules that bind to DR6. In certain embodiments, the DR6 aptamer antagonist promotes proliferation, differentiation, or survival of oligodendrocytes; promotes, oligodendrocyte-mediated myelination of neurons, or prevents demyelination, *e.g.*, in a mammal.

[0355] Nucleic acid aptamers are selected using methods known in the art, for example via the Systematic Evolution of Ligands by Exponential Enrichment (SELEX) process. SELEX is a method for the in vitro evolution of nucleic acid molecules with highly specific binding to target molecules as described in *e.g.* U.S. Pat. Nos. 5,475,096, 5,580,737, 5,567,588, 5,707,796, 5,763,177, 6, 011,577, and 6,699,843, incorporated herein by reference in their entirety. Another screening method to identify aptamers is described in U.S. Pat. No. 5,270,163 (also incorporated herein by reference). The SELEX process is based on the capacity of nucleic acids for forming a variety of two- and three- dimensional structures, as well as the chemical versatility available within the nucleotide monomers to act as ligands (form specific binding pairs) with virtually any

chemical compound, whether monomeric or polymeric, including other nucleic acid molecules and polypeptides. Molecules of any size or composition can serve as targets.

[0356] The SELEX method involves selection from a mixture of candidate oligonucleotides and step-wise iterations of binding, partitioning and amplification, using the same general selection scheme, to achieve desired binding affinity and selectivity. Starting from a mixture of nucleic acids, which can comprise a segment of randomized sequence, the SELEX method includes steps of contacting the mixture with the target under conditions favorable for binding; partitioning unbound nucleic acids from those nucleic acids which have bound specifically to target molecules; dissociating the nucleic acid-target complexes; amplifying the nucleic acids dissociated from the nucleic acid-target complexes to yield a ligand enriched mixture of nucleic acids. The steps of binding, partitioning, dissociating and amplifying are repeated through as many cycles as desired to yield highly specific high affinity nucleic acid ligands to the target molecule.

[0357] Nucleotide aptamers can be used, for example, as diagnostic tools or as specific inhibitors to dissect intracellular signaling and transport pathways (James, *Curr. Opin. Pharmacol. 1*:540-546 (2001)). The high affinity and specificity of nucleotide aptamers makes them good candidates for drug discovery. For example, aptamer antagonists to the toxin ricin have been isolated and have IC50 values in the nanomolar range (Hesselberth JR *et al.*, *J Biol Chem 275*:4937-4942 (2000)). Nucleotide aptamers can also be used against infectious disease, malignancy and viral surface proteins to reduce cellular infectivity.

[0358] Nucleotide aptamers for use in the methods described herein can be modified (*e.g.*, by modifying the backbone or bases or conjugated to peptides) as described herein for other polynucleotides.

[0359] Using the protein structure of DR6, screening for aptamers that act on DR6 using the SELEX process would allow for the identification of aptamers that inhibit DR6-mediated processes.

[0360] Polypeptide aptamers for use in the methods described herein are random peptides selected for their ability to bind to and thereby block the action of DR6. Polypeptide aptamers can include a short variable peptide domain attached at both ends to a protein scaffold. This double structural constraint greatly increases the binding affinity of the peptide aptamer to levels comparable to an antibody's (nanomolar range). *See, e.g.*, Hoppe-Seyler F *et al.*, *J Mol Med 78*(8):426-430 (2000). The length of the short variable peptide is typically about 10 to 20 amino

acids, and the scaffold can be any protein which has good solubility and compacity properties. One non-limiting example of a scaffold protein is the bacterial protein Thioredoxin-A. *See, e.g.*, Cohen BA *et al.*, *PNAS 95*(24): 14272-14277 (1998).

[0361] Polypeptide aptamers are peptides or small polypeptides that act as dominant inhibitors of protein function. Peptide aptamers specifically bind to target proteins, blocking their functional ability (Kolonin *et al.* (1998) *Proc. Natl. Acad. Sci. 95*: 14,266-14,271). Peptide aptamers that bind with high affinity and specificity to a target protein can be isolated by a variety of techniques known in the art. Peptide aptamers can be isolated from random peptide libraries by yeast two-hybrid screens (Xu, C.W., *et al.* (1997) *Proc. Natl. Acad. Sci. 94*:12,473-12,478) or by ribosome display (Hanes *et al.* (1997) *Proc. Natl. Acad. Sci. 94*:4937-4942). They can also be isolated from phage libraries (Hoogenboom, H.R., et al. (1998) Immunotechnology 4:1-20) or chemically generated peptide libraries. Additionally, polypeptide aptamers can be selected using the selection of Ligand Regulated Peptide Aptamers (LiRPAs). *See, e.g.*, Binkowski BF *et al.*, (2005) *Chem & Biol 12*(7): 847-855, incorporated herein by reference. Although the difficult means by which peptide aptamers are synthesized makes their use more complex than polynucleotide aptamers, they have unlimited chemical diversity. Polynucleotide aptamers are limited because they utilize only the four nucleotide bases, while peptide aptamers would have a much-expanded repertoire (*i.e.*, 20 amino acids).

[0362] Peptide aptamers for use in the methods described herein can be modified (*e.g.*, conjugated to polymers or fused to proteins) as described for other polypeptides elsewhere herein.

P75 Antagonists

[0363] Antagonists of p75 to be used in accordance with the methods described herein include, for example, (i) p75 antagonists compounds; (ii) p75 antagonist polypeptides; (iii) p75 antagonist antibodies or fragments thereof; (iv) -75 antagonist polynucleotides; (v) p75 aptamers; and (vi) combinations of two or more of said p75 antagonists. In some embodiments, the p75 antagonist inhibits interaction of p75 with DR6.

[0364] P75 antagonists are known in the art, and one of ordinary skill in the art would know how to screen for and test p75 antagonists which would inhibit the interaction of p75 and DR6. For example, a cyclic decapeptide antagonist of p75 is described in Turner *et al. J. Neuroscience Research 78*: 193-199 (2004), which is herein incorporated by reference in its entirety.

Vectors and Host Cells

[0365] Host-expression systems represent vehicles by which the coding sequences of interest can be produced and subsequently purified, but also represent cells which can, when transformed or transfected with the appropriate nucleotide coding sequences, express a DR6 and/or p75 antagonist polypeptide or antibody *in situ*. These include but are not limited to microorganisms such as bacteria (*e.g.*, *E. coli*, *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing DR6 and/or p75 antagonist polypeptide or antibody coding sequences; yeast (*e.g.*, *Saccharomyces*, *Pichia*) transformed with recombinant yeast expression vectors containing DR6 and/or p75 antagonist polypeptide or antibody coding sequences; insect cell systems infected with recombinant virus expression vectors (*e.g.*, baculovirus) containing DR6 and/or p75 antagonist polypeptide or antibody coding sequences; plant cell systems infected with recombinant virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (*e.g.*, Ti plasmid) containing DR6 and/or p75 antagonist polypeptide or antibody coding sequences; or mammalian cell systems (*e.g.*, COS, CHO, BLK, 293, 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (*e.g.*, metallothionein promoter) or from mammalian viruses (*e.g.*, the adenovirus late promoter; the vaccinia virus 7.5K promoter). Bacterial cells such as *Escherichia coli*, or eukaryotic cells, *e.g.*, for the expression of DR6 and/or p75 antagonist polypeptide or whole recombinant antibody molecules, are used for the expression of a recombinant DR6 and/or p75 antagonist polypeptide or antibody molecule. For example, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for DR6 and/or p75 antagonist polypeptide or antibodies (Foecking *et al.*, *Gene 45*:101 (1986); Cockett *et al.*, *Bio/Technology 8*:2 (1990)).

[0366] In bacterial systems, a number of expression vectors can be advantageously selected depending upon the use intended for the DR6 and/or p75 antagonist polypeptide or antibody molecule being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions of the DR6 and/or p75 antagonist polypeptide or antibody molecule, vectors which direct the expression of high levels of fusion protein products that are readily purified can be desirable. Such vectors include, but are not limited, to the *E. coli* expression vector pUR278 (Ruther *et al.*, *EMBO J. 2*:1791 (1983)), in

which the DR6 and/or p75 antagonist polypeptide or antibody coding sequence can be ligated individually into the vector in frame with the lacZ coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, *Nucleic Acids Res. 13*:3101-3109 (1985); Van Heeke & Schuster, *J. Biol. Chem. 24*:5503-5509 (1989)); and the like. pGEX vectors can also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption and binding to a matrix glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

[0367] In an insect system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is typically used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The DR6 and/or p75 antagonist polypeptide or antibody coding sequence can be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter).

[0368] In mammalian host cells, a number of viral-based expression systems can be utilized. In cases where an adenovirus is used as an expression vector, the DR6 and/or p75 antagonist polypeptide or antibody coding sequence of interest can be ligated to an adenovirus transcription/translation control complex, *e.g.*, the late promoter and tripartite leader sequence. This chimeric gene can then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non- essential region of the viral genome (*e.g.*, region E1 or E3) will result in a recombinant virus that is viable and capable of expressing the DR6 and/or p75 antagonist polypeptide or antibody molecule in infected hosts. (*e.g.*, see Logan & Shenk, *Proc. Natl. Acad. Sci. USA 81*:355-359 (1984)). Specific initiation signals can also be required for efficient translation of inserted antibody coding sequences. These signals include the ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression can be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (*see* Bittner *et al.*, *Methods in Enzymol. 153*:51-544 (1987)).

[0369] In addition, a host cell strain can be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired.

Such modifications (*e.g.*, glycosylation) and processing (*e.g.*, cleavage) of protein products can be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product can be used. Such mammalian host cells include but are not limited to CHO, VERY, BHK, HeLa, COS, MDCK, 293, 3T3, WI38, and in particular, breast cancer cell lines such as, for example, BT483, Hs578T, HTB2, BT20 and T47D, and normal mammary gland cell line such as, for example, CRL7030 and Hs578Bst.

[0370] For long-term, high-yield production of recombinant proteins, stable expression is typically used. For example, cell lines which stably express the DR6 and/or p75 antagonist polypeptide or antibody molecule can be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (*e.g.*, promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells can be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method can advantageously be used to engineer cell lines which stably express the DR6 and/or p75 antagonist polypeptide or antibody molecule.

[0371] A number of selection systems can be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler *et al.*, *Cell 11*:223 (1977)), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, *Proc. Natl. Acad. Sci. USA 48*:202 (1992)), and adenine phosphoribosyltransferase (Lowy *et al.*, *Cell 22*:817 1980) genes can be employed in tk-, hgprt- or aprt-cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler *et al.*, *Natl. Acad. Sci. USA 77*:357 (1980); O'Hare *et al.*, *Proc. Natl. Acad. Sci. USA 78*:1527 (1981)); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, *Proc. Natl. Acad. Sci. USA 78*:2072 (1981)); neo, which confers resistance to the aminoglycoside G-418 *Clinical Pharmacy 12*:488-505; Wu and Wu, *Biotherapy 3*:87-95 (1991); Tolstoshev, *Ann. Rev.*

Pharmacol. Toxicol. 32:573-596 (1993); Mulligan, *Science 260*:926-932 (1993); and Morgan and Anderson, *Ann. Rev. Biochem. 62*:191-217 (1993); *TIB TECH 11(5)*:155-215 (May, 1993); and hygro, which confers resistance to hygromycin (Santerre *et al.*, *Gene 30*:147 (1984). Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel *et al.* (eds.), *Current Protocols in Molecular Biology*, John Wiley & Sons, NY (1993); Kriegler, *Gene Transfer and Expression*, A Laboratory Manual, Stockton Press, NY (1990); and in Chapters 12 and 13, Dracopoli *et al.* (eds), *Current Prolocols in Human Genetics*, John Wiley & Sons, NY (1994); Colberre-Garapin *et al.*, *J. Mol. Biol. 150*:1 (1981), which are incorporated by reference herein in their entireties.

[0372] The expression levels of a DR6 and/or p75 antagonist polypeptide or antibody can be increased by vector amplification (for a review, *see* Bebbington and Hentschel, *The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning*, Academic Press, New York, Vol. 3. (1987)). When a marker in the vector system expressing a DR6 and/or p75 antagonist polypeptide or antibody is amplifiable, increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the antibody or other polypeptide gene, production of the antibody or other polypeptide will also increase (Crouse *et al.*, *Mol. Cell. Biol. 3*:257 (1983)).

[0373] Vectors comprising nucleic acids encoding DR6 and/or p75 antagonists, *e.g.*, soluble polypeptides, antibodies, antagonist polynucleotides, or aptamers, can be used to produce antagonists for use in the methods described herein. The choice of vector and expression control sequences to which such nucleic acids are operably linked depends on the functional properties desired, *e.g.*, protein expression, and the host cell to be transformed.

[0374] Expression control elements useful for regulating the expression of an operably linked coding sequence are known in the art. Examples include, but are not limited to, inducible promoters, constitutive promoters, secretion signals, and other regulatory elements. When an inducible promoter is used, it can be controlled, *e.g.*, by a change in nutrient status, or a change in temperature, in the host cell medium.

[0375] The vector can include a prokaryotic replicon, *i.e.*, a DNA sequence having the ability to direct autonomous replication and maintenance of the recombinant DNA molecule extra-chromosomally in a bacterial host cell. Such replicons are well known in the art. In addition, vectors that include a prokaryotic replicon can also include a gene whose expression

confers a detectable marker such as a drug resistance. Examples of bacterial drug-resistance genes are those that confer resistance to ampicillin or tetracycline.

[0376] Vectors that include a prokaryotic replicon can also include a prokaryotic or bacteriophage promoter for directing expression of the coding gene sequences in a bacterial host cell. Promoter sequences compatible with bacterial hosts are typically provided in plasmid vectors containing convenient restriction sites for insertion of a DNA segment to be expressed. Examples of such plasmid vectors are pUC8, pUC9, pBR322 and pBR329 (BioRad), pPL and pKK223 (Pharmacia). Any suitable prokaryotic host can be used to express a recombinant DNA molecule encoding a protein used in the methods described herein.

[0377] For the purposes described herein, numerous expression vector systems can be employed. For example, one class of vector utilizes DNA elements which are derived from animal viruses such as bovine papilloma virus, polyoma virus, adenovirus, vaccinia virus, baculovirus, retroviruses (RSV, MMTV or MOMLV) or SV40 virus. Others involve the use of polycistronic systems with internal ribosome binding sites. Additionally, cells which have integrated the DNA into their chromosomes can be selected by introducing one or more markers which allow selection of transfected host cells. The marker can provide for prototrophy to an auxotrophic host, biocide resistance (*e.g.*, antibiotics) or resistance to heavy metals such as copper. The selectable marker gene can either be directly linked to the DNA sequences to be expressed, or introduced into the same cell by cotransformation. The neomycin phosphotransferase (neo) gene is an example of a selectable marker gene (Southern *et al., J. Mol. Anal. Genet. 1*:327-341 (1982)). Additional elements can also be needed for optimal synthesis of mRNA. These elements can include signal sequences, splice signals, as well as transcriptional promoters, enhancers, and termination signals.

[0378] In one embodiment, a proprietary expression vector of Biogen IDEC, Inc., referred to as NEOSPLA (U.S. patent 6,159,730) can be used. This vector contains the cytomegalovirus promoter/enhancer, the mouse beta globin major promoter, the SV40 origin of replication, the bovine growth hormone polyadenylation sequence, neomycin phosphotransferase exon 1 and exon 2, the dihydrofolate reductase gene and leader sequence. This vector has been found to result in very high level expression upon transfection in CHO cells, followed by selection in G418 containing medium and methotrexate amplification. Of course, any expression vector which is capable of eliciting expression in eukaryotic cells can be used. Examples of suitable vectors include, but are not limited to plasmids pcDNA3, pHCMV/Zeo, pCR3.1,

pEF1/His, pIND/GS, pRc/HCMV2, pSV40/Zeo2, pTRACER-HCMV, pUB6/V5-His, pVAX1, and pZeoSV2 (available from Invitrogen, San Diego, CA), and plasmid pCI (available from Promega, Madison, WI). Additional eukaryotic cell expression vectors are known in the art and are commercially available. Typically, such vectors contain convenient restriction sites for insertion of the desired DNA segment. Exemplary vectors include pSVL and pKSV-10 (Pharmacia), pBPV-1, pml2d (International Biotechnologies), pTDT1 (ATCC 31255), retroviral expression vector pMIG and pLL3.7, adenovirus shuttle vector pDC315, and AAV vectors. Other exemplary vector systems are disclosed *e.g.*, in U.S. Patent 6,413,777.

[0379] In general, screening large numbers of transformed cells for those which express suitably high levels of the antagonist is routine experimentation which can be carried out, for example, by robotic systems.

[0380] Frequently used regulatory sequences for mammalian host cell expression include viral elements that direct high levels of protein expression in mammalian cells, such as promoters and enhancers derived from retroviral LTRs, cytomegalovirus (CMV) (such as the CMV promoter/enhancer), Simian Virus 40 (SV40) (such as the SV40 promoter/enhancer), adenovirus, (*e.g.*, the adenovirus major late promoter (AdmlP)), polyoma and strong mammalian promoters such as native immunoglobulin and actin promoters. For further description of viral regulatory elements, and sequences thereof, *see e.g.*, Stinski, U.S. Pat. No. 5,168,062; Bell, U.S. Pat. No. 4,510,245; and Schaffner, U.S. Pat. No. 4,968,615.

[0381] The recombinant expression vectors can carry sequences that regulate replication of the vector in host cells (*e.g.*, origins of replication) and selectable marker genes. The selectable marker gene facilitates selection of host cells into which the vector has been introduced (*see, e.g.*, Axel, U.S. Pat. Nos. 4,399,216; 4,634,665 and 5,179,017). For example, typically the selectable marker gene confers resistance to a drug, such as G418, hygromycin or methotrexate, on a host cell into which the vector has been introduced. Frequently used selectable marker genes include the dihydrofolate reductase (DHFR) gene (for use in dhfr- host cells with methotrexate selection/amplification) and the neo gene (for G418 selection).

[0382] Vectors encoding DR6 and/or p75 antagonists can be used for transformation of a suitable host cell. Transformation can be by any suitable method. Methods for introduction of exogenous DNA into mammalian cells are well known in the art and include dextran-mediated transfection, calcium phosphate precipitation, polybrene-mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of

the DNA into nuclei. In addition, nucleic acid molecules can be introduced into mammalian cells by viral vectors.

[0383] Host cells for expression of a DR6 and/or p75 antagonist for use in a method described herein can be prokaryotic or eukaryotic. Exemplary eukaryotic host cells include, but are not limited to, yeast and mammalian cells, *e.g.*, Chinese hamster ovary (CHO) cells (ATCC Accession No. CCL61), NIH Swiss mouse embryo cells NIH-3T3 (ATCC Accession No. CRL1658), and baby hamster kidney cells (BHK). Other useful eukaryotic host cells include insect cells and plant cells. Exemplary prokaryotic host cells are *E. coli* and *Streptomyces*.

[0384] Transformation of host cells can be accomplished by conventional methods suited to the vector and host cell employed. For transformation of prokaryotic host cells, electroporation and salt treatment methods can be employed (Cohen *et al., Proc. Natl. Acad. Sci. USA 69*:2110-14 (1972)). For transformation of vertebrate cells, electroporation, cationic lipid or salt treatment methods can be employed. *See, e.g.*, Graham *et al., Virology 52*:456-467 (1973); Wigler *et al., Proc. Natl. Acad. Sci. USA 76*:1373-76 (1979).

[0385] In certain embodiments, the host cell line used for protein expression is of mammalian origin; those skilled in the art are credited with ability to determine particular host cell lines which are best suited for the desired gene product to be expressed therein. Exemplary host cell lines include, but are not limited to NSO, SP2 cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (*e.g.*, Hep G2), A549 cells DG44 and DUXB11 (Chinese Hamster Ovary lines, DHFR minus), HELA (human cervical carcinoma), CVI (monkey kidney line), COS (a derivative of CVI with SV40 T antigen), R1610 (Chinese hamster fibroblast) BALBC/3T3 (mouse fibroblast), HAK (hamster kidney line), SP2/O (mouse myeloma), P3x63-Ag3.653 (mouse myeloma), BFA-1c1BPT (bovine endothelial cells), RAJI (human lymphocyte) and 293 (human kidney). Host cell lines are typically available from commercial services, the American Tissue Culture Collection or from published literature.

[0386] Expression of polypeptides from production cell lines can be enhanced using known techniques. For example, the glutamine synthetase (GS) system is commonly used for enhancing expression under certain conditions. *See, e.g.*, European Patent Nos. 0 216 846, 0 256 055, and 0 323 997 and European Patent Application No. 89303964.4.

Gene Therapy

[0387] A DR6 and/or p75 antagonist can be produced *in vivo* in a mammal, *e.g.*, a human patient, using a gene-therapy approach to treatment of a nervous-system disease, disorder or

injury in which promoting survival, proliferation and differentiation of oligodendrocytes or promoting myelination of neurons would be therapeutically beneficial. This involves administration of a suitable DR6 and/or p75 antagonist-encoding nucleic acid operably linked to suitable expression control sequences. Generally, these sequences are incorporated into a viral vector. Suitable viral vectors for such gene therapy include an adenoviral vector, an alphavirus vector, an enterovirus vector, a pestivirus vector, a lentiviral vector, a baculoviral vector, a herpesvirus vector, an Epstein Barr viral vector, a papovaviral vector, a poxvirus vector, a vaccinia viral vector, adeno-associated viral vector and a herpes simplex viral vector. The viral vector can be a replication-defective viral vector. Adenoviral vectors that have a deletion in its E1 gene or E3 gene are typically used. When an adenoviral vector is used, the vector usually does not have a selectable marker gene.

Pharmaceutical Compositions

[0388] The DR6 and/or p75 antagonists used in the methods described herein can be formulated into pharmaceutical compositions for administration to mammals, including humans. The pharmaceutical compositions used in the methods described herein comprise pharmaceutically acceptable carriers, including, *e.g.*, ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, polyethylene glycol and wool fat.

[0389] The compositions used in the methods described herein can be administered by any suitable method, *e.g.*, parenterally, intraventricularly, orally, by inhalation spray, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir. The term "parenteral" as used herein includes subcutaneous, intravenous, intramuscular, intra-articular, intra-synovial, intrasternal, intrathecal, intrahepatic, intralesional and intracranial injection or infusion techniques. As described previously, DR6 and/or p75 antagonists used in the methods described herein act in the nervous system to promote survival and prevent apoptosis of nervous system cells. Accordingly, in certain methods described herein, the DR6 and/or p75 antagonists are administered in such a way that they cross the blood-brain barrier. This crossing can result from

the physico-chemical properties inherent in the DR6 and/or p75 antagonist molecule itself, from other components in a pharmaceutical formulation, or from the use of a mechanical device such as a needle, cannula or surgical instruments to breach the blood-brain barrier. Where the DR6 and/or p75 antagonist is a molecule that does not inherently cross the blood-brain barrier, *e.g.*, a fusion to a moiety that facilitates the crossing, suitable routes of administration are, *e.g.*, intrathecal or intracranial, *e.g.*, directly into a chronic lesion of MS. Where the DR6 and/or p75 antagonist is a molecule that inherently crosses the blood-brain barrier, the route of administration can be by one or more of the various routes described below.

[0390] Sterile injectable forms of the compositions described herein can be aqueous or oleaginous suspension. These suspensions can be formulated according to techniques known in the art using suitable dispersing or wetting agents and suspending agents. The sterile, injectable preparation can also be a sterile, injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent, for example as a suspension in 1,3-butanediol. Among the acceptable vehicles and solvents that can be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil can be employed including synthetic mono- or di-glycerides. Fatty acids, such as oleic acid and its glyceride derivatives are useful in the preparation of injectables, as are natural pharmaceutically acceptable oils, such as olive oil or castor oil, especially in their polyoxyethylated versions. These oil solutions or suspensions can also contain a long-chain alcohol diluent or dispersant, such as carboxymethyl cellulose or similar dispersing agents which are commonly used in the formulation of pharmaceutically acceptable dosage forms including emulsions and suspensions. Other commonly used surfactants, such as Tweens, Spans and other emulsifying agents or bioavailability enhancers which are commonly used in the manufacture of pharmaceutically acceptable solid, liquid, or other dosage forms can also be used for the purposes of formulation.

[0391] Parenteral formulations can be a single bolus dose, an infusion or a loading bolus dose followed with a maintenance dose. These compositions can be administered at specific fixed or variable intervals, *e.g.*, once a day, or on an "as needed" basis.

[0392] Certain pharmaceutical compositions used in the methods described herein can be orally administered in an acceptable dosage form including, *e.g.*, capsules, tablets, aqueous suspensions or solutions. Certain pharmaceutical compositions also can be administered by nasal aerosol or inhalation. Such compositions can be prepared as solutions in saline, employing

benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, and/or other conventional solubilizing or dispersing agents.

[0393] The amount of a DR6 and/or p75 antagonist that can be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated, the type of antagonist used and the particular mode of administration. The composition can be administered as a single dose, multiple doses or over an established period of time in an infusion. Dosage regimens also can be adjusted to provide the optimum desired response (*e.g.*, a therapeutic or prophylactic response).

[0394] In some cases, the methods described herein use a "therapeutically effective amount" or a "prophylactically effective amount" of a DR6 and/or p75 antagonist. Such a therapeutically or prophylactically effective amount can vary according to factors such as the disease state, age, sex, and weight of the individual. A therapeutically or prophylactically effective amount is also one in which any toxic or detrimental effects are outweighed by the therapeutically beneficial effects.

[0395] A specific dosage and treatment regimen for any particular patient will depend upon a variety of factors, including the particular DR6 and/or p75 antagonist used, the patient's age, body weight, general health, sex, and diet, and the time of administration, rate of excretion, drug combination, and the severity of the particular disease being treated. Judgment of such factors by medical caregivers is within the ordinary skill in the art. The amount will also depend on the individual patient to be treated, the route of administration, the type of formulation, the characteristics of the compound used, the severity of the disease, and the desired effect. The amount used can be determined by pharmacological and pharmacokinetic principles well known in the art.

[0396] In the methods described herein the DR6 and/or p75 antagonists are generally administered directly to the nervous system, intracerebroventricularly, or intrathecally, *e.g.* into a chronic lesion. Compositions for administration according to the methods described herein can be formulated so that a dosage of 0.001 – 10 mg/kg body weight per day of the DR6 and/or p75 antagonist is administered. In some embodiments, the dosage is 0.01 – 1.0 mg/kg body weight per day. In some embodiments, the dosage is 0.001 – 0.5 mg/kg body weight per day.

[0397] For treatment with a DR6 and/or p75 antagonist antibody, the dosage can range, *e.g.*, from about 0.0001 to 100 mg/kg, and more usually 0.01 to 5 mg/kg (*e.g.*, 0.02 mg/kg, 0.25 mg/kg, 0.5 mg/kg, 0.75 mg/kg, 1mg/kg, 2 mg/kg, etc.), of the host body weight. For example

dosages can be 1 mg/kg body weight or 10 mg/kg body weight or within the range of 1-10 mg/kg, for example, at least 1 mg/kg. Doses intermediate in the above ranges can also be used. Subjects can be administered such doses daily, on alternative days, weekly or according to any other schedule determined by empirical analysis. An exemplary treatment entails administration in multiple dosages over a prolonged period, for example, of at least six months. Additional exemplary treatment regimes entail administration once per every two weeks or once a month or once every 3 to 6 months. Exemplary dosage schedules include 1-10 mg/kg or 15 mg/kg on consecutive days, 30 mg/kg on alternate days or 60 mg/kg weekly. In some methods, two or more monoclonal antibodies with different binding specificities are administered simultaneously, in which case the dosage of each antibody administered falls within the ranges indicated.

[0398] In certain embodiments, a subject can be treated with a nucleic acid molecule encoding a DR6 and/or p75 antagonist polynucleotide. Doses for nucleic acids range from about 10 ng to 1 g, 100 ng to 100 mg, 1 μg to 10 mg, or 30-300 μg DNA per patient. Doses for infectious viral vectors vary from 10-100, or more, virions per dose.

[0399] Supplementary active compounds also can be incorporated into the compositions used in the methods described herein. For example, a soluble polypeptide or a fusion protein can be coformulated with and/or coadministered with one or more additional therapeutic agents.

[0400] The delivery methods encompass any suitable delivery method for a DR6 and/or p75 antagonist to a selected target tissue, including bolus injection of an aqueous solution or implantation of a controlled-release system. Use of a controlled-release implant reduces the need for repeat injections.

[0401] The DR6 and/or p75 antagonists described herein can be directly infused into the brain. Various implants for direct brain infusion of compounds are known and are effective in the delivery of therapeutic compounds to human patients suffering from neurological disorders. These include chronic infusion into the brain using a pump, stereotactically implanted, temporary interstitial catheters, permanent intracranial catheter implants, and surgically implanted biodegradable implants. *See, e.g.*, Gill *et al.*, *supra*; Scharfen *et al.*, "High Activity Iodine-125 Interstitial Implant For Gliomas," *Int. J. Radiation Oncology Biol. Phys. 24*(4):583-591 (1992); Gaspar *et al.*, "Permanent 125I Implants for Recurrent Malignant Gliomas," *Int. J. Radiation Oncology Biol. Phys. 43*(5):977-982 (1999); chapter 66, pages 577-580, Bellezza *et al.*, "Stereotactic Interstitial Brachytherapy," in Gildenberg *et al.*, Textbook of Stereotactic and Functional Neurosurgery, McGraw-Hill (1998); and Brem *et al.*, "The Safety of Interstitial

Chemotherapy with BCNU-Loaded Polymer Followed by Radiation Therapy in the Treatment of Newly Diagnosed Malignant Gliomas: Phase I Trial," *J. Neuro-Oncology 26*:111-23 (1995).

[0402] The compositions can also comprise a DR6 and/or p75 antagonist dispersed in a biocompatible carrier material that functions as a suitable delivery or support system for the compounds. Suitable examples of sustained release carriers include semipermeable polymer matrices in the form of shaped articles such as suppositories or capsules. Implantable or microcapsular sustained release matrices include polylactides (U.S. Patent No. 3,773,319; EP 58,481), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate (Sidman *et al.*, *Biopolymers 22*:547-56 (1985)); poly(2-hydroxyethyl-methacrylate), ethylene vinyl acetate (Langer *et al., J. Biomed. Mater. Res. 15*:167-277 (1981); Langer, *Chem. Tech. 12*:98-105 (1982)) or poly-D-(-)-3hydroxybutyric acid (EP 133,988).

[0403] In some embodiments of the methods described herein, a DR6 and/or p75 antagonist is administered to a patient by direct infusion into an appropriate region of the brain. *See, e.g.*, Gill *et al.*, *Nature Med. 9*: 589-95 (2003). Alternative techniques are available and can be applied to administer a DR6 and/or p75 antagonist. For example, stereotactic placement of a catheter or implant can be accomplished using the Riechert-Mundinger unit and the ZD (Zamorano-Dujovny) multipurpose localizing unit. A contrast-enhanced computerized tomography (CT) scan, injecting 120 ml of omnipaque, 350 mg iodine/ml, with 2 mm slice thickness can allow three-dimensional multiplanar treatment planning (STP, Fischer, Freiburg, Germany). This equipment permits planning on the basis of magnetic resonance imaging studies, merging the CT and MRI target information for clear target confirmation.

[0404] The Leksell stereotactic system (Downs Surgical, Inc., Decatur, GA) modified for use with a GE CT scanner (General Electric Company, Milwaukee, WI) as well as the Brown-Roberts-Wells (BRW) stereotactic system (Radionics, Burlington, MA) can be used for this purpose. Thus, on the morning of the implant, the annular base ring of the BRW stereotactic frame can be attached to the patient's skull. Serial CT sections can be obtained at 3 mm intervals though the (target tissue) region with a graphite rod localizer frame clamped to the base plate. A computerized treatment planning program can be run on a VAX 11/780 computer (Digital Equipment Corporation, Maynard, Mass.) using CT coordinates of the graphite rod images to map between CT space and BRW space.

[0405] The methods of treatment of nervous system disorders associated with increased cell death as described herein are typically tested *in vitro*, and then *in vivo* in an acceptable

animal model, for the desired therapeutic or prophylactic activity, prior to use in humans. Suitable animal models, including transgenic animals, are will known to those of ordinary skill in the art. For example, *in vitro* assays to demonstrate the survival effect of the DR6 and/or p75 antagonists are described herein. The effect of the DR6 and/or p75 antagonists on apoptosis can be tested *in vitro* as described in the Examples. Finally, *in vivo* tests can be performed by creating transgenic mice which express the DR6 and/or p75 antagonist or by administering the DR6 and/or p75 antagonist to mice or rats in models as described herein.

[0406] The practices described herein will employ, unless otherwise indicated, conventional techniques of cell biology, cell culture, molecular biology, transgenic biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature. See, for example, Molecular Cloning: A Laboratory Manual (3-Volume Set), J. Sambrook, D. W. Russell, Cold Spring Harbor Laboratory Press (2001); Genes VIII, B. Lewin, Prentice Hall (2003); PCR Primer, C.W. Dieffenbach and G.S. Dveksler, CSHL Press (2003); DNA Cloning, D. N. Glover ed., Volumes I and II (1985); Oligonucleotide Synthesis: Methods and Applications (Methods in Molecular Biology), P. Herdewijn (Ed.), Humana Press (2004); Culture of Animal Cells: A Manual of Basic Technique, 4th edition, R. I. Freshney, Wiley-Liss (2000); Oligonucleotide Synthesis, M. J. Gait (Ed.), (1984); Mullis *et al.* U.S. Pat. No: 4,683,195; Nucleic Acid Hybridization, B. D. Hames & S. J. Higgins eds. (1984); Nucleic Acid Hybridization, M. L. M. Anderson, Springer (1999); Animal Cell Culture and Technology, 2nd edition, M. Butler, BIOS Scientific Publishers (2004); Immobilized Cells and Enzymes: A Practical Approach (Practical Approach Series), J. Woodward, Irl Pr (1992); Transcription And Translation, B. D. Hames & S. J. Higgins (Eds.) (1984); Culture Of Animal Cells, R. I. Freshney, Alan R. Liss, Inc., (1987); Immobilized Cells And Enzymes, IRL Press, (1986); A Practical Guide To Molecular Cloning, 3rd edition, B. Perbal, John Wiley & Sons Inc. (1988); the treatise, Methods In Enzymology, Academic Press, Inc., N.Y.; Gene Transfer Vectors For Mammalian Cells, J. H. Miller and M. P. Calos eds., Cold Spring Harbor Laboratory (1987); Methods In Enzymology, Vols. 154 and 155, Wu *et al.* (Eds.); Immunochemical Methods In Cell And Molecular Biology, Mayer and Walker, (Eds.), Academic Press, London (1987); Handbook Of Experimental Immunology, Volumes I-IV, D. M. Weir and C. C. Blackwell (Eds.), (1986); Immunology Methods Manual: The Comprehensive Sourcebook of Techniques (4 Volume Set), 1st edition, I. Lefkovits, Academic Press (1997); Manipulating the Mouse Embryo: A Laboratory Manual, 3rd edition, Cold Spring Harbor Laboratory Press

(2002); and in Ausubel *et al.*, Current Protocols in Molecular Biology, John Wiley and Sons, Baltimore, Maryland (1989).

[0407] General principles of antibody engineering are set forth in Antibody Engineering: Methods and Protocols (Methods in Molecular Biology), B.L. Lo (Ed.), Humana Press (2003); Antibody engineering, R. Kontermann and S. Dubel (Eds.), Springer Verlag (2001); Antibody Engineering, 2nd edition, C.A.K. Borrebaeck (Ed.), Oxford Univ. Press (1995). General principles of protein engineering are set forth in Protein Engineering, A Practical Approach, Rickwood, D., *et al.* (Eds.), IRL Press at Oxford Univ. Press, Oxford, Eng. (1995). General principles of antibodies and antibody-hapten binding are set forth in: Antibodies: A Laboratory Manual, E. Harlow and D. Lane, Cold Spring Harbor Laboratory Press (1988); Nisonoff, A., Molecular Immunology, 2nd edition, Sinauer Associates, Sunderland, MA (1984); and Steward, M.W., Antibodies, Their Structure and Function, Chapman and Hall, New York, NY (1984). Additionally, standard methods in immunology known in the art and not specifically described are generally followed as in Current Protocols in Immunology, John Wiley & Sons, New York; Stites *et al.* (Eds.) , Immunochemical Protocols (Methods in Molecular Biology), 2nd edition, J. D. Pound (Ed.), Humana Press (1998), Weir's Handbook of Experimental Immunology, 5th edition, D. M. Weir (Ed.), Blackwell Publishers (1996), Methods in Cellular Immunology, 2nd edition, R. Fernandez-Botran, CRC Press (2001); Basic and Clinical Immunology, 8th edition, Appleton & Lange, Norwalk, CT (1994) and Mishell and Shiigi (Eds.), Selected Methods in Cellular Immunology, W.H. Freeman and Co., New York (1980).

[0408] Standard reference works setting forth general principles of immunology include Current Protocols in Immunology, John Wiley & Sons, New York; Klein, J.; Kuby Immunology, 4th edition, R. A. Goldsby, *et al.*, H. Freeman & Co. (2000); Basic and Clinical Immunology, M. Peakman, *et al.*, Churchill Livingstone (1997); Immunology, 6th edition, I. Roitt, *et al.*, Mosby, London (2001); Cellular and Molecular Immunology, 5th edition; A.K. Abbas, A.H. Lichtman, Elsevier - Health Sciences Division (2005); Immunology Methods Manual: The Comprehensive Sourcebook of Techniques (4 Volume Set), 1st edition, I. Lefkovits, Academic Press (1997) Immunology, 5th edition, R.A. Goldsby, *et al.*, W. H. Freeman (2002); Monoclonal Antibodies : Principles and Practice, 3rd Edition , J.W. Goding, Academic Press (1996); Immunology: The Science of Self-Nonself Discrimination, John Wiley & Sons, New York (1982); Kennett, R., *et al.* (Eds.), Monoclonal Antibodies, Hybridoma: A New Dimension in Biological Analyses, Plenum Press, New York (1980); Campbell, A., "Monoclonal Antibody Technology" in Burden,

R., *et al.* (Eds.), Laboratory Techniques in Biochemistry and Molecular Biology, Vol. 13, Elsevere, Amsterdam (1984).

[0409] All of the references cited above, as well as all references cited herein, are incorporated herein by reference in their entireties.

Examples

Example 1

DR6 is Expressed in the Nervous System

[0410] Tissue sections from adult mouse cerebral cortex and rat spinal cord were examined for expression of DR6 protein. Tissue sections were first penetrated with PBS containing 1% Triton X-100 (Sigma) for 30 minutes followed by incubation in blocking solution (PBS containing 0.1% Triton X-100 and 10% normal goat serum (NGS)) for 1 hour at room temperature. For primary antibody labeling, sections were incubated in blocking medium containing rabbit anti-DR6 (Santa Cruz, sc-13106, 1:200) and mouse anti-neuronal class III β-tubulin (Covance, MMS-435P, 1:500) at 4°C overnight. After three PBS rinses, sections were incubated in 5% NGS-PBS containing Alexa 594 anti-rabbit antibody (Invitrogen) (1:500) at room temperature for 1 hour. The results show that the colocalization of DR6 and neuronal class III β-tubulin, which indicates that DR6 is expressed in neurons (data not shown).

[0411] To understand the role of DR6 in the nervous system, DR6 mRNA expression levels were evaluated to determine if they were developmentally regulated across rat brain tissues using quantitative real-time polymerase chain reaction after reverse transcription (RT-PCR). mRNA was extracted from whole brain and spinal cord homogenates taken at embryonic day 18 (E18), postnatal days 1 (P1), 7 (P7), 14 (P14), and 21 (P21) and from adults. All mRNA were extracted using Absolutely RNA miniprep kit following the manufacturer's instructions (Stratagene). Purified RNA (High Capacity cDNA Archive Kit, Applied Biosystems) was then used to generate cDNA of DR6. The cDNAs served as the template for quantitative real-time PCR (Q-PCR), and TaqMan Gene Expression system (Mx3000P) was used to quantify the DR6 using Mm00446361_m1 premixed primer set with MGB probes (Applied Biosystems). As seen in Figures 1A-B, DR6 expression level is low at E18, peaks at postnatal day 7 or 14, then reaches lower levels in both brain and spinal cord after maturation. The developmental transcription profile agreed with protein expression profile based on Western blot using anti-DR6

antibody (Fig. 1C). Immunohistochemical staining of human and rat brain tissues section revealed that DR6 is expressed in both human and rat neurons based on colocalization with the βIII-tubulin neuron marker (data not shown).

[0412] In addition, several other cell types were examined for expression of DR6 mRNA. mRNA was extracted from purified cultures of P2 oligodendrocyte progenitor cells (OPCs), E18 cortical neurons, P2 microglias and P42 cerebral cortex astrocytes. All mRNA were extracted using Absolutely RNA miniprep kit following the manufacturer's instructions (Stratagene). Purified RNA (High Capacity cDNA Archive Kit, Applied Biosystems) was then used to generate cDNA of DR6 and of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as a control. Figure 1D shows that DR6 is expressed in all four cell types examined.

[0413] The expression of DR6 is temporally regulated in the oligodendrocyte lineage. Three different approaches were used to examine the DR6 expression in oligodendrocytes. First, semi-quantitive RT-PCR was performed to determine the mRNA level from three different stages of purified populations of oligodendrocyte (A2B5, O4 and MBP). As shown in the Figure 2A, DR6 mRNA was detected through all stage of the oligodendrocyte lineage with equivalent mRNA levels found in $A2B5^+$, $O4^+$and MBP^+ oligodendrocytes. Second, Western blot was performed to determine the DR6 protein level in the three different stage of oligodendrocytes. As shown in the Figure 2B, DR6 protein is detectable in all three stages of oligodendrocyte. Interestingly, it is 5-fold higher in pre-myelinating ($O4^+$) oligodendrocytes stage than earlier progenitor A2B5 and 10 fold higher than mature oligodendrocyte (MBP positive) suggesting that premyelinaitng oligodendrocytes are the predominant DR6 expressing cells. Third, the presence of DR6 protein in oligodendrocytes was confirmed using immunohistochemistry to show that $A2B5^+$, $O4^+$and MBP^+ oligodendrocytes were labeled by an anti-DR6 antibody (data not shown). Again, O4 positive cells show much more intensive fluorescence staining than MBP positive cells suggesting more DR6 expression in the premyelinating stage (O4 positive) than the mature oligodendrocytes. Pre-adsorption of the anti-DR6 antibody by addition of competing DR6-Fc completely ablated the signal.

Example 2

DR6 is Overexpressed in Brains of Patients with Alzheimer's Disease

[0414] Levels of DR6 mRNA in the brains of Alzhemier's disease patients were also examined. Quantitative real-time PCR was performed using six snap-frozen brain tissue blocks from four different donors with Alzhemier's disease. These results were compared to those obtained using three brain tissue blocks from 2 donors without neurological disease.

[0415] As shown in Figure 3, DR6 is expressed 1.2- to 1.8-fold higher in Alzheimer's samples (2 frontal lobes, 1 temporal lobe, 1 basal ganglion and 2 unspecified regions collected from 4 individual donors) when compared to 3 normal brain samples (Fig. 3). To determine if the up-regulated DR6 is neurons specific, immunohistochemistry staining was performed. Cells in the center of αβ amyloid plaque are DR6 positive. The cells near the plaque have significantly brighter DR6 staining, suggesting higher levels of DR6 expression. The mRNA extraction, cDNA production and Q-PCR were performed as described in Example 1.

Example 3

DR6 is Upregulated after Axotomy

[0416] The effect of axotomy on DR6 mRNA and protein levels was also examined. For these experiments, embryonic DRG neurons were prepared as previously described (Mi *et al.*, *Nat. Neurosci. 8*:745-51 (2005)). Briefly, DRGs were first dissected out from 2-week-old E16 Sprague Dawley rats (Charles River) and incubated in 0.25% Trypsin/EDTA (Invitrogen) at 37°C for 30 minutes. An equal volume of DMEM (Invitrogen) containing 20% fetal bovine serum (Invitrogen) was then added to the digestion mixture to stop the reaction. Cell pellets that were collected after spinning down at 1,000 rpm at room temperature for 5 minutes were mechanically dissociated by gently passing through a plastic pipette until no large fragments were visible. About $1x10^5$ DRG neurons were spotted in the center of each well of 4 well chamber-slides (LabTek) that were coated with 100 μg/ml Poly-D-lysine (Sigma). Cells were allowed to attach in growth medium (Neurobasal medium containing B27 supplement (Invitrogen) and 100 ng/ml nerve growth factor (BD Biosciences) at 37°C in humidified air with 5% CO_2. The next morning, the medium was replaced with fresh growth medium and cells were treated with 20 μM of fluorodeoxyuridine for 3 days to remove proliferating glial cells. Cultures were then maintained

in growth medium at 37°C in humidified air with 5% CO_2 with fresh medium change every 3-4 days.

[0417] These cultured axons were severed by blade, and then at 0, 24 and 48 hours after injury, mRNAs were extracted using the Absolutely RNA miniprep kit following manufacturer's instructions (Stratagene). Quantitation, as shown in Figure 4, revealed that DR6 mRNA levels were high at both 24 and 48 hours after axotomy compared to control uninjured axon cultures.

[0418] The mRNA extraction, cDNA production and Q-PCR were performed as described in Example 1.

Example 4

DR6 is Upregulated in Motor Neurons at the Lesion Site after Spinal Cord Injury

[0419] To create a model of spinal cord injury, dorsal hemisection of rat spinal cords were performed as described by Ji *et al.* (*Mol Cell Neurosci. 33*: 311-20 (2006)). The spinal cord tissues were fixed and stained using an anti-DR6 antibody. A significantly higher level of DR6 positive motor neurons was detected in rats with spinal cord injuries than in uninjured rats.

Example 5

Overexpression of DR6 Induces Neuronal Death

Cell Culture

[0420] Cerebral cortical neurons grown in cell cultures were infected with lentivirus expressing DR6, and the effects on cell death were examined. Cerebral cortical neurons were prepared from E18 Sprague Dawley rats (Charles River). Briefly, cerebral cortices from E18 rat embryos were dissected out, minced and incubated in 0.25% Trypsin/EDTA (Invitrogen) at 37°C for 10 minutes. The cells were triturated after adding 60 μg/ml DNase I (Sigma) and 10% fetal bovine serum (Invitrogen) to stop the reaction. Cell pellets collected after spinning down at 1,000 rpm at room temperature for 5 minutes were then mechanically dissociated by gently passing through a plastic pipette until no large fragments were visible. All surfaces of tissue culture plates (Costar) were coated with 100 μg/ml Poly-D-lysine (Sigma) prior to cell seeding. The plating densities for different experimental setups were as follows: $1x10^6$ /well of a 12 well plate for western blots, $1x10^5$ /well of a 24 well plate for time-lapse imaging and $2x10^4$ /well of a

96 well plate for LDH assay, homogeneous caspase assay and Q-PCR analysis. Cells were maintained in Neurobasal medium containing B27 supplement (Invitrogen) at 37°C in humidified air with 5% CO_2 with fresh medium change every 3-4 days.

Protein Expression Constructs

[0421] DNA encoding full-length DR6 (amino acids 1-655) was inserted into the Not I sites of HRST-IRESeGFP lentivirus vector. The sequence of full-length human DR6 in lentivirus was obtained and is the sequence of SEQ ID NO:154. Nucleotides 124-153 of SEQ ID NO:154 encode a Myc tag that is used to check protein expression. The nucleotide sequence encodes a full-length Myc-tagged human DR6 polypeptide of the sequence of SEQ ID NO:155. Amino acids 42-51 of SEQ ID NO:155 are the Myc tag.

[0422] DNA encoding dominant negative DR6 (amino acids 1-370) was inserted into the Not I sites of HRST-IRESeGFP lentivirus vector. The sequence of dominant negative DR6 in lentivirus is provided as SEQ ID NO: 156. Nucleotides 124-153 of SEQ ID NO:156 encode a Myc tag that is used to check protein expression. The polyeptide sequence of dominant negative human DR6 polypeptide is provided as SEQ ID NO: 157. Amino acids 42-51 of SEQ ID NO:157 are the Myc tag.

Infections

[0423] The resulting plasmids and a GFP control plasmid were transfected into 293 cells to produce lentivirus as previously described (Rubinson *et al. Nat. Genet. 33*:401-6 (2003)), and the cortical neurons were infected with lentivirus at a mutiplicity of infection (MOI) of 1. Ectopic expression of FL-DR6 induced cortical neurons apoptosis as visualized by cell morphology and cell count (Fig. 5A) compared to control infected cells after 92 hours.

[0424] DR6 induced cell death was further verified by XTT assay in parallel cultures that monitors mitochondrial activities of all living cells. The XTT assay is a colorimetric way to determine cell number by measuring mitochondrial activity. FL-DR6 infected cortical neurons exhibited a 2 fold reduction in XTT reading reflecting a significant decrease in cell number caused by DR6 overexpression -induced neuronal death (Fig. 5B). To determine if the Death domain (DD) is required for the cell death, a dominant-negative DR6 lentivirus (DN-DR6) which does not contain the DD, was introduced into cortical neurons by infection. As shown in Figure 5B, DN-DR6 failed to induce apoptosis in cultured neurons suggesting that DR6 death domain is essential for its neuronal death induction.

[0425] The effect of DR6 on caspase-3, a key mediator of apoptosis, was also analyzed. Cortical neurons were infected as described above, and cell lysates were collected 48 hours after infection. A fluorometric homogenous caspase assay kit (Roche, 03005372001) was used to measure caspase-3 activity. Cells were assayed 48 hours after infection. As shown in Figure 5C, free Rhodamine levels increased 2 fold after infection with the full-length DR6 lentivirus compared to uninfected cells, cells infected with the GFP control lentivirus or cells infected with the dominant negative DR6 lentivirus. This indicates that DR6, but not dominant negative DR6, increases the activity of caspase-3.

[0426] These results were confirmed by western blot. Cell lysates were subjected to PAGE, and the separated proteins were probed with anti-DR6 antibody (Santa Cruz). As shown in Figure 5D, active caspase-3 levels were higher in cells infected with the full-length DR6 lentivirus than with cells infected with the dominant negative DR6 lentivirus or the GFP control lentivirus. In contrast, levels of a control protein (βIII-tubulin) were similar in all three infections. Efficacy of each of the infections was comparable as demonstrated by similar levels of GFP produced by the lentivirus vector in each of the infections. (*See* Fig. 5D).

[0427] Each of these results suggests that DR6 is able to induce cell death in cortical neurons.

Example 6

DR6-FL Overexpression Induces Death of OPCs

[0428] The effect of DR6 on the viability of oligodendrocyte precursor cells (OPCs) was also examined. In these experiments, cultures of enriched oligodendrocytes were prepared as previously described (Mi *et al.*, *Nat. Neurosci. 8*:745-51 (2005)). Briefly, forebrains from P2 Sprague Dawley rats (Charles River) were dissected out, minced and incubated in 0.01% Trypsin (Sigma) and 10 μg/ml DNase (Sigma) at 37°C for 15 minutes. Dissociated cells were plated into 100 μg/ml poly-D-lysine T75 tissue culture flasks and were grown at 37°C for 10 days in Dulbecco's modified Eagle's medium (DMEM) containing 20% fetal bovine serum (Invitrogen). To get enriched oligodendrocyte progenitors, flasks were shaken at 200 rpm at 37°C overnight, resulting in a population of 95% purity. Oligodendrocyte progenitor cells were then plated at $2x10^4$/well of a 96 well plate and maintained in DMEM (Invitrogen) containing 10 ng/ml

platelet-derived growth factor ($PDGF_{AA}$) and 10 ng/ml fibroblast growth factor (FGF) at 37°C in humidified air with 5% CO_2.

[0429] OPCs were then infected with GFP, full-length DR6 and dominant negative DR6 lentivirus as described in Example 5, and 48 hours after infection, the number of dying cells was assessed. Untreated cells and cells treated with 2% Triton X-100 were also examined as negative and positive controls, respectively. First, cells were visualized using phase contrast microscopy and the total number of cells, as well as the number of dying cells, was counted. The percentage of dying cells is represented graphically in Figure 6A. Infection with full-length DR6 lentivirus led to a significant increase in the percentage of dying cells as compared to cells infected with dominant negative DR6 lentivirus or GFP lentivirus.

[0430] The effect of DR6 on the viability of OPCs was also evaluated using XTT and LDH assays. The XTT assay was performed as described in Example 5. Cells were treated with rotenone, an NADH inhibitor, as a positive control. As shown in Figure 6B, cells that were infected with either dominant negative DR6 lentivirus or GFP lentivirus showed similar levels of cell viability. In contrast, cells treated with rotenone or infected with full-length DR6 lentivirus showed significantly lower levels of cell viability.

[0431] Similar results were obtained using an LDH assay. LDH is an enzyme that is released upon cell lysis, so colorimetric assays for LDH activity can be used to measure cell damage. The LDH assay was performed using a cytotoxicity detection kit (LDH, Roche, 11644793001) by following the manufacture's instruction. The results are displayed graphically in Figure 6C and demonstrate that infection of OPCs with full-length DR6 lentivirus result in significantly greater cytotoxicity as compared to infection with either dominant negative DR6 lentivirus or GFP lentivirus.

[0432] Each of these experiments suggest that full-length DR6 is able to induce cell death in OPCs, in addition to cortical neurons.

Example 7

Blocking DR6 Signaling Pathway Promotes Oligodendrocyte Survival and Differentiation

[0433] Since oligodendrocyte survival is critical for their terminal differentiation, the ability of DR6 antagonists to promote oligodendrocyte survival, differentiation and myelination was evaluated. To address this issue, DR6-DN (deletion of death domain) was used to block DR6 function in oligodendrocytes. As shown in the figure 7A, cells infected with DR6 DN

exhibited 5-fold higher levels of MBP^+ cells and higher MBP and MOG protein determined by Western blot analysis using mouse anti-MBP antibody (SMI 94 and SMI 99, 1:4000, Convance), mouse anti-MOG antibody (1:500) and rabbit anti-beta actin antibody (1:2000, Sigma) (Fig. 7A). Cell infection by lentivirus was confirmed by Western blot detection of the GFP protein co-expression marker (Fig. 7A). In addition, a CNPase assay (Fig. 7B) was performed to measure the level of CNPase, a marker for both immature and mature oligodendrocytes, in an ELISA format. As shown in Figure 7B, the culture of DR6 FL infected oligodendrocytes expressed decreased CNPase activity compared to the control. In contrast, blocking the DR6 signaling pathwasy with DR6 DN increased CNPase activity (Fig. 7B). These data support the notion that endogenous DR6 negatively regulates oligodendrocyte survival and differentiation.

Example 8

DR6-Induced Neuronal Death is Reversed by DR6-Fc

[0434] Cortical neurons were infected with full-length DR6 lentivirus as described in Example 5. However, these cells were incubated in media that was supplemented with increasing amounts of recombinant soluble DR6. Recombinant soluble DR6 was produced by fusing amino acids 1-349 of DR6 to an Fc sequence. The nucleotide sequence of SEQ ID NO: 158 was used in these experiments. Nucleotides 1-1047 of SEQ ID NO: 158 encode DR6 amino acids, and nucleotides 1051-1731 of SEQ ID NO: 158 encode Fc amino acids. Nucleotides 1048-1050 of SEQ ID NO: 158 were inserted due to cloning procedures.

[0435] The sequence of the soluble DR6 polypeptide is provided as SEQ ID NO: 159. Amino acids 1-349 of SEQ ID NO: 159 are DR6 amino acids. Amino acids 351-576 of SEQ ID NO:159 are Fc amino acids, and amino acid 350 is an amino acid that was inserted due to cloning procedures.

[0436] The soluble DR6 coding sequence was inserted into a lenti viral vector and then used to produce and purify recombinant soluble DR6 from 293 cells. To directly monitor the survival effects of DR6-Fc on FL-DR6 expressing neurons, time-lapse images were obtained. In the presence of DR6-Fc, FL-DR6 failed to induce cortical neuron death (Fig 8A-B).

[0437] The effect of soluble DR6 on DRG neurons was also examined. DRG neurons were first dissected out from adult Sprague Dawley rats (Charles River) and incubated in 0.25% Trypsin/EDTA (Invitrogen) at 37 °C for 30 minutes. An equal volume of DMEM (Invitrogen)

containing 20% fetal bovine serum (Invitrogen) was then added to the digestion mixture to stop the reaction. Cell pellets were collected after spinning down at 1,000 rpm at room temperature for 5 minutes and were mechanically dissociated by gently passing through a plastic pipette until no large fragments were visible. About $1x10^5$ DRG neurons were spotted in the center of each well of 4 well chamber-slides (LabTek) that were coated with 100 μg/ml Poly-D-lysine (Sigma). Cells were allowed to attach in growth medium (Neurobasal medium containing B27 supplement (Invitrogen) and 100 ng/ml nerve growth factor (BD Biosciences)) at 37 °C in humidified air with 5% CO_2. The next morning, fresh growth medium was replaced, and cells were treated with 20 μM of fluorodeoxyuridine for 3 days to remove proliferating glial cells. Cultures were then maintained in growth medium at 37 °C in humidified air with 5% CO_2 with fresh medium change every 3-4 days. After 7 days of culture, DRGs were treated with either control Fc or soluble DR6-Fc for 3 days. DRGs were fixed with 4% paraformaldehyde, and then stained with mouse anti-neuronal class III β-tubulin (Covance, MMS-435P, 1:500). Soluble DR6-Fc increased the total number of neurons bearing processes. In addition, the number of neurons with large and complex processes increased when treated with soluble DR6-Fc (Fig 9A-B).

[0438] In another experiment, cortical neurons were incubated in media containing 0, 1, 3, 10 or 30 μg/ml soluble DR6 protein. After 48 hours, cell lysates were collected and levels of activated caspase-3 protein were measured using rabbit anti-cleaved caspase-3 antibody (91:1000; Cell Signaling). As shown in Figure 10A, activated caspase-3 was not detectable in uninfected cells, but cells infected with full-length DR6 lentivirus showed high levels of activated caspase-3. However, levels of activated caspase-3 decreased when infected cells were incubated in media containing soluble DR6 (Fig. 10 A-B). Cell lysates were also probed with anti-GFP and anti-β-actin antibodies to control for efficacy of infection and quantity and quality of cell lysates, respectively. These data support the notion that blocking DR6 expression promotes neuronal survival and axon integrity.

Example 9

The Effect of DR6-Fc on Cell Death in Animal Model of Alzheimer's Disease

[0439] The effect of DR6-Fc *in vivo* can be studied using a mouse model of Alzheimer's disease, for example, APPswe/PS-1ΔE9 mice (Park *et al., J. Neurosci 26*:1386-1395 (2006)) from Jackson laboratories (Bar Harbor, ME) (Stock #04462).

[0440] Mice are divided into several treatment groups. The first groups serves as a normal control. Each additional group is treated with DR6-Fc, for example, by intracranial injection or by systemic administration. The amount administered varies for each treatment group. For example, a group can receive 1, 10, 25, 50, 75, 100, 200, 300, 400 or 500 μg/kg per day. Administration can be a one time administration or can occur repeatedly for a specified period of time. Administration can occur before the onset of Alzheimer's symptoms, such that a delay or lack of development of symptoms is indicative of successful prevention and/or treatment. Alternatively, administration can begin after the onset of symptoms (*i.e.* at 7 months of age), such that a decrease or lack of increase in symptoms is indicative of successful treatment.

[0441] Efficacy of treatment can be evaluated symptomatically in live mice, for example by comparison of treated and untreated mice in a water mice. Efficacy can also be evaluated by molecular, biochemical and histological analysis of tissues, such as brain tissue, from sacrificed mice. For example, the number of apoptotic cells, *e.g.* cortical neurons, in a predetermined size and region of the brain can be compared in treated and untreated mice. The number of apoptotic cells can be determined using any known method in the art including for example, the TUNEL (TdT-mediated dUTP Nick-End Labeling) assay or anti-PARP (poly(ADP-ribose) polymerase) staining. In addition, the total number of surviving cortical neurons in a predetermined size and region of the brain can be compared in treated and untreated mice.

Example 10

The Effect of DR6-Fc on Cell Death in Animal Model of ALS

[0442] The effect of DR6-Fc *in vivo* can also be studied using an animal model of Amyotrophic Lateral Sclerosis (ALS), for example, mice, rats, flies, or worms expressing a mutant superoxide dismutase (SOD1).

[0443] For example, mice expressing mutant SOD1 (G37R) are treated with DR6-Fc by any mode of administration such as parenteral administration, subcutaneous administration etc. The amounts and times of administration can be varied. Administration can occur before the onset of ALS symptoms (*i.e.* at 7 to 9 months of age), such that a delay or lack of development of symptoms is indicative of successful prevention and/or treatment. In addition, administration can begin after the onset of symptoms, such that a decrease or lack of increase in symptoms is indicative of successful treatment.

[0444] Efficacy of treatment can be evaluated symptomatically, for example by comparison of muscle strength or longevity of treated and untreated mice. Efficacy can also be evaluated by molecular, biochemical and histological analysis of tissues, such as sections of motor neurons, from sacrificed mice. For example, the number of apoptotic cells, *e.g.* motor neurons, from a predetermined location, for example, along the spinal cord, can be compared in treated and untreated mice. The number of apoptotic cells can be determined using any known method in the art including for example, the TUNEL (TdT-mediated dUTP Nick-End Labeling) assay or anti-PARP (poly(ADP-ribose) polymerase) staining. In addition, the total number of surviving motor neurons in a predetermined size and region of the spinal cord can be compared in treated and untreated mice.

Example 11

DR6 RNAi promotes neuron survival

[0445] Neocortical neurons were removed from embryonic 18 rats, and three million cells were transfected with either 200 nM DR6 siRNAs or scramble control siRNAs using Rat Neuron Nucleofector Kit (Amaxa Inc.). The DR6 siRNAs were a mixture of 4 siRNAs obtained from Dharmacon. The sequences of the 4 siRNAs were: AGAAACGGCUCCUUUAUUA (SEQ ID NO:160), GGAAGGACAUCUAUCAGUU (SEQ ID NO:161), GGCCGAUGAUUGAGAGAUU (SEQ ID NO:162), GCAGUUGGAAACAGACAAA (SEQ ID NO:163). The sequence of the control siRNA was: GGUGACAUGAUCGACAGCCAU (SEQ ID NO:164).

[0446] Transfected cells were plated in one 96-well plate and cultured for 6 days. At day 7, half of the culture media (100 µl) was removed and replaced with 100 µl fresh Neurobasal media containing different concentrations of glutamate, Aβ42 or TNFα. Triplicate cultures were set up for each treatment condition. Cultures were treated for 24 hours. 100 µl of supernatant was removed from each well, and LDH assays were performed using a cytotoxicity kit (LDH Cytotoxicity Detection Kit, Clontech Laboratories, Inc.) according to the manufacturer's instruction. As shown in Fig. 11, knocking down DR6 using RNAi promotes neocortical neuronal survival. Moreover, reducing DR6 expression attenuates Aβ42, glutamate, and TNFα-induced neuronal cytotoxicity. The data also suggests that blocking DR6 expression using RNAi promotes neuronal survival and prevents neuronal death.

Example 12

Blocking DR6 Signaling Pathway by siRNA Promotes Oligodendrocte Differentiation

[0447] The effect of DR6 antagonism by DR6 RNAi on oligodendrocyte survival, differentiation and myelination was also evaluated. In these experiments, A2B5 cells were transfected with DR6 RNAi or control RNAi. The cells were harvested and half of the lysis was used for RT-PCR and analyzed by 15% agarose gel. The rest of the cell lysis was used for MBP and MOG western. As shown in Figures 12A-B, cells exposed to DR6 RNAi exhibited 2 fold higher levels of MBP^+ cells, and higher MBP and MOG protein levels were shown by Western blot analysis (Fig. 12B). Cell infection by lentivirus was confirmed by Western blot detection of the GFP protein co-expression marker. These data further support the notion that endogenous DR6 negatively regulates oligodendrocyte survival and differentiation.

Example 13

Generation of Phage-Display-Derived Fab Antibodies

[0448] Recombinant human DR6 ectodomain was used to screen a human naïve phagemid Fab library containing 3.5×10^{10} unique clones (Nat Biotechnol. 2005 Mar;23(3):344-8.) Biotinylated AP-DR6 protein was captured on steptavidin-coated magnetic beads prior to incubation with the phage library. Selections were performed as described previously, with depletion on a AP-p75 to eliminate AP specific binders (Nat Biotechnol. 2005 Mar;23(3):344-8.). After 3 rounds of panning, the 479 bp gene III stump was removed by *MluI* digestion, and the vector was religated for soluble Fab expression in TG1 cells. ELISA analysis of 2496 clones yielded 212 positive clones, containing 49 unique sequences. Unique clones were purified and binding was reconfirmed at a single concentration to recombinant human DR6 ectodomain by ELISA as well as by FACS on 293E cells transiently transfected with full-length human DR6. Twenty-four unique clones were selected from this analysis for further characterization. The 24 Fabs were tested at multiple concentrations by ELISA on human DR6-Fc to confirm specificity for the DR6 ectodomain versus the AP-DR6 fusion protein, as well as by FACS on full-length human DR6-293E cells, full-length rat DR6-293E cells, and untransfected 293E cells to check

for species cross-reactivity. Based on specificity and cross-reactivity data, ten Fabs were selected.

Example 14

Cloning of murine anti-human DR6 monoclonal antibody variable domains

[0449] Total cellular RNA from murine hybridoma cells was prepared using a Qiagen RNeasy mini kit following the manufacturer's recommended protocol. cDNAs encoding the variable regions of the heavy and light chains were cloned by RT-PCR from total cellular RNA, using random hexamers for priming of first strand cDNA. For PCR amplification of the murine immunoglobulin variable domains with intact signal sequences, a cocktail of degenerate forward primers hybridizing to multiple murine immunoglobulin gene family signal sequences and a single back primer specific for the 5' end of the murine constant domain were used. The PCR products were gel-purified and subcloned into Invitrogen's pCR2.1TOPO vector using their TOPO cloning kit following the manufacturer's recommended protocol. Inserts from multiple independent subclones were sequenced to establish a consensus sequence. Deduced mature immunoglobulin N-termini were identical to those determined by Edman degradation of the purified immunoglobulins from the hybridomas. Assignment to specific subgroups is based upon BLAST analysis using consensus immunoglobulin variable domain sequences from the Kabat database. CDRs are designated using the Kabat definitions.

[0450] Shown below as SEQ ID NO:107 is the 1P1D6.3 mature heavy chain variable domain protein sequence, with CDRs (Kabat definitions) underlined:

```
  1  QVQLQQSGTE LARPGASVKL SCKASGYTFT DYYLNWMKQG TGQGLEWIGE
 51  IYPGGDHTYY NEKFKGKATL TADKSSNTAF MQLSSLTSED SAVYFCTRGV
101  IKWGQGTLVT VSL
```

[0451] This is a murine subgroup II(A) heavy chain. The DNA sequence of the 1P1D6.3 heavy chain variable domain (from pYL466) is provided as SEQ ID NO:106.

[0452] Shown below as SEQ ID NO:112 is the 1P1D6.3 mature light chain variable domain protein sequence, with CDRs underlined:

```
  1  DILMTQSPPS MSVSLGDTVS ITCHASQGIS SNIGWLQQKP GKSFKGLIYH
```

```
 51  GSTLEDGVPS RFSGSGSGAE FSLTISSLES EDFADYYCVQ YAQFPYTFGG

101  GTKLEIK
```

[0453] This is a murine subgroup V kappa light chain. The DNA sequence of the mature light chain variable domain (from pYL469) is provided as SEQ ID NO:111.

[0454] Shown below as SEQ ID NO:117 is the mature 1P2F2.1 heavy chain variable domain protein sequence, with CDRs underlined:

```
  1  QVQLQQSGPE VARPGASVKL SCKASGYTFT DYYLNWVKQR TGQGLEWIGE

 51  IYPGNNHTYY NEKFKGKATL TADNSSSTAY LQFSSLTSED SAVYFCTRGV

101  IKWGQGTLVT VSV
```

[0455] This is a murine subgroup II(A) heavy chain. Note the potential N-linked glycosylation site in CDR2 indicated as double underlined above. The DNA sequence of the 1P2F2.1 heavy chain variable domain (from pYL467) is provided as SEQ ID NO:116.

[0456] The heavy chains of 1P1D6.3 and 1P2F2.1 are related, sharing 89.4% identity at the protein level, with identical CDR1 and CDR3 sequences. IgBLAST analyses suggest that they were derived from the same recombinational event. Shown below is the alignment of the heavy chains of 1P1D6.3 (top) and 1P2F2.1 (bottom):

```
  1 QVQLQQSGTELARPGASVKLSCKASGYTFTDYYLNWMKQGTGQGLEWIGE 50
    |||||||| |.|||||||||||||||||||||||||.|| ||||||||||
  1 QVQLQQSGPEVARPGASVKLSCKASGYTFTDYYLNWVKQRTGQGLEWIGE 50
              .          .          .          .          .
 51 IYPGGDHTYYNEKFKGKATLTADKSSNTAFMQLSSLTSEDSAVYFCTRGV 100
    |||| .|||||||||||||||||| ||.||::| |||||||||||||||||
 51 IYPGNNHTYYNEKFKGKATLTADNSSSTAYLQFSSLTSEDSAVYFCTRGV 100
              .
101 IKWGQGTLVTVSL 113  (SEQ ID NO:107)
    ||||||||||||.
101 IKWGQGTLVTVSV 113  (SEQ ID NO:117)
```

[0457] Shown below as SEQ ID NO:122 is the 1P2F2.1 mature light chain variable domain protein sequence, with CDRs underlined:

```
  1  DILMTQSPSS MSVSLGDTVS ITCHASQGIR NSIGWLQQKP GKSFKGLIYH

 51  ATTLEDGVPS RFTGSGSGAD FSLTISSLES EDFADYYCVQ YAQFPYTFGG
```

```
101  GTKLEIK
```

[0458] This is a murine subgroup V kappa light chain. The DNA sequence of the mature light chain variable domain (from pYL470) is provided as SEQ ID NO:121.

[0459] The light chains of 1P1D6.3 and 1P2F2.1 are related, sharing 92.5% identity at the protein level, with identical CDR3 sequences. IgBLAST analyses suggest that they were derived from the same recombinational event. Shown below is the alignment of the light chains of 1P1D6.3 (top) and 1P2F2.1 (bottom):

```
  1 DILMTQSPPSMSVSLGDTVSITCHASQGISSNIGWLQQKPGKSFKGLIYH 50
    |||||||| |||||||||||||||||||| ..||||||||||||||||||
  1 DILMTQSPSSMSVSLGDTVSITCHASQGIRNSIGWLQQKPGKSFKGLIYH 50
            .         .         .         .         .
 51 GSTLEDGVPSRFSGSGSGAEFSLTISSLESEDFADYYCVQYAQFPYTFGG 100
     .||||||||||.||||||:||||||||||||||||||||||||||||||
 51 ATTLEDGVPSRFTGSGSGADFSLTISSLESEDFADYYCVQYAQFPYTFGG 100

101 GTKLEIK 107  (SEQ ID NO:112)
    |||||||
101 GTKLEIK 107  (SEQ ID NO:122)
```

[0460] Shown below as SEQ ID NO:127 is the mature 1P5D10.2 heavy chain variable domain protein sequence, with CDRs underlined:

```
  1  EVQLVESGGG LVKPGGSLKL SCAASGFTFS DYYMYWVRQT PEKRLEWVAT

 51  ISDGGLYTYY QDSVKGRFTI SRDNAKNNLY LQMSSLKSED TAMYYCARED

101  DYDGDFYTMD YWGQGTSVTV SS
```

[0461] This is a murine subgroup III(D) heavy chain. The DNA sequence of the 1P5D10.2 heavy chain variable domain (from pYL468) is provided as SEQ ID NO:126.

[0462] Shown below as SEQ ID NO:132 is the 1P5D10.2 mature light chain variable domain protein sequence, with CDRs underlined:

```
  1  QIVLTQSPAI MSASPGEKVT ITCSASSSVS YMHWFQQKPG TSPKLWIYST

 51  SNLASGVPAR FSGSGSGTSY SLTISRMEAE DAATYYCQQR SSYPLTFGAG
```

101 TKLELK

[0463] This is a murine subgroup VI kappa light chain. The DNA sequence of the mature 1P5D10.2 light chain variable domain (from pYL471) is provided as SEQ ID NO:131.

Example 15

Anti-DR6 Antibodies Bind to Rat, Mouse and Human DR6.

[0464] Six million HEK293 cells were transfected with 10 ug of plasmid DNA, which encoded full length human, rat, or mouse DR6. Three days after transfection, approximately 50,000 cells in 200 μL of PBS, 1% BSA, 0.1% NaN3 (FACS buffer) were analyzed. Cells were pelleted and resuspended in 150 μL of serial dilutions of anti-DR6 antibodies in FACS buffer. Samples were incubated for 1 hour on ice with occasional agitation and then washed three times. Bound DR6 antibody was visualized with PE-labeled goat $F(ab)_2$ anti-human Fab (for Dyax Fabs) or anti-mouse IgG specific antibody (for monoclonal antibodies) (Jackson Labs). The results, shown in Figure 13, demonstrate that 5D10 and 1E6 antibodies each bind to human, rat, and mouse DR6. The results of binding assays using 5D10 and M53E04 are shown in Figure 30.

Example 16

Blocking DR6 by anti-DR6 antibodies promotes oligodendrocyte differentiation and inhibit apoptosis

[0465] To further validate the role of DR6 function in oligodendrocyte survival, anti-DR6 antibodies were used to block DR6 function in the oligodendrocytes culture. As shown in Figures 14A-B, anti-DR6 antibody treatment reduced caspase 3^+ cells about 3 fold (Fig. 14A) and increased MBP^+ cells by 10 fold (Fig. 14B). These results were confirmed by the Western blot analysis (Fig 14C) where the anti-DR6 antibody reduced the caspase 3 production about 3 fold. In contrast, a 10 fold increase of MBP protein production was seen in the cell cultures treated with the anti-DR6 antibody (Fig 14C). The results of an oligodendrocyte-DRG co-culture assay using DR6 antibodies M53E04 and 5D10 are shown in Figure 31 and compared to the results obtained using anti-LINGO-1 antibody Li81 as a positive control.

Example 17

Blocking DR6 by anti-DR6 antibodies promotes oligodendrocyte/DRG myelination in co-culture

[0466] To test the hypothesis that blocking DR6 promotes myelination, co-cultures of rat primary oligodendrocytes and DRG neurons were used to ascertain the effects of anti-DR6 antibody on myelination. Such co-cultures normally exhibit low basal levels of myelination which was profoundly enhanced by the addition of anti-DR6 antibody. The cocultures were infected with lentivirus at a multiplicity of infection of two per cell (2 MOI). Treatment with anti-DR6 antibody for 10 days resulted in robust axonal myelination as evident by the presence of MBP^+ myelinated axons, 10 fold higher than control Ig treated cells culture. Western blot analysis using mouse anti-MBP antibody (SMI 94 and SMI 99, 1:4000, Convance), mouse anti-MOG antibody (1:500), and rabbit anti-beta actin antibody (1:2000, Sigma) demonstrates that anti-DR6 antibody promotes myelination in a dosage dependent manner, the higher concentration of anti-DR6 antibody added to the co-culture, the higher level of MBP and MOG protein was produce (Fig 15). These studies demonstrate that blocking DR6 function promotes oligodendrocyte survival, differentiation and myelination.

Example 18

Blocking DR6 by anti-DR6 antibodies promote remylination in rat brain slice culture

[0467] The brain slice culture system provides a powerful *in vitro* model for the analyses of the pathology of demyelination and mechanisms of remyelination. Three day treatment of P17 brain slices with the bioactive lipid, lysophosphatidylcholine (LPC), results in a rapid and near-complete demyelination as visualized by the absence of black gold staining for myelination (Fig 16A). Exposure to anti-DR6 antibody for 4 days after LPC removal resulted in 15-fold more black gold staining, whereas the control antibody treatment had no effect (Figs 16A-B). We next determined whether remyelination could be achieved *in vivo* in the adult LPC induced demyelination model. LPC was injected into the dorsal columns of the 9 week old young adult (250 gms) rat spinal cords at day 0, followed by anti-DR6 antibody administration 3 days later. The extent of the LPC induced lesion and remyelination was next determined by black gold

staining. Myelinated white matter appears dark red in the black gold stained sections and demyelinated lesions appear as pale red or white. Sections from control antibody-treated animals (n=3) showed large lesions with extensive areas of demyelination, whereas substantially smaller lesions were apparent in the anti-DR6 treated group (n=3) 7 days after LPC injection. The black gold staining pattern of anti-DR6-treated and control lesions differed. In anti-DR6 treated lesions, lace-like structures were present throughout the lesion indicative of remyelination. Both brain slice culture and *in vivo* lysolecithin studies demonstrated that blocking DR6 function by anti-DR6 antibody promote remyelination.

Example 19

Anti-DR6 antibodies promote functional recovery in rat EAE model

[0468] Adult 9-week old Brown Norway rats (150 g) were anaesthetized with isoflourine, followed by an injection at the base of the tail with 200 µl of cocktail solution containing: 100 µl of CFA (complete Freund's adjuvant from Chondrex Inc.) and 100 µl of 100 µg recombinant rat MOG corresponding to the N-terminal sequence of rat MOG (amino acids 1-125) in DPBS (MP Biomedicals, LCC). Animals developed signs of EAE 10-15 days after injections. After MOG induction, each animal was assessed by a behavioral test based on motor functions. EAE scores were used as a surrogate clinical metric for demyelination. Rats were scored for clinical signs of EAE daily. The signs were scored as follows: grade 0.5, distal paresis of the tail; grade 1, complete tail paralysis; grade 1.5, paresis of the tail and mild hind leg paresis; grade 2.0, unilateral severe hind leg paresis; grade 2.5, bilateral severe hind limb paresis; grade 3.0, complete bilateral hind limb paralysis; grade 3.5, complete bilateral hind limb paralysis and paresis of one front limb; and grade 4, complete paralysis (tetraplegia), moribund state, or death. After 15 day immunization, rats were randomly assigned to one of two groups. One group of rats (n=10) was injected with isotype control antibodies, and another group (n=10) was injected with DR6 antibodies. The rats were injected twice a week at 6 mg/kg for a total of 5 treatments. EAE scores were measured daily, and the statistical significance was assessed using an unpaired t-test (two-tailed). As shown in Fig. 17A, the EAE scores in DR6 antibody treated rats were significantly lower compared with control animals. For electrophysiological recordings, after 40 day immunization, motor potentials (MEPs) were induced by magnetic cortical stimulation (Magstim) and recorded from the gatrocnemius muscles (Cadwell). The onset of the first, usually

negative, deflection was taken as the cortical MEP latency. As shown in Fig. 17B, the DR6-treated rats showed faster nerve conduction velocity.

Example 20

Lymphocyte number is not affected in EAE rats treated by anti-DR6 antibodies

[0469] EAE is a complex model for demyelination, as it involves both immune and neurological components. To determine whether treatment with anti-DR6 antibodies affects lymphocytes, after 40 day MOG immunization, peripheral blood was drawn from the facial vein of EAE rats treated with anti-DR6 antibody or control antibody. The total whole blood cells and subset numbers were measured using Hemavet. As shown in Fig. 18, the total lymphocyte numbers and the percentage of lymphocytes in total white blood cells did not show significant differences between the control and anti-DR6 antibody treated animals.

Example 21

Anti-DR6 antibodies inhibit T-cell infiltration into spinal cord in EAE rats

[0470] To determine whether anti-DR6 antibodies affect T cell infiltration into the CNS, EAE rats were euthanized with CO_2, then perfused with 0.1 M phosphate buffer after six weeks of MOG immunizations. The lumbar region of spinal cords were dissected out and fixed in 4 % paraformaldehyde overnight at 4 °C followed by incubation in 25% sucrose in 0.1 M PBS. 15 μm transverse and longitude frozen sections (Leica microtome) were cut and standard fluoresce immunohistochemistry was performed using anti-CD4 antibodies (BD Pharmingen). Images were taken under Leica fluorescence microscopy and analyzed using Openlab. As shown in Fig. 19, systemic administration of anti-DR6 antibodies significantly reduced the infiltration of T cells into the spinal cord of the EAE rats, suggesting that the decreased T cell infiltration at least partially explains the decreased severity of the EAE symptoms (lower EAE scores) in anti-DR6 treated animals.

Example 22

TNFalpha promotes neuron death through NFκb

[0471] TNF has been reported to induce DR6 expression in tumor cells to activate apoptosis by activating the NF-κB, Caspase-3 pathway and down regulating Iκb protein level. In order to determine if TNFalpha induces DR6 expression in cortical neurons, neurons were exposed to 24 hour treatment with TNFalpha, and immunohistochemistry staining was performed. The results demonstrated that DR6 expression was increased significantly. TNFalpha induced neuronal death and was well correlated with DR6 positive cells (Fig. 20A-C). The study was confirmed by Western blot (Fig 21A). Treatment with TNFalpha induced a 2 fold increase in DR6 expression after a 24 hour treatment, which also correlated with a 10 fold NF-κB increase and a 2 fold Iκb protein down regulation (Figs. 21A-D). DR6 RNAi transfected neurons showed a 2 fold reduction of DR6 and NFκb level (Figs. 22B and C). In contrast, DR6 RNAi tranfected neurons showed increased (2 fold) Iκb protein expression (Figs. 22A-D). (Control and DR6 siRNAs used were as described in Example 11). These data suggest that DR6 up-regulation correlated with NFκb expression and inversely correlated with Iκb expression.

Example 23

DR6 antagonists promote schwann cell myelination of DRG axons

[0472] In order to determine if DR6 antagonists affect schwann cells, the effect of anti-DR6 antibodies on schwann cell and DR6 neuron co-cultures was examined. In these experiments, DRG neurons from E16 rats were plated (50,000/well) in 4 well slides with Neurobasal medium plus B27 and NGF. The cultures were treated by FDUR for 4-6 days to removed the dividing cells. After day 7, the DRG cells were treated with Neurobasal medium plus B27 and NGF (100 ng/ml) for an additional 7-10 days. Then, purified schwann cells (50,000/well) were added to the DRG neurons (50,000/well) in Neurobasal medium with B27 and 100 ng/ml NGF. The medium was changed weekly. The co-cultures were harvested and assayed by IHC or Western blot for MBP protein after 10 days. IHC staining and Western blots (Fig. 23) both showed increased levels of MBP protein in cultures treated with anti-DR6

antibodies compared to cultures treated with a control antibody. These data indicate that DR6 antagonists promote schwann cell myelination of neurons.

Example 24

DR6 is upregulated in apoptotic cortical neurons

[0473] In order to examine DR6 expression in neocortical neurons, neurons were first separated from E18 Sprague Dawley rats (Charles River). Briefly, cerebral cortices from E18 rat embryos were dissected out, minced, and incubated in 0.25% Trypsin/EDTA (Invitrogen) at 37 °C for 10 minutes. The cells were then triturated after adding 20 ug/ml DNase I (Sigma) and 10% fetal bovine serum (Invitrogen) to stop the reaction. Cell pellets were collected and then mechanically dissociated by gently passing through a plastic pipette until no large fragments were visible. Cells were plated in 8-well slide chambers (NUNC) that were pre-coated with 100 ug/ml Poly-D-Lysine (Sigma) at $4x10^4$ cells per well. Cells were maintained in Neurobasal medium containing B27 supplement (Invitrogen) at 37 °C in humidified air with 5% CO_2 with fresh medium changed every 3-4 days. Cells were cultured for 3 weeks and fixed with 4% paraformaldehyde in PBS for 30 minutes. After three washes with PBS, cells were penetrated with PBS containing 1% Triton X-100 (PBST, Sigma) for 30 minutes followed by incubation in blocking solution (PBS containing 0.1% Triton X-100 and 10% normal goat serum (NGS)) for 30 minutes at room temperature. For primary labeling, cells were then incubated in blocking medium containing rabbit anti-DR6 (Santa Cruz, sc-13106, 1:200) and mouse anti-neuronal class III β-tubulin (Covance, MMS-435P, 1:500) at 4 °C overnight. After three PBST rinses, cells were incubated in 5% NGS-PBS containing Alexa 594 anti-rabbit IgG (1:500) and Alexa 488 anti-mouse IgG (1:500) secondary antibodies at room temperature for 1 hour in the dark. After three PBST washes, cells were mounted with antifade with DAPI reagents (Invitrogen) and observed under fluorescence microscope. Apoptosis, shown by nuclear condensation, was visible in neocortical neurons after 3 weeks in culture. Levels of DR6 were compared in apoptotic and non-apoptotic neurons. The expression level of DR6 was up-regulated in the apoptotic neurons compared to non-apoptotic neurons. These results suggest than an increase in DR6 expression can contribute to aged neuron apoptosis.

Example 25

DR6 antagonists promote axon integrity

[0474] In order to examine the effect of DR6 antagonists on the axonal integrity of neocortical neurons, neurons were separated from E18 Sprague Dawley rats (Charles River) and cultured in neurobasal medium containing B27 supplement (Invitrogen) at 37 °C in humidified air with 5% CO_2 as described in Example 24. After 7 days of culture, cells were treated with β-amyloid (aggregated Aβ-42) at a concentration of (50 μg/ml) and either 10 μg/ml soluble DR6-Fc or 10 μg/ml soluble control antibody. After 48 hours, neurons were fixed with 4% paraformaldehyde and then stained with mouse anti-neuronal class III β-tubulin (Covance, MMS-435P, 1:500) as described in Example 24. Treatment with β-amyloid induced neuronal cell death and axon degeneration as compared to untreated controls. However, soluble DR6-Fc treatment significantly attenuated β-amyloid induced axonal degeneration and neuronal cell death. Soluble DR6-Fc treatment also led to increased survival of neurons and decreased axon beading, which is typical of axon degeneration morphology. These results suggest that DR6 antagonists diminish the negative effects of β-amyloid on neocortical neurons.

Example 26

DR6 and AKT expression are inversely correlated

[0475] Phosphorylated AKT (phospho-AKT) is a well-known survival signal. Therefore, the relationship of DR6 and phospho-AKT levels was examined in neocortical neurons. In these experiments, neocortical neurons were separated as described above. After 3, 7, 14, and 20 days of culture, neocortical neurons were lysed in 80 μl lysis buffer (50mM HEPES, pH 7.5, 150mM NaCl, 1.5mM $MgCl_2$, 1mM EGTA, 1% Triton X-100, and 10% glycerol) for 30 minutes at 4 °C. After centrifugation at 14,000xg for 15 minutes, the supernatants were boiled in Laemmli sample buffer, subjected to 4-20% SDS-PAGE, and analyzed by Western blotting with rabbit anti-phospho-AKT antibody (1:500, cell signaling), rabbit anti-AKT antibody (1:1000, cell signaling), goat anti-DR6 antibody (1:1000, Santa Cruz), and rabbit anti-beta actin antibody (1:2000, Sigma). Primary antibodies were visualized using anti-rabbit IgG-HRP (1:5000) and anti-goat IgG-HRP (1:5000, Bio-Rad) accordingly. The expression levels of DR6 were high in day 7 cultures and low in day 20 cultures (Fig. 24). In contrast, the levels of phospho-AKT were

low at day 7 and high at day 20 (Fig. 24). The inverse correlation of DR6 and phospho-AKT levels suggests that DR6 could induce cell death through the AKT signaling pathway.

Example 27

DR6 and p75 Form a Complex

[0476] In order to determine if p75 could interact with DR6, recombinant cell lines expressing (i) DR6, p75, and TrkA, (ii) DR6 and TrkA, (iii) p75 and TrkA, and (iv) DR6 and p75 were created. In these experiments, human TrkA was expressed with a Flag tag fused to the N-terminal of the mature TrkA protein (amino acids 34-796). Rat p75 was expressed with a His tag fused to the N-terminus of the mature p75 protein (amino acids 30-431), and human DR6 was expressed with a Myc tag fused to the N-terminus of the mature DR6 protein (amino acids 32-655). DR6 was immunoprecipitated from cell lysates using a Myc antibody, and the levels of DR6 and p75 in the samples was assessed. Western blots (Figure 25 A) show that p75 co-immunoprecipitated with DR6 in the presence or absence of TrkA.

[0477] In addition, cells containing a vector encoding DR6 or a negative control vector (pV90) were assayed for binding to p75. Alkaline phosphatase-p75 protein was added to the cells and cell surface binding was measured. The results are shown in Figure 25B.

[0478] Similar results were obtained using samples obtained from human fetal spinal cords obtained from BioChain®. Immunoprepitation experiments demonstrated that p75 co-immunoprecipiates with DR6 (Figure 25 C).

[0479] These data demonstrate that DR6 and p75 form a complex both in cell culture assays and in human samples.

Example 28

The Expression Patterns of DR6 and p75 Overlap

[0480] The expression levels of DR6 and p75 mRNA were obtained from the publically available database mouse.brain-map.org, made available by the Allen Institute for Brain Science. Both DR6 and p75 were highly expressed in various regions of the brain, and expression levels were well correlated (Figure 26). These results suggest that DR6 and p75 co-localize, and therefore can interact and function together *in vivo*.

Example 29

DR6 Antibodies Can Block Interaction of DR6 with p75

[0481] In order to determine if DR6 antibodies block the interaction of DR6 with p75, recombinant DR6 was immunoprepitated from cells using either the 2A9 anti-DR6 antibody or the 5D10 anti-DR6 antibody. Western analysis demonstrated that while both anti-DR6 antibodies were able to pull down DR6 protein, p75 only co-immunoprecipiated with DR6 when the 2A9 antibody was used (Figure 27A). Thus, the 5D10 DR6 antibody disrupted interaction of DR6 with p75.

[0482] The ability of 5D10 to disrupt the interaction of DR6 and p75 was also confirmed by a functional assay. In this assay cells CHO cells containing either a control vector or a vector encoding p75 were incubated with alkaline phosphatase-DR6 and assayed for binding. High levels of cell surface binding were observed when the p75-expressing cells were incubated with DR6. However, the addition of 5D10 prevented DR6 from binding to p75-expressing cells (Figure 27B). These results indicate that the DR6 antibody 5D10 can disrupt binding of DR6 to p75 and promote cell survival.

Example 30

The TNFR-Cys Repeats 3 and 4 of DR6 Bind to Antibodies that Disrupt the DR6-p75 Interaction

[0483] In order to identify domains of DR6 that bind to DR6 antibodies that disrupt the DR6-p75 interaction, cells expressing DR6 deletion constructs were created. The deletion constructs were tagged with Myc. The constructs tested included deletions of amino acids 168-189 of SEQ ID NO:2 (#123); amino acids 134-168 of SEQ ID NO:2 (#124); amino acids 109-131 of SEQ ID NO:2 (#134); amino acids 49-108 of SEQ ID NO:2 (#234); amino acids 133-189 of SEQ ID NO:2 (#12); amino acids 49-131 of SEQ ID NO:2 (#34); and amino acids 49-108 and 168-189 of SEQ ID NO:2 (#23).

[0484] FACS analysis was used to demonstrate that each of the deletion constructs expressed in cells (Figure 28A). Then, samples obtained from the recombinant cells were immunoprecipiated with an anti-Myc antibody or an anti-DR6 antibody. The immunoprecipitates were assayed for p75 protein by Western, and the results showed that

antibody 5D10 binds to the Cys3 and Cys4 domain of DR6 (amino acids 133-189) (Figure 28B). Antibody 2A9 binds to the Cys1 domain of DR6 (amino acids 49-131) (Figure 28B). The binding of a panel of DR6 antibodies to DR6 deletion constructs was also assayed (Figure 28C). The results demonstrated that 5D10 and 4A4 bind to the Cys3 and Cys4 domains of DR6, and 2A9, 1D6, and 2F2 bind to the Cys1 domain of DR6.

Example 31

The TNFR-Cys Repeats 3 and 4 of DR6 Bind to p75

[0485] In order to indentify the domains of DR6 that bind to p75, cells expressing the DR6 deletion constructs described in Example 30 were assayed for binding to p75. Recombinant cells were incubated with p75, and then cell lysates were immunoprecipitated using an anti-Myc antibody. Levels of p75 were assessed by Western, and the results demonstrated that deletion of the Cys3 and Cys4 domain of DR6 (amino acids 133-189) resulted in a decreased ability of DR6 to interact with p75.

***

[0486] The present invention is not to be limited in scope by the specific embodiments described which are intended as single illustrations of individual aspects of the invention, and any compositions or methods which are functionally equivalent are within the scope of this invention. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications are intended to fall within the scope of the appended claims.

[0487] All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

[0488] It is to be appreciated that the Detailed Description section, and not the Summary and Abstract sections, is intended to be used to interpret the claims. The Summary and Abstract sections can set forth one or more but not all exemplary embodiments of the present invention as contemplated by the inventor(s), and thus, are not intended to limit the present invention and the appended claims in any way.

WHAT IS CLAIMED IS:

1. An isolated antibody or antigen-binding fragment thereof that can specifically bind to a DR6 polypeptide, wherein the antibody can promote survival of a cell of the nervous system.

2. An isolated antibody or antigen-binding fragment thereof that can specifically bind to a DR6 polypeptide, wherein the antibody can promote proliferation, differentiation or survival of an oligodendrocyte.

3. An isolated antibody or antigen-binding fragment thereof that can specifically bind to a DR6 polypeptide, wherein the antibody can promote myelination.

4. An isolated antibody or antigen-binding fragment thereof that can specifically bind to the same DR6 epitope as a reference monoclonal Fab antibody fragment selected from the group consisting of M50-H01, M51-H09, M53-E04, M53-F04, M62-B02, M63-E10, M66-B03, M67-G02, M72-F03, and M73-C04 or a reference monoclonal antibody selected from the group consisting of 1P1D6.3, 1P2F2.1, and 1P5D10.2.

5. An isolated antibody or antigen-binding fragment thereof that can specifically bind to a DR6 polypeptide, wherein said antibody or fragment thereof competitively inhibits a reference monoclonal Fab antibody fragment selected from the group consisting of M50-H01, M51-H09, M53-E04, M53-F04, M62-B02, M63-E10, M66-B03, M67-G02, M72-F03, and M73-C04 or a reference monoclonal antibody selected from the group consisting of 1P1D6.3, 1P2F2.1, and 1P5D10.2 from binding to DR6.

6. An isolated antibody or antigen-binding fragment thereof that can specifically bind to a DR6 polypeptide, wherein said antibody or fragment thereof comprises an antigen binding domain identical to that of a monoclonal Fab antibody fragment selected from the group consisting of M50-H01, M51-H09, M53-E04, M53-F04, M62-B02, M63-E10, M66-B03, M67-G02, M72-F03, and M73-C04 or a reference monoclonal antibody selected from the group consisting of 1P1D6.3, 1P2F2.1, and 1P5D10.2.

7. An isolated antibody or fragment thereof that can specifically bind to a DR6 polypeptide, wherein the heavy chain variable region (VH) of said antibody or fragment thereof comprises an amino acid sequence at least 90% identical to a reference amino acid

sequence selected from the group consisting of: SEQ ID NO:7, SEQ ID NO:17, SEQ ID NO:27, SEQ ID NO:37, SEQ ID NO:47, SEQ ID NO:57, SEQ ID NO:67, SEQ ID NO:77, SEQ ID NO:87, SEQ ID NO: 97, SEQ ID NO:107, SEQ ID NO:117, and SEQ ID NO:127.

8. An isolated antibody or fragment thereof that can specifically bind to a DR6 polypeptide, wherein the light chain variable region (VL) of said antibody or fragment thereof comprises an amino acid sequence at least 90% identical to a reference amino acid sequence selected from the group consisting of: SEQ ID NO:12, SEQ ID NO:22, SEQ ID NO:32, SEQ ID NO:42, SEQ ID NO:52, SEQ ID NO:62, SEQ ID NO:72, SEQ ID NO:82, SEQ ID NO:92, SEQ ID NO:102 SEQ ID NO:112, SEQ ID NO:122, and SEQ ID NO:132.

9. An isolated antibody or fragment thereof that can specifically bind to a DR6 polypeptide, wherein the VH of said antibody or fragment thereof comprises an amino acid sequence that is identical, except for 20 or fewer conservative amino acid substitutions, to a reference amino acid sequence selected from the group consisting of: SEQ ID NO:7, SEQ ID NO:17, SEQ ID NO:27, SEQ ID NO:37, SEQ ID NO:47, SEQ ID NO:57, SEQ ID NO:67, SEQ ID NO:77, SEQ ID NO:87, SEQ ID NO: 97, SEQ ID NO:107, SEQ ID NO:117, and SEQ ID NO:127.

10. An isolated antibody or fragment thereof that can specifically bind to a DR6 polypeptide, wherein the VL of said antibody or fragment thereof comprises an amino acid sequence identical, except for 20 or fewer conservative amino acid substitutions, to a reference amino acid sequence selected from the group consisting of: SEQ ID NO:12, SEQ ID NO:22, SEQ ID NO:32, SEQ ID NO:42, SEQ ID NO:52, SEQ ID NO:62, SEQ ID NO:72, SEQ ID NO:82, SEQ ID NO:92, SEQ ID NO:102 SEQ ID NO:112, SEQ ID NO:122, and SEQ ID NO:132.

11. An isolated antibody or fragment thereof that can specifically bind to a DR6 polypeptide, wherein the VH of said antibody or fragment thereof comprises an amino acid sequence selected from the group consisting of: SEQ ID NO:7, SEQ ID NO:17, SEQ ID NO:27, SEQ ID NO:37, SEQ ID NO:47, SEQ ID NO:57, SEQ ID NO:67, SEQ ID NO:77, SEQ ID NO:87, SEQ ID NO: 97, SEQ ID NO:107, SEQ ID NO:117, and SEQ ID NO:127.

12. An isolated antibody or fragment thereof that can specifically bind to a DR6 polypeptide, wherein the VL of said antibody or fragment thereof comprises an amino acid sequence selected from the group consisting of: SEQ ID NO:12, SEQ ID NO:22, SEQ ID NO:32, SEQ ID NO:42, SEQ ID NO:52, SEQ ID NO:62, SEQ ID NO:72, SEQ ID NO:82, SEQ ID NO:92, SEQ ID NO:102 SEQ ID NO:112, SEQ ID NO:122, and SEQ ID NO:132.

13. An isolated antibody or fragment thereof that can specifically bind to a DR6 polypeptide, wherein the VH and VL of said antibody or fragment thereof comprise, respectively, amino acid sequences at least 90% identical to reference amino acid sequences selected from the group consisting of: SEQ ID NO:7 and SEQ ID NO:12; SEQ ID NO:17 and SEQ ID NO:22; SEQ ID NO:27 and SEQ ID NO:32; SEQ ID NO:37 and SEQ ID NO:42; SEQ ID NO:47 and SEQ ID NO:52; SEQ ID NO:57 and SEQ ID NO:62; SEQ ID NO:67 and SEQ ID NO:72; SEQ ID NO:77 and SEQ ID NO:82; SEQ ID NO:87 and SEQ ID NO:92; SEQ ID NO:97 and SEQ ID NO:102; SEQ ID NO:107 and SEQ ID NO:112; SEQ ID NO117 and SEQ ID NO:122; and SEQ ID NO:127 and SEQ ID NO:132.

14. An isolated antibody or fragment thereof that can specifically bind to a DR6 polypeptide, wherein the VH and VL of said antibody or fragment thereof comprise, respectively, amino acid sequences identical, except for 20 or fewer conservative amino acid substitutions each, to reference amino acid sequences selected from the group consisting of: SEQ ID NO:7 and SEQ ID NO:12; SEQ ID NO:17 and SEQ ID NO:22; SEQ ID NO:27 and SEQ ID NO:32; SEQ ID NO:37 and SEQ ID NO:42; SEQ ID NO:47 and SEQ ID NO:52; SEQ ID NO:57 and SEQ ID NO:62; SEQ ID NO:67 and SEQ ID NO:72; SEQ ID NO:77 and SEQ ID NO:82; SEQ ID NO:87 and SEQ ID NO:92; SEQ ID NO:97 and SEQ ID NO:102; SEQ ID NO:107 and SEQ ID NO:112; SEQ ID NO117 and SEQ ID NO:122; and SEQ ID NO:127 and SEQ ID NO:132.

15. An isolated antibody or fragment thereof that can specifically bind to a DR6 polypeptide, wherein the VH and VL of said antibody or fragment thereof comprise, respectively, amino acid sequences selected from the group consisting of: SEQ ID NO:7 and SEQ ID NO:12; SEQ ID NO:17 and SEQ ID NO:22; SEQ ID NO:27 and SEQ ID NO:32; SEQ ID NO:37 and SEQ ID NO:42; SEQ ID NO:47 and SEQ ID NO:52; SEQ ID NO:57 and SEQ ID NO:62; SEQ ID NO:67 and SEQ ID NO:72; SEQ ID NO:77 and SEQ ID NO:82; SEQ ID NO:87 and SEQ ID NO:92; SEQ ID NO:97 and SEQ ID NO:102; SEQ ID

NO:107 and SEQ ID NO:112; SEQ ID NO117 and SEQ ID NO:122; and SEQ ID NO:127 and SEQ ID NO:132.

16. An isolated antibody or fragment thereof that can specifically bind to a DR6 polypeptide, wherein the VH of said antibody or fragment thereof comprises a Kabat heavy chain complementarity determining region-1 (VH-CDR1) amino acid sequence identical, except for two or fewer amino acid substitutions, to a reference VH-CDR1 amino acid sequence selected from the group consisting of: SEQ ID NO: 8, SEQ ID NO: 18, SEQ ID NO: 28, SEQ ID NO: 38, SEQ ID NO: 48, SEQ ID NO: 58, SEQ ID NO: 68, SEQ ID NO: 78, SEQ ID NO: 88, SEQ ID NO: 98, SEQ ID NO: 108 SEQ ID NO: 118, and SEQ ID NO: 128.

17. The antibody or fragment thereof of claim 16, wherein said VH-CDR1 amino acid sequence is selected from the group consisting of: SEQ ID NO: 8, SEQ ID NO: 18, SEQ ID NO: 28, SEQ ID NO: 38, SEQ ID NO: 48, SEQ ID NO: 58, SEQ ID NO: 68, SEQ ID NO: 78, SEQ ID NO: 88, SEQ ID NO: 98, SEQ ID NO: 108 SEQ ID NO: 118, and SEQ ID NO: 128.

18. An isolated antibody or fragment thereof that can specifically bind to a DR6 polypeptide, wherein the VH of said antibody or fragment thereof comprises a Kabat heavy chain complementarity determining region-2 (VH-CDR2) amino acid sequence identical, except for four or fewer amino acid substitutions, to a reference VH-CDR2 amino acid sequence selected from the group consisting of: SEQ ID NO: 9, SEQ ID NO: 19, SEQ ID NO: 29, SEQ ID NO: 39, SEQ ID NO: 49, SEQ ID NO: 59, SEQ ID NO: 69, SEQ ID NO: 79, SEQ ID NO: 89, SEQ ID NO: 99, SEQ ID NO: 109, SEQ ID NO: 119 and SEQ ID NO: 129.

19. The antibody or fragment thereof of claim 18, wherein said VH-CDR2 amino acid sequence is selected from the group consisting of: SEQ ID NO: 9, SEQ ID NO: 19, SEQ ID NO: 29, SEQ ID NO: 39, SEQ ID NO: 49, SEQ ID NO: 59, SEQ ID NO: 69, SEQ ID NO: 79, SEQ ID NO: 89, SEQ ID NO: 99, SEQ ID NO: 109, SEQ ID NO: 119 and SEQ ID NO: 129.

20. An isolated antibody or fragment thereof that can specifically bind to a DR6 polypeptide, wherein the VH of said antibody or fragment thereof comprises a Kabat heavy chain

complementarity determining region-3 (VH-CDR3) amino acid sequence identical, except for four or fewer amino acid substitutions, to a reference VH-CDR3 amino acid sequence selected from the group consisting of: SEQ ID NO: 10, SEQ ID NO: 20, SEQ ID NO: 30, SEQ ID NO: 40, SEQ ID NO: 40, SEQ ID NO: 60, SEQ ID NO: 70, SEQ ID NO: 80, SEQ ID NO: 90, SEQ ID NO: 100, SEQ ID NO: 110, SEQ ID NO: 120 and SEQ ID NO:130.

21. The antibody or fragment thereof of claim 20, wherein said VH-CDR3 amino acid sequence is selected from the group consisting of: SEQ ID NO: 10, SEQ ID NO: 20, SEQ ID NO: 30, SEQ ID NO: 40, SEQ ID NO: 40, SEQ ID NO: 60, SEQ ID NO: 70, SEQ ID NO: 80, SEQ ID NO: 90, SEQ ID NO: 100, SEQ ID NO: 110, SEQ ID NO: 120 and SEQ ID NO:130.

22. An isolated antibody or fragment thereof that can specifically bind to a DR6 polypeptide, wherein the VL of said antibody or fragment thereof comprises a Kabat light chain complementarity determining region-1 (VL-CDR1) amino acid sequence identical, except for four or fewer amino acid substitutions, to a reference VL-CDR1 amino acid sequence selected from the group consisting of: SEQ ID NO: 13, SEQ ID NO: 23, SEQ ID NO: 33, SEQ ID NO: 43, SEQ ID NO: 53, SEQ ID NO: 63, SEQ ID NO: 73, SEQ ID NO: 83, SEQ ID NO: 93, SEQ ID NO: 103, SEQ ID NO: 113, SEQ ID NO: 123 and SEQ ID NO: 133.

23. The antibody or fragment thereof of claim 22, wherein said VL-CDR1 amino acid sequence is selected from the group consisting of: SEQ ID NO: 13, SEQ ID NO: 23, SEQ ID NO: 33, SEQ ID NO: 43, SEQ ID NO: 53, SEQ ID NO: 63, SEQ ID NO: 73, SEQ ID NO: 83, SEQ ID NO: 93, SEQ ID NO: 103, SEQ ID NO: 113, SEQ ID NO: 123 and SEQ ID NO: 133.

24. An isolated antibody or fragment thereof that can specifically bind to a DR6 polypeptide, wherein the VL of said antibody or fragment thereof comprises a Kabat light chain complementarity determining region-2 (VL-CDR2) amino acid sequence identical, except for two or fewer amino acid substitutions, to a reference VL-CDR2 amino acid sequence selected from the group consisting of: SEQ ID NO: 14, SEQ ID NO: 24, SEQ ID NO: 34, SEQ ID NO: 44, SEQ ID NO: 54, SEQ ID NO: 64, SEQ ID NO: 74, SEQ ID NO: 84,

SEQ ID NO: 94, SEQ ID NO: 104, SEQ ID NO: 114, SEQ ID NO: 124, and SEQ ID NO: 134.

25. The antibody or fragment thereof of claim 24, wherein said VL-CDR2 amino acid sequence is selected from the group consisting of: SEQ ID NO: 14, SEQ ID NO: 24, SEQ ID NO: 34, SEQ ID NO: 44, SEQ ID NO: 54, SEQ ID NO: 64, SEQ ID NO: 74, SEQ ID NO: 84, SEQ ID NO: 94, SEQ ID NO: 104, SEQ ID NO: 114, SEQ ID NO: 124, and SEQ ID NO: 134.

26. An isolated antibody or fragment thereof that can specifically bind to a DR6 polypeptide, wherein the VL of said antibody or fragment thereof comprises a Kabat light chain complementarity determining region-3 (VL-CDR3) amino acid sequence identical, except for four or fewer amino acid substitutions, to a reference VL-CDR3 amino acid sequence selected from the group consisting of: SEQ ID NO: 15, SEQ ID NO: 25, SEQ ID NO: 35, SEQ ID NO: 45, SEQ ID NO: 55, SEQ ID NO: 65, SEQ ID NO: 75, SEQ ID NO: 85, SEQ ID NO: 95, SEQ ID NO: 105, SEQ ID NO: 115, SEQ ID NO: 125, and SEQ ID NO: 135.

27. The antibody or fragment thereof of claim 26, wherein said VL-CDR3 amino acid sequence is selected from the group consisting of: SEQ ID NO: 15, SEQ ID NO: 25, SEQ ID NO: 35, SEQ ID NO: 45, SEQ ID NO: 55, SEQ ID NO: 65, SEQ ID NO: 75, SEQ ID NO: 85, SEQ ID NO: 95, SEQ ID NO: 105, SEQ ID NO: 115, SEQ ID NO: 125, and SEQ ID NO: 135.

28. An isolated antibody or fragment thereof that can specifically bind to a DR6 polypeptide, wherein the VH of said antibody or fragment thereof comprises VH-CDR1, VH-CDR2, and VH-CDR3 amino acid sequences selected from the group consisting of: SEQ ID NOs: 8, 9, and 10; SEQ ID NOs: 18, 19, and 20; SEQ ID NOs: 28, 29, and 30; SEQ ID NOs: 38, 39, and 40; SEQ ID NOs: 48, 49, and 50; SEQ ID NOs: 58, 59, and 60; SEQ ID NOs: 68, 69, and 70; SEQ ID NOs: 78, 79, and 80; SEQ ID NOs: 88, 89, and 90; SEQ ID NOs: 98, 99, and 100; SEQ ID NOs: 108, 109, and 110; SEQ ID NOs: 118, 119, and 120; and SEQ ID NOs: 128, 129, and 130, except for one, two, three, or four amino acid substitutions in at least one of said VH-CDRs.

29. An isolated antibody or fragment thereof that can specifically bind to a DR6 polypeptide, wherein the VH of said antibody or fragment thereof comprises VH-CDR1, VH-CDR2, and VH-CDR3 amino acid sequences selected from the group consisting of: SEQ ID NOs: 8, 9, and 10; SEQ ID NOs: 18, 19, and 20; SEQ ID NOs: 28, 29, and 30; SEQ ID NOs: 38, 39, and 40; SEQ ID NOs: 48, 49, and 50; SEQ ID NOs: 58, 59, and 60; SEQ ID NOs: 68, 69, and 70; SEQ ID NOs: 78, 79, and 80; SEQ ID NOs: 88, 89, and 90; SEQ ID NOs: 98, 99, and 100; SEQ ID NOs: 108, 109, and 110; SEQ ID NOs: 118, 119, and 120; and SEQ ID NOs: 128, 129, and 130.

30. An isolated antibody or fragment that can specifically bind to a DR6 polypeptide, wherein the VL of said antibody or fragment thereof comprises VL-CDR1, VL-CDR2, and VL-CDR3 amino acid sequences selected from the group consisting of: SEQ ID NOs: 13, 14, and 15; SEQ ID NOs: 23, 24, and 25; SEQ ID NOs: 33, 34, and 35; SEQ ID NOs: 43, 44, and 45; SEQ ID NOs: 53, 54, and 55; SEQ ID NOs: 63, 64, and 65; SEQ ID NOs: 73, 74, and 75; SEQ ID NOs: 83, 84, and 85; SEQ ID NOs: 93, 94, and 95; SEQ ID NOs: 103, 104, and 105; SEQ ID NOs: 113, 114, and 115; SEQ ID NOs: 123, 124, and 125; and SEQ ID NOs: 133, 134, and 135, except for one, two, three, or four amino acid substitutions in at least one of said VL-CDRs.

31. An isolated antibody or fragment thereof that can specifically bind to a DR6 polypeptide, wherein the VL of said antibody or fragment thereof comprises VL-CDR1, VL-CDR2, and VL-CDR3 amino acid sequences selected from the group consisting of: SEQ ID NOs: 13, 14, and 15; SEQ ID NOs: 23, 24, and 25; SEQ ID NOs: 33, 34, and 35; SEQ ID NOs: 43, 44, and 45; SEQ ID NOs: 53, 54, and 55; SEQ ID NOs: 63, 64, and 65; SEQ ID NOs: 73, 74, and 75; SEQ ID NOs: 83, 84, and 85; SEQ ID NOs: 93, 94, and 95; SEQ ID NOs: 103, 104, and 105; SEQ ID NOs: 113, 114, and 115; SEQ ID NOs: 123, 124, and 125; and SEQ ID NOs: 133, 134, and 135.

32. The antibody or fragment thereof of any one of claims 1 to 31, wherein the VH framework regions are human, except for five or fewer amino acid substitutions.

33. The antibody or fragment thereof of any one of claims 1 to 32, wherein the VL framework regions are human, except for five or fewer amino acid substitutions.

34. The antibody or fragment thereof of any one of claims 1 to 33, which binds to a linear epitope of a DR6 polypeptide.

35. The antibody or fragment thereof of any one of claims 1 to 33, which binds to a non-linear conformational epitope if a DR6 polypeptide.

36. The antibody or fragment thereof of any one of claims 1 to 35, wherein the antibody or fragment thereof is multivalent, and comprises at least two heavy chains and at least two light chains.

37. The antibody or fragment thereof of any one of claims 1 to 36, wherein the antibody or fragment thereof is multispecific.

38. The antibody or fragment thereof of claim 37, wherein the antibody or fragment thereof is bispecific.

39. The antibody or fragment thereof of any one of claims 1 to 38, wherein the antibody or fragment thereof is bispecific.

40. The antibody or fragment thereof of any one of claims 1 to 39, wherein the heavy and light chain variable domains are murine in origin.

41. The antibody or fragment thereof of claim 40, wherein said heavy and light chain variable domains are derived from a monoclonal antibody selected from the group consisting of 1P1D6.3, 1P2F2.1, and 1P5D10.2

42. The antibody or fragment thereof of any one of claims 1 to 39, wherein the heavy and light chain variable domains are fully human in origin.

43. The antibody or fragment thereof of claim 42, wherein said heavy and light chain variable domains are from a monoclonal Fab antibody fragment selected from the group consisting of M14-H06, M15-E10, M16-C07, M23-F10, and M80-B03 M50-H01, M51-H09, M53-E04, M53-F04, M62-B02, M63-E10, M66-B03, M67-G02, M72-F03, and M73-C04

44. The antibody or fragment thereof of any one of claims 1 to 39, which is humanized.

45. The antibody or fragment thereof of any one of claims 1 to 39, which is chimeric.

46. The antibody or fragment thereof of any one of claims 1 to 39, which is primatized.

47. The antibody or fragment thereof of any one of claims 1 to 39, which is fully human.

48. The antibody or fragment thereof of any one of claims 1 to 47, which is an Fab fragment.

49. The antibody or fragment thereof of any one of claims 1 to 47, which is an Fab' fragment.

50. The antibody or fragment thereof of any one of claims 1 to 47, which is an F(ab)2 fragment.

51. The antibody or fragment thereof of any one of claims 1 to 47, which is an Fv fragment.

52. The antibody or fragment thereof of any one of claims 1 to 47, which is a single chain antibody.

53. The antibody or fragment thereof of any one of claimsm 48 to 52, wherein said antibody or fragment thereof is conjugated to a polymer.

54. The antibody or fragment thereof of claim 53, wherein the polymer is a polyalkylene glycol.

55. The antibody or fragment thereof of claim 54, wherein the polyalkylene glycol is a polyethylene glycol (PEG).

56. The antibody or fragment thereof of any one of claims 1 to 49 and 52-55, which comprises a light chain constant region selected from the group consisting of a human kappa constant region and a human lambda constant region.

57. The antibody or fragment thereof of any one of claims 1 to 49 and 52-55, which comprises at a heavy chain constant region or fragment thereof.

58. The antibody or fragment thereof of claim 57, wherein said heavy chain constant region or fragment thereof is selected from the group consisting of human IgG4, IgG4 agly, IgG1 and IgG1 agly.

59. The antibody or fragment thereof of any one of claims 4 to 58, which can specifically bind to a DR6 polypeptide or fragment thereof, or a DR6 variant polypeptide, with an

affinity characterized by a dissociation constant (KD) that is less than the KD for said reference monoclonal antibody.

60. The antibody or fragment thereof of any one of claims 1 to 59, that can specifically bind to a DR6 polypeptide or fragment thereof, or a DR6 variant polypeptide with an affinity characterized by a dissociation constant (KD) no greater than 5×10^{-2} M, 10^{-2} M, 5×10^{-3} M, 10^{-3} M, 5×10^{-4} M, 10^{-4} M, 5×10^{-5} M, 10^{-5} M, 5×10^{-6} M, 10^{-6} M, 5×10^{-7} M, 10^{-7} M, 5×10^{-8} M, 10^{-8} M, 5×10^{-9} M, 10^{-9} M, 5×10^{-10} M, 10^{-10} M, 5×10^{-11} M, 10^{-11} M, 5×10^{-12} M, 10^{-12} M, 5×10^{-13} M, 10^{-13} M, 5×10^{-14} M, 10^{-14} M, 5×10^{-15} M, or 10^{-15} M.

61. The antibody or fragment thereof of any one of claims 1 to 60, that can preferentially bind to a human DR6 polypeptide or fragment thereof, relative to a murine DR6 polypeptide or fragment thereof.

62. The antibody or fragment thereof of any one of claims 1 to 61 that inhibits binding of DR6 to p75.

63. The antibody or fragment thereof of any one of claims 1 to 62 that does not prevent binding of DR6 to APP.

64. A method of promoting survival of cells of the nervous system comprising contacting said cells with a DR6 antagonist.

65. The method of claim 64, wherein said cells are in a mammal and said contacting comprises administering a therapeutically effective amount of a DR6 antagonist to a mammal in need thereof.

66. A method of treating a condition associated with death of cells of the nervous system in a subject, the method comprising administering a therapeutically effective amount of a DR6 antagonist.

67. The method of any one of claims 64 to 66, wherein the cells of the nervous system are cells of the central nervous system (CNS).

68. The method of claim 67, wherein the cells of the CNS are selected from the group consiting of cortical neurons, motor neurons, oligodendrocytes, microglia and astrocytes.

69. The method of any one of claims 64 to 66, wherein the cells of the nervous system are cells of the peripheral nervous system (PNS).

70. The method of claim 69, wherein the cells of the PNS are selected from the group consiting of dorsal root ganglion (DRG) neurons and schwann cells.

71. The method of any one of claims 64 to 66, wherein the cells of the nervous system are neurons.

72. The method of claim 71, wherein the neurons are cortical neurons, DRG neurons or motor neurons.

73. The method of any one of claims 64 to 66, wherein the cells of the nervous system are glial cells.

74. The method of claim 73, wherein the glial cells are selected from the group consisting of oligodendrocyte precursor cells (OPCs), schwann cells, astrocytes and microglial cells.

75. A method of promoting oligodendrocyte proliferation, differentiation or survival comprising contacting oligodendrocyte cells or oligodendrocyte precursor cells with a DR6 antagonist.

76. The method of claim 75, wherein said cells are in a mammal and said contacting comprises administering an effective amount of a DR6 antagonist to a mammal in need thereof.

77. A method of treating a condition associated with oligodendrocyte death or lack of differentiation comprising administering a therapeutically effective amount of a DR6 antagonist.

78. A method of promoting myelination comprising contacting contacing a mixture of neuronal cells and glial cells with a DR6 antagonist.

79. The method of claim 78, wherein the glial cells are oligodendrocyte cells or oligodendrocyte precursor cells.

80. The method of claim 78, wherein the glial cells are schwann cells.

81. The method of any one of claims 78-80, wherein said neuronal cells and said glial cells are in a mammal and said contacting comprises administering an effective amount of a DR6 antagonist to a mammal in need thereof.

82. A method of treating a condition associated with dysmyelination or demyelination comprising administering a therapeutically effective amount of a DR6 antagonist.

83. A method of inhibiting the binding of DR6 to p75 comprising contacting a DR6 polypeptide and/or p75 polypeptide with a DR6 antagonist under conditions wherein binding of DR6 to p75 is inhibited.

84. The method of any of claims 64-83, wherein the DR6 antagonist is a soluble DR6 polypeptide.

85. The method of claim 84, wherein the soluble DR6 polypeptide comprises a DR6 region selected from the group consisting of:

(i) a DR6 TNFR-like cysteine rich domain or a fragment, variant or derivative thereof

(ii) a DR6 extracellular domain or a fragment, variant or derivative thereof

(iii) a DR6 cytoplasmic domain or a fragment, variant or derivative thereof

(iv) a DR6 transmembrane domain or a fragment, variant or derivative thereof

(v) a DR6 death domain or fragment, variant or derivative thereof

(vi) a combination of at least two of said DR6 domains or fragments, variants or derivatives thereof of (i) to (v).

86. The method of claim 84, wherein the soluble DR6 polypeptide lacks at least one DR6 region selected from the group consisting of

(i) a DR6 TNFR-like cysteine rich domain

(ii) a DR6 extracellular domain

(iii) a DR6 cytoplasmic domain

(iv) a DR6 transmembrane domain

(v) a DR6 death domain

(vi) a combination of at least two of said DR6 domains of (i) to (v).

87. The method of claim 84, wherein said soluble DR6 polypeptide lacks a DR6 transmembrane domain and a DR6 cytoplasmic domain.

88. The method of claim 84, wherein said soluble DR6 polypeptide comprises a polypeptide fragment selected from the group consisting of:

(i) amino acids 1 to 40 of SEQ ID NO:2;

(ii) amino acids 1 to 64 of SEQ ID NO:2;

(iii) amino acids 1 to 105 of SEQ ID NO:2;

(iv) amino acids 1 to 145 of SEQ ID NO:2;

(v) amino acids 1 to 185 of SEQ ID NO:2;

(vi) amino acids 1 to 212 of SEQ ID NO:2;

(vii) amino acids 1 to 349 of SEQ ID NO:2;

(viii) amino acids 42 to 64 of SEQ ID NO:2;

(ix) amino acids 42 to 105 of SEQ ID NO:2;

(x) amino acids 42 to 145 of SEQ ID NO:2;

(xi) amino acids 42 to 185 of SEQ ID NO:2;

(xii) amino acids 42 to 212 of SEQ ID NO:2;

(xiii) amino acids 42 to 349 of SEQ ID NO:2;

(xiv) amino acids 65 to 212 of SEQ ID NO:2;

(xv) amino acids 65 to 349 of SEQ ID NO:2;

(xvi) amino acids 65 to 105 of SEQ ID NO:2;

(xvii) amino acids 106 to 145 of SEQ ID NO:2;

(xviii) amino acids 146 to 185 of SEQ ID NO:2;

(xix) amino acids 186 to 212 of SEQ ID NO:2;

(xx) amino acids 106 to 212 of SEQ ID NO:2;

(xxi) amino acids 106 to 349 of SEQ ID NO:2;

(xxii) amino acids 146 to 212 of SEQ ID NO:2;

(xxiii) amino acids 146 to 349 of SEQ ID NO:2;

(xxiv) amino acids 213 to 349 of SEQ ID NO:2;

(xxv) variants or derivatives of any of said polypeptide fragments; and

(xxvi) a combination of at least two of any of said polypeptide fragments or variants or derivatives thereof.

89. The method of claim 84, wherein said soluble DR6 polypeptide comprises amino acids 1 to 349 or amino acids 41 to 349 or amino acids 42 to 349 of SEQ ID NO:2.

90. The method of any one of claims 84 to 89 wherein said soluble DR6 polypeptide comprises a DR6 TNFR-like cysteine rich motif or fragment, variant or derivative thereof

91. The method of any one of claims 84 to 90, wherein said soluble DR6 polypeptide further comprises a non-DR6 moiety.

92. The method of claim 91, wherein said non-DR6 moiety is a heterologous polypeptide fused to said soluble DR6 polypeptide.

93. The method of claim 92, wherein heterologous polypeptide is selected from the group consisting of an immunoglobulin fragment, serum albumin, a targeting protein, a reporter protein, and a purification-facilitating protein.

94. The method of claim 93, wherein said heterologous polypeptide is an immunoglobulin fragment.

95. The method of claim 94, wherein said immunoglobulin fragment comprises a hinge and Fc region.

96. The method of any one of claims 91-95, wherein said soluble DR6 polypeptide is conjugated to a polymer.

97. The method of claim 96, wherein the polymer is selected from the group consisting of a polyalkylene glycol, a sugar polymer, and a polypeptide.

98. The method of claim 97, wherein the polymer is a polyalkylene glycol.

99. The method of claim 98, wherein the polyalkylene glycol is polyethylene glycol (PEG).

100. The method of claim 99, wherein said soluble DR6 polypeptide is conjugated to 1, 2, 3 or 4 polymers.

101. The method of claim 100, wherein the total molecular weight of said polymers is from 5,000 Da to 100,000 Da.

102. The method of any one of claims 64 to 83, wherein said DR6 antagonist comprises a DR6 antibody, or fragment thereof.

103. The method of claim of claim 102, wherein said DR6 antibody, or fragment thereof is the antibody or fragment thereof of any one of claims 1-63.

104. The method of claim 102 wherein said DR6 antibody or fragment thereof specifically binds to an epitope consisting essentially of a polypeptide fragment selected from the group consisting of:

(i) amino acids 65 to 105 of SEQ ID NO:2;

(ii) acids 106 to 145 of SEQ ID NO:2;

(iii) amino acids 146 to 185 of SEQ ID NO:2;

(iv) amino acids 186 to 212 of SEQ ID NO:2;

(v) amino acids 65 to 145 of SEQ ID NO:2;

(vi) amino acids 65 to 185 of SEQ ID NO:2;

(vii) amino acids 65 to 212 of SEQ ID NO:2;

(viii) amino acids 106 to 185 of SEQ ID NO:2;

(ix) amino acids 106 to 212 of SEQ ID NO:2;

(x) amino acids 134-189 of SEQ ID NO:2;

(xi) amino acids 134-168 of SEQ ID NO:2;

(xii) amino acids 168-189 of SEQ ID NO:2;

(xiii) amino acids 146 to 212 of SEQ ID NO:2;

(xiv) variants or derivatives of any of said polypeptide fragments; and

(xv) a combination of two or more of any of said polypeptide fragments or variants or derivatives thereof.

105. The method of any one of claims 64-83, wherein said DR6 antagonist comprises a DR6 antagonist polynucleotide.

106. The method of claim 105, wherein said DR6 antagonist polynucleotide is selected from the group consisting of:

(i) an antisense polynucleotide;

(ii) a ribozyme;

(iii) a small interfering RNA (siRNA); and

(iv) a small-hairpin RNA (shRNA).

107. The method of claim 106, wherein said DR6 antagonist polynucleotide is an antisense polynucleotide comprising at least 10 bases complementary to DR6 mRNA.

108. The method of claim 107, wherein said DR6 antagonist polynucleotide is a ribozyme.

109. The method of claim 105, wherein said DR6 antagonist polynucleotide is an siRNA.

110. The method of claim 109, wherein said siRNA comprises a sequence selected from the group consisting of AGAAACGGCUCCUUUAUUA (SEQ ID NO:160), GGAAGGACAUCUAUCAGUU (SEQ ID NO:161), GGCCGAUGAUUGAGAGAUU (SEQ ID NO:162), GCAGUUGGAAACAGACAAA (SEQ ID NO:163).

111. The method of claim 106 wherein said DR6 antagonist polynucleotide is an shRNA.

112. The method of any one of claims 64-83, wherein said LINGO-1 antagonist is an aptamer.

113. The method of any one of claims 64-65, 67-76 or 78-83 wherein the contacting comprises (a) introducing into said cells a polynucleotide which encodes said DR6 antagonist through operable association with an expression control sequence, and (b) allowing expression of said DR6 antagonist.

114. The method of claim 113, wherein said polynucleotide is introduced into said cell by a method selected from the group consisting of:

(a) transfection;

(b) electroporation;

(c) transduction; and

(d) direct microinjection.

115. The method of any one of claims 113 to 114, wherein said polynucleotide is administered as an expression vector.

116. The method of claim 115, wherein said expression vector is a viral vector.

117. The method of claim 116, wherein the viral vector is selected from the group consisting of an adenoviral vector, an alphavirus vector, an enterovirus vector, a pestivirus vector, a lentiviral vector, a baculoviral vector, a herpesvirus vector a papovaviral vector, a poxvirus vector and a parvovirus vector.

118. The method of claim 117, wherein said herpesvirus vector is selected from the group consisting of a herpes simplex virus vector and an Epstein Barr virus vector.

119. The method of claim 117, wherein said poxvirus vector is a vaccinia virus vector.

120. The method of claim 117, wherein said lentivirus vector is pLL3.7.

121. The method of claim 117, wherein said parvovirus vector is an adeno-associated virus (AAV) vector.

122. The method of any one of claims 66-74, 77, or 81-82, wherein said DR6 antagonist is administered by a route selected from the group consisting of topical administration, intraocular administration, intravitreal administration, parenteral administration, intrathecal administration, subdural administration, subcutaneous administration or via a capsule implant.

123. The method of any one of claims 65, 76, or 81 wherein said mammal has been diagnosed with or is suspected of having a condition associated death of cells of the central nervous system.

124. The method of claim 66 or 123, wherein said condition is selected from the group consisting of Alzheimer's disease, Parkinson's disease, Huntington's disease, motor neuron disease, (*e.g.* amyotrophic lateral sclerosis), multiple sclerosis, neuronal trauma and cerebral ischemia (*e.g.* stroke).

125. The method of claim 66, wherein the cells are schwann cells and the condition is neuropathic pain.

126. The method of claim 81, wherein said mammal has been diagnosed with or is suspected of having neuropathic pain.

127. The method of claim 82, wherein the condition is neuropathic pain.

128. The method of any one of claims 64 to 127, wherein said DR6 antagonist is used in combination with a p75 antagonist.

129. The method of claim 128, wheein the DR6 antagonist and the p75 antagonist are used simultaneously.

130. The method of claim 128, wherein the DR6 antagonist and the p75 antagonist are used sequentially.

131. A method of inhibiting the binding of DR6 to p75 comprising contacting a p75 polypeptide or a DR6 polypeptide with a p75 antagonist under conditions wherein binding of DR6 to p75 is inhibited.

132. The method of claim 131, wherein the p75 antagonist is selected from the group consisting of (i) a p75 antagonist compound; (ii) p75 antagonist polypeptide; (iii) a p75 antagonist antibody or fragment thereof; (iv) a p75 antagonist polynucleotide; (v) a p75 aptamer; and (vi) a combination of two or more of said p75 antagonists.