

METHODS FOR THE TREATMENT OF AMYOTROPHIC LATERAL SCLEROSIS

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Prov. App. No. 63/365458, filed May 27, 2022, which is incorporated by reference in its entirety.

REFERENCE TO SEQUENCE LISTING

[0002] The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled "ELDN.010WO.xml" created on April 20, 2023, which is 27,094 bytes in size. The information in the electronic format of the Sequence Listing is incorporated herein by reference in its entirety.

BACKGROUND

Field

[0003] The present disclosure relates to methods for treating neurodegenerative diseases such as Amyotrophic Lateral Sclerosis. Also provided are methods of predicting and/or measuring response to a treatment, including measuring levels of one or more biomarker. Embodiments also relate to methods of modulating a level of one or more biomarker.

Description of the Related Art

[0004] Amyotrophic lateral sclerosis (ALS), sometimes called Lou Gehrig's Disease, is a progressive, fatal, neurological disorder characterized by muscle fiber atrophy resulting from the degeneration of motor neurons in the spinal column and brain. ALS affects approximately 30,000 US citizens with only about 10% of the cases being classified as the familial form of ALS. In a subset of familial patients with mutations in the metabolic enzyme superoxide dismutase 1 (*SOD1*), the pathological progression may be attributed to an unknown gain of function associated with a mutant form of the enzyme (*SOD1* dependent) (Rosen, 1993). However, in the majority of ALS cases the *SOD1* gene contains no mutations, the activity of the *SOD1* enzyme is normal, and the mechanism of disease pathology is unknown (*SOD1* independent). Therefore, the remaining 90% of ALS cases

are classified as sporadic cases with no well characterized genetic component or causal agent.

[0005] Because the cause of the sporadically occurring form of the disease is unknown, researchers have turned to transgenic strategies to create laboratory models of the disease. Identification role of the *SOD1* gene has led to the generation of transgenic rodent models of ALS. A transgenic mouse strain carrying 23 copies of the human *SOD1*^{G93A} transgene (the “G93A mouse”) is the most widely used murine model of ALS and is accepted as a standard model for ALS therapeutic studies (the “G93A mouse”) (*see* Tu PH et. al.(1996) *Proc Natl Acad Sci U S A* 93:3155-3160 and Gurney ME (1997) *J Neuro Sci* 152 Suppl 1: S67-S73)

[0006] Although ALS is characterized by loss of motor neurons in the spinal cord resulting in muscle atrophy, the disease also manifests itself with changes in axon transport, protein aggregation, excitotoxicity, astrocytosis, mitochondrial dysfunction, microglial activation, and synaptic remodeling. Microglial activation, astrocytosis and the presence of infiltrating inflammatory cells from the periphery has been well described. There is accumulation of IgG immunoreactive deposits in the spinal cord of ALS patients, infiltration of lymphocytes, dendritic cells, monocytes, and macrophages into the spinal cord in ALS. Although the role of infiltrating immune cells is poorly understood, recent work would suggest that infiltrating T cell populations are neuroprotective and not cytotoxic. Although ALS has an immune component mediated by activation of microglia and astrocytes it is not considered to be an autoimmune disorder. Unlike diseases such as rheumatoid arthritis or systemic lupus erythematosus in which involvement of specific immune modulatory pathways (e.g., the costimulatory pathway) has been described, involvement of such pathways has not been described for ALS.

[0007] Currently physicians have limited choices for treating ALS. At this time, riluzole is the only drug that has been approved by the FDA for treatment of ALS. In clinical trials, riluzole has shown only a slight benefit in modestly increasing survival time. Thus, there is an urgent need for effective therapies for ALS.

SUMMARY

[0008] Disclosed herein is a method of predicting the response of a subject with a disease or disorder to treatment with a compound against CD40L or CD40. In some embodiments, the method comprises collecting a sample from the subject; measuring a concentration of at least one biomarker present in the sample, wherein the at least one

biomarker is NFL, MMP9, MMP3, IL6, IL18, IgA, TNFR2, TNFa, IL2ra, FactorVIII, CRP, SAP, MCP1, En-Raged, MIG, vWF, IgE, IL8, IP10, C3, and MMP2; wherein the concentration of the at least one biomarker is predictive that the subject is likely to be responsive to treatment against the disease or disorder with a compound against CD40L or CD40. In some embodiments, the disease or disorder is inflammation, a neurodegenerative disease, or neuromuscular disease. In some embodiments, the neurodegenerative disease or neuromuscular disease is Amyotrophic Lateral Sclerosis, Alzheimer's Disease, Parkinson's Disease, Myasthenia Gravis, Multifocal Motor Neuropathy, Primary Lateral Sclerosis, Spinal Muscular Atrophy, Kennedy's Disease, or Spinocerebellar Ataxia. In some embodiments, the neurodegenerative disease or neuromuscular disease is Amyotrophic Lateral Sclerosis (ALS). In some embodiments, the subject is mammalian and/or human. In some embodiments, the compound blocks the interaction of CD40 and CD40L. In some embodiments, the compound is an anti-CD40L antibody or an anti-CD40 antibody. In some embodiments, the compound is tegoprubart. In some embodiments, the compound is MR1 or 5c8.

[0009] Also disclosed herein is a method of treating a subject with a disease or disorder. In some embodiments, the method comprises administering a therapeutically effective dose of a compound against CD40L or CD40, wherein administering the therapeutically effective dose maintains the compound in plasma of the subject at a concentration between 5 µg/mL and 100 µg/mL; preferably at a dose that is between 30 µg/mL and 60 µg/mL. In some embodiments, the disease or disorder is inflammation, a neurodegenerative disease, or neuromuscular disease. In some embodiments, the neurodegenerative disease or neuromuscular disease is Amyotrophic Lateral Sclerosis, Alzheimer's Disease Parkinson's Disease, Myasthenia Gravis, Multifocal Motor Neuropathy, Primary Lateral Sclerosis, Spinal Muscular Atrophy, Kennedy's Disease, or Spinocerebellar Ataxia. In some embodiments, the neurodegenerative disease or neuromuscular disease is Amyotrophic Lateral Sclerosis (ALS). In some embodiments, the subject is mammalian and/or human. In some embodiments, the compound blocks the interaction of CD40 and CD40L. In some embodiments, the compound is an anti-CD40L antibody or an anti-CD40 antibody. In some embodiments, the compound is tegoprubart. In some embodiments, the compound is MR1 or 5c8. In some embodiments, the compound is administered at least once every two weeks. In some embodiments, the compound is administered for a period of at least 12 weeks. In some embodiments, the compound is administered at a dose of between 200 mg/kg and 100 mg/kg; preferably at a dose between

1 mg/kg and 8 mg/kg; most preferably at a dose between 2 mg/kg and 4 mg/kg. In some embodiments, method further comprises administering a second pharmaceutically effective compound. In some embodiments, the second compound blocks the interaction between CD28 and CD86 or between CD28 and CD80. In some embodiments, the second compound targets at least one of the biomarkers selected from the group consisting of: interleukins, cytokines, and proinflammatory markers. In some embodiments, the second compound targets at least one of the biomarkers selected from the group consisting of: NFL, CXCL13, CD40L, CXCL9, TNF-alpha, En-Raged, TNFR2, IgM, IgA, Il2r, CXCL10, CD40, B2M, VCAM, Il-18, Il-16, SAP, MIP1beta, MDC, C3, CRP, Fibrogen, IgE, ICAM, FactorVIII, Ena-78, Pall, Rantes, TIMP1, vWF, Il-6, MCP1, MMP3, MMP9, and MMP2. In some embodiments, the second compound is a CTLA4-Ig fusion protein, an abatacept, a belatacept, or a galiximab. In some embodiments, the compound is administered orally, parenterally, or topically. In some embodiments, the compound is administered parenterally. In some embodiments, the compound is administered by injection, most preferably intravenous, intramuscular, or subcutaneous injection. In some embodiments, the disease or disorder is an inflammatory or immune disease or disorder selected from the group consisting of colitis, drug induced lupus nephritis, graft versus host disease, immune graft response, transplant rejection and atherosclerosis. In some embodiments, the disease or disorder is an autoimmune disease, selected from the group consisting of systemic lupus erythematosus, type-1 diabetes, myasthenia gravis, inflammatory bowel disease, immune thrombocytopenic purpura, rheumatoid arthritis, psoriasis, Addison's disease, Crohn's disease, uveitis, multiple sclerosis, hemolytic anemia, and Graves' disease. In some embodiments, the compound that targets CD40L or CD40 is an antibody that comprises a heavy chain variable region (V_H) comprising: (1) a CDRH1 domain comprising the sequence set forth in SEQ ID NO: 9; (2) a CDRH2 domain comprising the sequence set forth in SEQ ID NO: 10, 11, 12, 13, or 14; and (3) a CDRH3 domain comprising the sequence set forth in SEQ ID NO: 15; and also comprises a light chain variable region (V_L) comprising: (4) a CDRL1 domain comprising the sequence set forth in SEQ ID NO: 16 or 17; (5) a CDRL2 domain comprising the sequence set forth in SEQ ID NO: 18 or 19; and (6) a CDRL3 domain comprising the sequence set forth in SEQ ID NO: 20. In some embodiments, the compound that targets CD40L or CD40 is an antibody that comprises a heavy chain variable region (V_H) having the amino acid sequence as set forth in SEQ ID NOs: 1, 2, 3, or 4; and a light chain variable region (V_L) having the amino acid sequence as set forth in SEQ ID NOs: 5, 6, 7, or 8.

[0010] Also disclosed herein is a method of modulating a concentration of at least one biomarker in a subject. In some embodiments, the method comprises administering to the subject a therapeutically effective amount of a compound against CD40L or CD40, wherein the at least one biomarker is NFL, CXCL13, CD40L, CXCL9, TNF-alpha, En-Raged, TNFR2, IgM, IgA, IL2r, CXCL10, CD40, B2M, VCAM, IL-18, IL-16, SAP, MIP1beta, MDC, C3, CRP, Fibrogen, IgE, ICAM, FactorVIII, Ena-78, Pal1, Rantes, TIMP1, vWF, IL-6, MCP1, MMP3, MMP9, MCP1, MIG, vWF, IL-8, IP10, or MMP2. In some embodiments, the concentration of the at least one biomarker increases following administration of the compound. In some embodiments, the concentration of the at least one biomarker decreases following administration of the compound. In some embodiments, the concentration of the at least one biomarker increases, and the concentration of at least one other biomarker decreases, following administration of the compound. In some embodiments, the subject has a disease or disorder. In some embodiments, the disease or disorder is inflammation, a neurodegenerative disease, or neuromuscular disease. In some embodiments, the neurodegenerative disease or neuromuscular disease is Amyotrophic Lateral Sclerosis, Alzheimer's Disease Parkinson's Disease, Myasthenia Gravis, Multifocal Motor Neuropathy, Primary Lateral Sclerosis, Spinal Muscular Atrophy, Kennedy's Disease, or Spinocerebellar Ataxia. In some embodiments, the neurodegenerative disease or neuromuscular disease is Amyotrophic Lateral Sclerosis (ALS). In some embodiments, the subject is mammalian and/or human. In some embodiments, the compound blocks the interaction of CD40 and CD40L. In some embodiments, the compound is an anti-CD40L antibody or an anti-CD40 antibody. In some embodiments, the compound is tegoprubart. In some embodiments, the compound is MR1 or 5c8. In some embodiments, the method comprises administering the therapeutically effective dose maintains the compound in plasma of the subject at a concentration between 5 µg/mL and 100 µg/mL; preferably at a dose that is between 30 µg/mL and 60 µg/mL. In some embodiments, the compound is administered at least once every two weeks. In some embodiments, the compound is administered for a period of at least 12 weeks. In some embodiments, the compound is administered at a dose of between 200 mg/kg and 100 mg/kg; preferably at a dose between 1 mg/kg and 8 mg/kg; most preferably at a dose between 2 mg/kg and 4 mg/kg. In some embodiments, the compound is administered orally, parenterally, or topically. In some embodiments, the compound is administered parenterally. In some embodiments, the compound is administered by injection, most preferably intravenous, intramuscular, or subcutaneous injection. In some embodiments,

the disease or disorder is an inflammatory or immune disease or disorder selected from the group consisting of colitis, drug induced lupus nephritis, graft versus host disease, immune graft response, transplant rejection and atherosclerosis. In some embodiments, the disease or disorder is an autoimmune disease, selected from the group consisting of systemic lupus erythematosus, type-1 diabetes, myasthenia gravis, inflammatory bowel disease, immune thrombocytopenic purpura, rheumatoid arthritis, psoriasis, Addison's disease, Crohn's disease, uveitis, multiple sclerosis, hemolytic anemia, and Graves' disease. In some embodiments, the compound that targets CD40L or CD40 is an antibody that comprises a heavy chain variable region (V_H) comprising: (1) a CDRH1 domain comprising the sequence set forth in SEQ ID NO: 9; (2) a CDRH2 domain comprising the sequence set forth in SEQ ID NO: 10, 11, 12, 13, or 14; and (3) a CDRH3 domain comprising the sequence set forth in SEQ ID NO: 15; and further comprises a light chain variable region (V_L) comprising: (4) a CDRL1 domain comprising the sequence set forth in SEQ ID NO: 16 or 17; (5) a CDRL2 domain comprising the sequence set forth in SEQ ID NO: 18 or 19; and (6) a CDRL3 domain comprising the sequence set forth in SEQ ID NO: 20. In some embodiments, the compound that targets CD40L or CD40 is an antibody that comprises a heavy chain variable region (V_H) having the amino acid sequence as set forth in SEQ ID NOs: 1, 2, 3, or 4: and a light chain variable region (V_L) having the amino acid sequence as set forth in SEQ ID NOs: 5, 6, 7, or 8.

[0011] Also disclosed herein is a method of maintaining or improving the ALS-FRS scores of a subject with ALS. In some embodiments, the method comprises administering a therapeutically effective dose of a compound against CD40L or CD40 wherein administering the therapeutically effective dose maintains the compound in plasma of the subject at a concentration between 5 $\mu\text{g/mL}$ and 100 $\mu\text{g/mL}$; preferably at a dose that is between 30 $\mu\text{g/mL}$ and 60 $\mu\text{g/mL}$.

[0012] Also disclosed herein is a method of pre-screening and treating a subject with a disease or disorder with a compound against CD40L or CD40. In some embodiments, the method comprises collecting a sample from the subject; detecting a concentration of at least one biomarker present in the fluid, wherein the at least one biomarker is NFL, MMP9, MMP3, IL6, IL18, IgA, TNFR2, TNFa, IL2ra, FactorVIII, CRP, SAP, MCP1, En-Raged, MIG, vWF, IgE, IL8, IP10, C3, or MMP2; screening the concentration of the at least one biomarker to the concentration of the at least one biomarker in a subject that does not have that disease or disorder; and administering a therapeutically effective dose of a compound against CD40L or CD40 such that the plasma of the subject

maintains a concentration of the compound at a dose that is between 5 µg/mL and 100 µg/mL; preferably at a dose that is between 30 µg/mL and 60 µg/mL. In some embodiments, the compound is administered to the subject if the concentration of the at least one biomarker in the subject is significantly different than the concentration of the at least one biomarker in a subject that does not have the disease or disorder.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] FIG. 1A shows MR1 concentration over time using a linear concentration (Y) axis.

[0014] FIG. 1B shows MR1 concentration over time using a log concentration (Y) axis.

[0015] FIG. 2 is a graph of the mean daily body weight measurements starting at day 40 to attainment of peak body weight for the control and the treatment group.

[0016] FIG. 3 depicts the mean daily body weight measurements from peak body weight to death for the control and treatment group.

[0017] FIG. 4 depicts the time to disease onset based on the time to progress to a neurological score of 2 from the start of the study.

[0018] FIG. 5 is a graph representing the median age (X-axis) each group was at a given neurological score 0 to 4 as well as the number of days that each group was stable at a given score (Y-axis)

[0019] FIG. 6 shows a Kaplan Meier Survival Plot for the control and treatment group as calculated by a Cox proportional hazard model.

[0020] FIG. 7A is a graph of the mean daily body weight measurements starting at day 40 to attainment of peak body weight for the control group and the MR1 treated group.

[0021] FIG. 7B is a graph depicting the mean daily body weight measurements from peak body weight to death for the control group and the MR1 treated group.

[0022] FIG. 7C shows the time to disease onset based on the time to progress to a neurological score of 2 in the control group and the MR1 treated group.

[0023] FIG. 7D shows the mean daily body weight measurements from peak body weight to death for the control and the MR1 treated group.

[0024] FIG. 8 is a graph showing the relative binding to human CD40L, of JB5 antibody (circles, dotted line), hu5c8 antibody (squares-solid line), and the control CTLA4-IgG1 (triangles).

[0025] FIG. 9 provides the amino acid sequences for the hu5c8 and JB5 VL region (SEQ ID NO: 1), hu5c8 and JB5 VH region (SEQ ID NO: 2), the Fc region (SEQ ID NO: 3), the JB5 Fc region (SEQ ID NO: 4), the JB5-R28K VL region (SEQ ID NO: 5), the JB5-K74R VH region (SEQ ID NO: 6), and the JB5 light chain Amino acid sequence (SEQ ID NO: 7).

[0026] FIG. 10 provides the light chain synthetic nucleotide sequence that encodes the anti-CD40L antibody JB5 (SEQ ID NO: 8), upper case letters represent the exons and the lower case letters represent the intron sequences of the synthetic gene, and also provides the heavy chain amino acid sequence of the anti-CD40L antibody JB5 (SEQ ID NO: 9).

[0027] FIG. 11 provides a synthetic nucleic acid sequence that encodes the heavy chain of the anti-CD40L antibody JB5 (SEQ ID NO: 10), upper case letters represent the exons, and the lower-case letters represent the intron sequences of the synthetic gene.

[0028] FIG. 12 provides the amino acid sequence of the anti-CD40L antibody JB5-R28K (SEQ ID NO: 11), a synthetic nucleic acid sequence that encodes the light chain of the anti-CD40L antibody JB5-R28K (SEQ ID NO: 12), upper case letters represent the exons and the lower case letters represent the intron sequences of the synthetic gene, and also provides the heavy chain amino acid sequence of the anti-CD40L antibody JB5-K74R (SEQ ID NO: 13).

[0029] FIG. 13 provides a synthetic nucleic acid sequence that encodes the heavy chain of the anti-CD40L antibody JB5-K74R (SEQ ID NO: 14) upper case letters represent the exons, and the lower-case letters represent the intron sequences of the synthetic gene.

[0030] FIG. 14 provides the amino acid sequences of the CDRs of the heavy and light chain of the anti-CD40L antibody JB5 (SEQ ID NOs: 15-20, respectively) and the amino acid sequence of the hu5C8 heavy chain (SEQ ID NO: 21).

[0031] FIG. 15A is a bar graph showing the ranked potency of 16 antibody clones versus the anti-CD40L antibody 5c8. Ranked potency is $IC_{50} \text{ clone} / IC_{50} \text{ of } 5c8 \times 100$.

[0032] FIG. 15B is a bar graph showing the ranked potency of 16 antibody clones versus the anti-CD40L antibody AT-1501. Ranked potency is $IC_{50} \text{ clone} / IC_{50} \text{ of } AT-1501 \times 100$.

[0033] FIGs. 16A-16G and 16I-16Q show the binding curves of the antibodies from each of the clones with the binding curve for 5c8. FIG. 16H shows the binding curves of AT-1501 and 5c8.

[0034] FIGs. 17A-17G and 17I-17Q show the binding curves of the antibodies from each of the clones with the binding curve for AT-1501. FIG. 17H shows the binding curves of AT-1501 and 5c8.

[0035] FIGs. 18A and 18B are graphs, each of which shows binding of eight of the antibody clones, 5c8, AT-1501 and abatacept to FcγRI. The only antibody having significant binding is the 5c8 antibody.

[0036] FIGs. 19A and 19B are graphs, each of which shows binding of eight of the antibody clones, 5c8, AT-1501 and abatacept to FcγRIIa. The only antibody having significant binding is the 5c8 antibody.

[0037] FIGs. 20A and 20B are graphs, each of which shows binding of eight of the antibody clones, 5c8, AT-1501 and abatacept to FcγRIIIa. None of the antibodies showed significant binding to FcγRIIIa.

[0038] FIGs. 20C and 20D are graphs, each of which shows binding of eight of the antibody clones, 5c8, AT-1501 and abatacept to FcγRIIIb. None of the antibodies showed significant binding to FcγRIIIb.

[0039] FIGs. 21A and 21B are graphs, each showing the binding of eight of the antibody clones, 5c8, AT-1501 and abatacept to C1q. The only antibody having significant binding to C1q was the 5c8 antibody.

[0040] FIG. 22 shows a schematic illustration of the timepoints in which AT-1501 was administered to human subjects as part of the clinical trial outlined in Example 1. The subjects had blood (biosamples) collected for a “prescreening” baseline assessment at days -28 to -4, then dosing was administered to the subjects once every two weeks for a total of 101 days. On each dosing day, blood was collected for prescreening prior to the drug administration, and again collected 2 hours post-transfusion.

[0041] FIG. 23 shows the concentration of AT-1501 in subjects dosed with either 1 mg/kg, 2 mg/kg, 4 mg/kg, or 8 mg/kg, over time. The X axis is days since initial dosing, and the Y axis is the concentration of AT-1501 present in plasma (in µg/mL).

[0042] FIGs. 24A-24I show the nine ADA-positive samples, the concentration of AT-1501 in blood, and the times in which ADA was detected (circles). The figures show the positive detection of ADA titer in the plasma of patients 103202 (FIG. 24A), 104202

(FIG. 24B), 105202 (FIG. 24C), 105205 (FIG. 24D), 105206 (FIG. 24E), 108202 (FIG. 24F), 108203 (FIG. 24G), 108204 (FIG. 24H), and 108205 (FIG. 24I).

[0043] FIG. 25 shows a heatmap for the changes in proteins of subjects after exposure to 1, 2, 4, or 8 mg/kg of AT-1501.

[0044] FIGs. 26A-26B show the percent change of the biomarker CXCL13 in human subjects after exposure to 1, 2, 4, or 8 mg/kg of AT-1501 at 4, 6, 8, 10, and 12 weeks compared to baseline. FIG. 26B is a close-up view of the data at week 12.

[0045] FIGs. 27A-27B show the percent change of the biomarker CD40L in human subjects after exposure to 1, 2, 4, or 8 mg/kg of AT-1501 at 4, 6, 8, 10, and 12 weeks compared to baseline. FIG. 27B is a close-up view of the data at week 12. An “*” denotes a p-value between 0.05 and 0.01; an “**” denotes a p-value between 0.01 and 0.001; an “***” denotes a p-value that is less than 0.001.

[0046] FIGs. 28A-28B show the percent change of biomarker CD40 in human subjects after exposure to 1, 2, 4, or 8 mg/kg of AT-1501 at 4, 6, 8, 10, and 12 weeks compared to baseline. FIG. 28B is a close-up view of the data at week 12. An “*” denotes a p-value between 0.05 and 0.01; an “**” denotes a p-value between 0.01 and 0.001; an “***” denotes a p-value that is less than 0.001.

[0047] FIGs. 29A-29L show the percent change in protein concentration in the plasma of human subjects, following 12 weeks of treatment with a compound targeting CD40L/CD40. Each graph shows the average change of proteins in patients dosed with 1, 2, 4, or 8 mg/kg of AT-1501. The respective graphs each show the highest reduction of total protein concentration out of the proteins assessed, which were for CD40L (FIG. 29A), CXCL13 (FIG. 29B), CXCL9 (FIG. 29C), TNF-alpha (FIG. 29D), En-Raged (FIG. 29E), TNFR2 (FIG. 29F), IgM (FIG. 29G), IgA (FIG. 29H), IL2r (FIG. 29I), CXCL10 (FIG. 29J), CD40 (FIG. 29K), and B2M (FIG. 29L), respectively. An “*” denotes a p-value between 0.05 and 0.01; an “**” denotes a p-value between 0.01 and 0.001; an “***” denotes a p-value that is less than 0.001.

[0048] FIGs. 30A-30L show the percent change in protein concentration in the plasma of human subjects, following 12 weeks of treatment with a compound targeting CD40L/CD40. Each graph shows the average change of proteins in patients dosed with 1, 2, 4, or 8 mg/kg of AT-1501. The respective graphs each show a reduction of total protein concentration for VCAM (FIG. 30A), IL-18 (FIG. 30B), IL-16 (FIG. 30C), SAP (FIG. 30D), MIP1-beta (FIG. 30E), MDC (FIG. 30F), C3 (FIG. 30G), CRP (FIG. 30H), Fibrinogen (FIG. 30I), IgE (FIG. 30J), ICAM (FIG. 30K), and MCP1 (FIG. 30L),

respectively. An “*” denotes a p-value between 0.05 and 0.01; an “**” denotes a p-value between 0.01 and 0.001; an “***” denotes a p-value that is less than 0.001.

[0049] FIGs. 31A-31E show the percent change in protein concentration in the plasma of human subjects, following 12 weeks of treatment with a compound targeting CD40L/CD40. Each graph shows the average change of proteins in patients dosed with 1, 2, 4, or 8 mg/kg of AT-1501. The respective graphs each show an increase of total protein concentration for IL-6 (FIG. 31A), ENA-78 (FIG. 31B), RANTES (FIG. 31C), MMP3 (FIG. 31D), and MMP9 (FIG. 31E), respectively. An “*” denotes a p-value between 0.05 and 0.01; an “**” denotes a p-value between 0.01 and 0.001; an “***” denotes a p-value that is less than 0.001.

[0050] FIG. 32 shows the percent change in concentration of NFL in the plasma of human subjects, following 12 weeks of treatment with AT-1501 at either 1, 2, 4, or 8 mg/kg dosages.

[0051] FIGs. 33A-33D show the total change in ALS-FRS score for human subject cohorts over 11 weeks. The ALS-FRS scores were assessed by clinicians. The data is separated by those receiving 1 mg/kg treatment of AT-1501, 2 mg/kg treatment of AT-1501, 4 mg/kg treatment of AT-1501, 8 mg/kg of AT-1501, or no treatment “Proact.” FIG. 33A is the combined scores of all patients involved in the clinical trial. FIG. 33B removed the scores of patients who no longer met the enrollment criteria. FIG. 33C removed the scores of patients who reported treatment-related adverse effects. FIG. 33D removed all patient scores, except those who responded best to treatment.

[0052] FIG. 34 shows the change in ALS-FRS score for human subjects over 7 weeks. In this case, the subjects are not combined, and the data is grouped by Responders (grey) and non-Responders (dark grey) to treatment with AT-1501.

[0053] FIG. 35 shows a nested dot plot for the concentration of proteins (in log2) of subjects before treatment. The values are separated by the type of protein (X-axis), and for non-Responders (left, light grey) and Responders (right, dark grey) to later treatment with AT-1501. The horizontal lines in each cluster signifies the mean value for that cluster.

DETAILED DESCRIPTION

[0054] In the following detailed description, reference is made to the accompanying drawings, which form a part hereof. In the drawings, similar symbols typically identify similar components, unless context dictates otherwise. The illustrative

embodiments described in the detailed description, drawings, and claims are not meant to be limiting. Other embodiments may be utilized, and other changes may be made, without departing from the spirit or scope of the subject matter presented herein. It will be readily understood that the aspects of the present disclosure, as generally described herein, and illustrated in the Figures, can be arranged, substituted, combined, separated, and designed in a wide variety of different configurations, all of which are explicitly contemplated herein. All references cited herein are expressly incorporated by reference herein in their entirety and for the specific disclosure referenced herein.

[0055] The present disclosure describes methods of treating a patient with a neurodegenerative or neuromuscular disorder by administering a therapeutically effective amount of a compound that blocks the interaction of CD40 and CD40L. The disclosure also describes methods of treating patients by co-administering a compound that blocks the interaction of CD40 and CD40L with a compound that blocks the interactions blocks the interaction between CD28 and CD86 or between CD28 and CD80. Some embodiments relate to methods of predicting and/or measuring response to a treatment, including measuring levels of one or more biomarker. Embodiments also relate to methods of modulating a level of one or more biomarker.

Abbreviations and definitions

[0056] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of ordinary skill in the art. All patents, applications, published applications and other publications referenced herein are incorporated by reference in their entirety unless stated otherwise. In the event that there is a plurality of definitions for a term herein, those in this section prevail unless stated otherwise.

[0057] One of ordinary skill in the art will appreciate that starting materials, biological and chemical materials, biological and chemical reagents, synthetic methods, purification methods, analytical methods, assay methods, and biological methods other than those specifically exemplified can be employed in the practice of embodiments without resort to undue experimentation. All art-known functional equivalents, of any such materials and methods are intended to be included in this disclosure.

[0058] As used herein, “a” or “an” can mean one or more than one.

[0059] As used herein, the term “about” or “approximately” has its usual meaning as understood by those skilled in the art and thus indicates that a value includes

the inherent variation of error for the method being employed to determine a value, or the variation that exists among multiple determinations.

[0060] The use of the conjunction “or” is used interchangeably with at “least one of”. For example: where a composition comprises A or B, the method must comprise at least one of A and B but may also comprise both A and B. Likewise a composition comprising “A, B, C or D” must comprise at least one of the group of A, B, C and D, but may also comprise all or any combination of A, B, C and D.

[0061] Amino acid substitutions are denoted by the convention in which the original amino acid, the position of the amino acid in the specified sequence and the replacement amino acid are identified, for example, C11S would indicate that the cysteine at position 11 of the polypeptide sequence is replaced with a serine.

[0062] Humanized antibodies are antibodies produced from non-human species whose protein sequences have been modified to increase their similarity to antibody variants produced naturally in humans. The process of “humanization” is usually applied to monoclonal antibodies developed for administration to humans (for example, antibodies developed as anti-cancer drugs).

[0063] Currently it is common to humanize a non-human antibody by insertion of relevant CDRs from antibodies created in a non-human animal into a human antibody “scaffold”. “Direct” creation of a humanized antibody can be accomplished by inserting the appropriate CDR coding segments (responsible for the desired binding properties) into a human antibody “scaffold.” This may be achieved through recombinant DNA methods using an appropriate vector and expression in mammalian cells. That is, after an antibody is developed to have the desired properties in a mouse (or other non-human), the DNA coding for that antibody can be isolated, cloned into a vector and sequenced. The DNA sequence corresponding to the antibody CDRs can then be determined. Once the precise sequence of the desired CDRs is known, a strategy can be devised for inserting these sequences appropriately into a construct containing the DNA for a human antibody variant. The CDRs may also be varied, e.g., to increase specificity, prior to insertion into the scaffold.

[0064] The term “human” antibody refers to an antibody which possesses an amino acid sequence which corresponds to that of an antibody produced by a human and/or has been made using any technique for making human antibodies. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen binding residues.

[0065] The term “variant” as used herein is defined as a modified or altered form of a wildtype sequence, e.g., where one or more amino acids may be replaced by other amino acid(s) or non-amino acid(s) which do not substantially affect function. In some embodiments, the variant may contain an altered side chain for at least one amino acid residue.

[0066] The term “antigen” as used herein is defined as an entity that can stimulate the production of antibodies and specifically combine with them and/or an entity which elicits an immune system response. For example, a cell surface protein or a specific linear or non-linear portion thereof. The term herein may be abbreviated to “Ag.”

[0067] An “antigen binding antibody fragment” refers to a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen to which the intact antibody binds. Examples of antibody fragments include but are not limited to Fv, Fab, Fab', Fd fragments, dAb fragments, Fab'-SH, F(ab')₂; diabodies; triabodies; linear antibodies; single-chain antibody molecules (e.g., scFv); and multispecific antibodies formed from antibody fragments, minimal recognition units consisting of the amino acid residues that mimic the hypervariable region of an antibody (e.g., an isolated complementarity determining region (CDR) such as a CDR3 peptide). An antigen binding fragment as disclosed in the present application binds to the antigen CD40L.

[0068] An antigen-binding fragment of an antibody will typically comprise at least one variable domain. The variable domain may be of any size or amino acid composition and will generally comprise at least one CDR which is adjacent to or in frame with one or more framework sequences. In antigen-binding fragments having a VH domain associated with a VL domain, the VH and VL domains may be situated relative to one another in any suitable arrangement. For example, the variable region may be dimeric and contain VH-VH, VH-VL or VL-VL dimers. Alternatively, the antigen-binding fragment of an antibody may contain a monomeric VH or VL domain.

[0069] The isolated antibody includes bispecific antibodies in which each arm of the antibody or the antigen binding fragment binds to a different target or epitope.

[0070] The terms “full length antibody,” “intact antibody,” and “whole antibody” are used herein interchangeably to refer to an antibody having a structure substantially similar to a native antibody structure or having heavy chains that contain an Fc region.

[0071] “5c8” refers to the mouse anti-human antibody that binds CD40L and is produced by the hybridoma that is available from the ATCC having the accession number

HB10916 and is described in U.S. Pat. No. 5,474,771. “hu5c8” refers to a humanized version of 5c8 the sequence of which is disclosed in Karpusas, et al., Structure vol. 9, pp 321-329, (2001).

[0072] The term “specifically binds,” or the like, means that an antibody or antigen-binding fragment thereof forms a complex with an antigen that is relatively stable under physiological conditions. Specific binding may be characterized by an equilibrium dissociation constant (KD) of about 3000 nM or less (i.e., a smaller KD denotes a tighter binding), about 2000 nM or less, about 1000 nM or less; about 500 nM or less; about 300 nM or less; about 200 nM or less; about 100 nM or less; about 50 nM or less; about 1 nM or less; or about 0.5 nM.

[0073] Specific binding for a particular antigen or an epitope may be exhibited, for example, by an antibody having a KD for an antigen or epitope of at least about 1×10^{-4} M, at least about 1×10^{-5} M, at least about 1×10^{-6} M, at least about 1×10^{-7} M, at least about 1×10^{-8} M, at least about 1×10^{-9} M, alternatively at least about 1×10^{-10} M, at least about 1×10^{-11} M, at least about 1×10^{-12} M, or greater, where KD refers to an equilibrium dissociation constant of a particular antibody-antigen interaction. Typically, an antibody that specifically binds an antigen will have a KD that is 20-, 50-, 100-, 500-, 1000-, 5,000-, 10,000- or more times greater for a control molecule relative to the antigen or epitope. Also, specific binding for a particular antigen or an epitope may be exhibited, for example, by an antibody having a Ka for an antigen or epitope of at least 20-, 50-, 100-, 500-, 1000-, 5,000-, 10,000- or more times greater for the epitope relative to a control, where Ka refers to an association rate of a particular antibody-antigen interaction.

[0074] The term “neutralizing antibody” includes an antibody that is capable of inhibiting and/or neutralizing the biological activity of CD40L, for example an anti-CD40L antibody or antigen fragment thereof that inhibits or prevents or diminishes the binding of CD40L to CD40, and thus inhibiting or reducing the signaling pathway triggered by CD40L and/or inhibiting or reducing the binding of CD40L to CD40.

[0075] The terms “antagonistic antibody” or “antagonist antibody” are used herein equivalently and include an antibody that is capable of inhibiting and/or neutralizing the biological signaling activity of CD40L, as described for a neutralizing antibody *supra*.

[0076] The terms and expressions which have been employed herein are used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the

scope of the embodiments claimed. Thus, it should be understood that although aspects of the present disclosure have been specifically disclosed by various embodiments which may include preferred embodiments, exemplary embodiments and optional features, modifications and variations of the concepts herein disclosed may be resorted to by those skilled in the art. Such modifications and variations are considered to be within the scope of embodiments of the disclosure as described and as may be defined by the appended claims.

[0077] Reference in the specification is made to percent identity between polypeptide or amino acid sequences. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences. Identity can be measured as “local identity” or “global identity.” Local identity refers the degree of sequence relatedness between polypeptides as determined by the match between strings of such sequences. Global identity refers to the degree of sequence relatedness of a polypeptide compared to the full-length of a reference polypeptide. Unless specified otherwise, as used herein, identity means global identity. For the purposes of this disclosure, the percentages for global identity are calculated using Needleman and Wunsch ((1970) J. Mol. Biol. 48:444-453) algorithm using a Blossum 62 scoring matrix with a gap penalty of 12, a gap extend penalty of 4, and a frameshift gap penalty of 5. There are many publicly available software programs that incorporate the Needleman and Wunsch algorithm, e.g., the GAP program in the GCG software package.

[0078] CD40L is also known as CD154, gp39, T-BAM, 5c8 antigen, or TNF related activation protein (TRAP).

[0079] Throughout this specification, unless the context requires otherwise, the words “comprise,” “comprises,” and “comprising” will be understood to imply the inclusion of a stated step or element or group of steps or elements but not the exclusion of any other step or element or group of steps or elements. By “consisting of” is meant including, and limited to, whatever follows the phrase “consisting of.” Thus, the phrase “consisting of” indicates that the listed elements are required or mandatory, and that no other elements may be present. By “consisting essentially of” is meant including any elements listed after the phrase and limited to other elements that do not interfere with or contribute to the activity or action specified in the disclosure for the listed elements. Thus, the phrase “consisting essentially of” indicates that the listed elements are required or

mandatory, but that other elements are optional and may or may not be present depending upon whether or not they materially affect the activity or action of the listed elements.

[0080] The terms “function” and “functional” as used herein have their plain and ordinary meaning as understood in light of the specification, and refer to a biological, enzymatic, or therapeutic function.

[0081] The term “yield” of any given substance, compound, or material as used herein has its plain and ordinary meaning as understood in light of the specification and refers to the actual overall amount of the substance, compound, or material relative to the expected overall amount. For example, the yield of the substance, compound, or material is, is about, is at least, is at least about, is not more than, or is not more than about, 80, 81, 82, 83, 84, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% of the expected overall amount, including all decimals in between. Yield may be affected by the efficiency of a reaction or process, unwanted side reactions, degradation, quality of the input substances, compounds, or materials, or loss of the desired substance, compound, or material during any step of the production.

[0082] As used herein, the term “isolated” has its plain and ordinary meaning as understood in light of the specification, and refers to a substance and/or entity that has been (1) separated from at least some of the components with which it was associated when initially produced (whether in nature and/or in an experimental setting), and/or (2) produced, prepared, and/or manufactured by the hand of man. Isolated substances and/or entities may be separated from equal to, about, at least, at least about, not more than, or not more than about, 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, about 98%, about 99%, substantially 100%, or 100% of the other components with which they were initially associated (or ranges including and/or spanning the aforementioned values). In some embodiments, isolated agents are, are about, are at least, are at least about, are not more than, or are not more than about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, substantially 100%, or 100% pure (or ranges including and/or spanning the aforementioned values). As used herein, a substance that is “isolated” may be “pure” (e.g., substantially free of other components). As used herein, the term “isolated cell” may refer to a cell not contained in a multi-cellular organism or tissue.

[0083] The term “isolated protein” or “isolated polypeptide” (e.g., an isolated antibody or isolated antigen binding fragment) is a protein or polypeptide that by virtue of

its origin or source of derivation is not associated with naturally associated components that accompany it in its native state; is substantially free of other proteins from the same species; is expressed by a cell from a different species; or does not occur in nature. Thus, a polypeptide that is chemically synthesized or synthesized in a cellular system different from the cell from which it naturally originates will be “isolated” from its naturally associated components. A protein may also be rendered substantially free of naturally associated components by isolation, using protein purification techniques well known in the art.

[0084] As used herein, “in vivo” is given its plain and ordinary meaning as understood in light of the specification and refers to the performance of a method inside living organisms, usually animals, mammals, including humans, and plants, or living cells which make up these living organisms, as opposed to a tissue extract or dead organism.

[0085] As used herein, “ex vivo” is given its plain and ordinary meaning as understood in light of the specification and refers to the performance of a method outside a living organism with little alteration of natural conditions.

[0086] As used herein, “in vitro” is given its plain and ordinary meaning as understood in light of the specification and refers to the performance of a method outside of biological conditions, e.g., in a petri dish or test tube.

[0087] As used herein, “nucleic acid”, “nucleic acid molecule”, or “nucleotide” refers to polynucleotides or oligonucleotides such as deoxyribonucleic acid (DNA) or ribonucleic acid (RNA), oligonucleotides, fragments generated by the polymerase chain reaction (PCR), and fragments generated by any of ligation, scission, endonuclease action, exonuclease action, and by synthetic generation. Nucleic acid molecules can be composed of monomers that are naturally occurring nucleotides (such as DNA and RNA), or analogs of naturally occurring nucleotides (e.g., enantiomeric forms of naturally occurring nucleotides), or a combination of both. Modified nucleotides can have alterations in sugar moieties and/or in pyrimidine or purine base moieties. Sugar modifications include, for example, replacement of one or more hydroxyl groups with halogens, alkyl groups, amines, and azido groups, or sugars can be functionalized as ethers or esters. Moreover, the entire sugar moiety can be replaced with sterically and electronically similar structures, such as aza-sugars and carbocyclic sugar analogs. Examples of modifications in a base moiety include alkylated purines and pyrimidines, acylated purines or pyrimidines, or other well-known heterocyclic substitutes. Nucleic acid monomers can be linked by phosphodiester bonds or analogs of such linkages. Analogs of phosphodiester linkages include phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphorodiselenoate,

phosphoroanilothioate, phosphoranilidate, phosphoramidate, and the like. The term “nucleic acid molecule” also includes so-called “peptide nucleic acids,” which comprise naturally occurring or modified nucleic acid bases attached to a polyamide backbone. Nucleic acids can be either single stranded or double stranded.

[0088] The terms “peptide”, “polypeptide”, and “protein” as used herein have their plain and ordinary meaning as understood in light of the specification and refer to macromolecules comprised of amino acids linked by peptide bonds. The numerous functions of peptides, polypeptides, and proteins are known in the art, and include but are not limited to enzymes, structure, transport, defense, hormones, or signaling. Peptides, polypeptides, and proteins are often, but not always, produced biologically by a ribosomal complex using a nucleic acid template, although chemical syntheses are also available. By manipulating the nucleic acid template, peptide, polypeptide, and protein mutations such as substitutions, deletions, truncations, additions, duplications, or fusions of more than one peptide, polypeptide, or protein can be performed. These fusions of more than one peptide, polypeptide, or protein can be joined in the same molecule adjacently, or with extra amino acids in between, e.g. linkers, repeats, epitopes, or tags, or any other sequence that is, is about, is at least, is at least about, is not more than, or is not more than about, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, 200, or 300 bases long, or any length in a range defined by any two of the aforementioned lengths. The term “downstream” on a polypeptide as used herein has its plain and ordinary meaning as understood in light of the specification and refers to a sequence being after the C-terminus of a previous sequence. The term “upstream” on a polypeptide as used herein has its plain and ordinary meaning as understood in light of the specification and refers to a sequence being before the N-terminus of a subsequent sequence.

[0089] The term “gene” as used herein have their plain and ordinary meaning as understood in light of the specification, and generally refers to a portion of a nucleic acid that encodes a protein or functional RNA; however, the term may optionally encompass regulatory sequences. It will be appreciated by those of ordinary skill in the art that the term “gene” may include gene regulatory sequences (e.g., promoters, enhancers, etc.) and/or intron sequences. It will further be appreciated that definitions of gene include references to nucleic acids that do not encode proteins but rather encode functional RNA molecules such as tRNAs and miRNAs. In some cases, the gene includes regulatory sequences involved in transcription, or message production or composition. In other embodiments,

the gene comprises transcribed sequences that encode for a protein, polypeptide, or peptide. In keeping with the terminology described herein, an “isolated gene” may comprise transcribed nucleic acid(s), regulatory sequences, coding sequences, or the like, isolated substantially away from other such sequences, such as other naturally occurring genes, regulatory sequences, polypeptide, or peptide encoding sequences, etc. In this respect, the term “gene” is used for simplicity to refer to a nucleic acid comprising a nucleotide sequence that is transcribed, and the complement thereof. As will be understood by those in the art, this functional term “gene” includes both genomic sequences, RNA or cDNA sequences, or smaller engineered nucleic acid segments, including nucleic acid segments of a non-transcribed part of a gene, including but not limited to the non-transcribed promoter or enhancer regions of a gene. Smaller engineered gene nucleic acid segments may express or may be adapted to express using nucleic acid manipulation technology, proteins, polypeptides, domains, peptides, fusion proteins, mutants and/or such like.

[0090] The terms “individual”, “subject”, “host,” or “patient” as used herein have their usual meaning as understood by those skilled in the art and thus includes a human or a non-human mammal. The term “mammal” is used in its usual biological sense. Thus, it specifically includes, but is not limited to, primates, including simians (chimpanzees, apes, monkeys), humans, cattle, horses, sheep, goats, swine, rabbits, dogs, cats, rodents, rats, mice, or guinea pigs.

[0091] The dimensions and values disclosed herein are not to be understood as being strictly limited to the exact numerical values recited. Instead, unless otherwise specified, each such dimension is intended to mean both the recited value and a functionally equivalent range surrounding that value. For example, a dimension disclosed as “20 mm” is intended to mean “about 20 mm”.

[0092] The following abbreviations are used herein: amyotrophic lateral sclerosis (ALS); super oxide dismutases-1 (*SOD1*); T cell receptor (TCR); major histocompatibility complex (MHC) antigen presenting cell (APC); Phosphate buffered saline (PBS), complementarity determining regions (CDR). “IP” means intraperitoneally and “IV” means intravenously.

[0093] MR1 is a hamster monoclonal antibody that binds to mouse CD40 ligand. “Wild type” as used herein means a non-transgenic mouse. As used herein “small molecule” means a compound having a molecular weight of less than 2000 Daltons. “Treatment” or “treating” as used herein includes prophylactic and therapeutic treatment. The phrase “therapeutically effective amount” means an amount of a compound, or a

pharmaceutically acceptable salt thereof, sufficient to inhibit, or allow an improvement in the disorder or condition being treated with administered alone or in conjunction with another pharmaceutical agent or treatment in a particular subject or subject population.

[0094] An “effective amount” of an agent, e.g., a pharmaceutical formulation, refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result.

[0095] “Affinity” refers to the strength of the sum total of noncovalent interactions between a single binding site of a molecule (e.g., an antibody) and its binding partner (e.g., an antigen). Unless indicated otherwise, as used herein, “binding affinity” refers to intrinsic binding affinity which reflects a 1:1 interaction between members of a binding pair (e.g., antibody and antigen). The affinity of a molecule X for its partner Y may generally be represented by the equilibrium dissociation constant (KD), a ratio of k_{off}/k_{on} , between the antibody and its antigen. KD and affinity are inversely related. The KD value relates to the concentration of antibody (the amount of antibody needed for a particular experiment) and so the lower the KD value (lower concentration) and thus the higher the affinity of the antibody. Affinity may be measured by common methods known in the art, including those described herein. Specific, illustrative, and exemplary embodiments for measuring binding affinity may be measured by radioimmunoassays (RIA), Surface Plasmon Resonance (SPR) on a BIAcore® instrument (GE Healthcare Europe GmbH, Glattbrugg, Switzerland) by capturing the antibody on a protein-A coupled CM5 research grade sensor chip (GE Healthcare Europe GmbH, Glattbrugg, Switzerland; BR-1000-14) with a human CD40 ligand polypeptide used as analyte. Other methods may include radioimmunoassays, and the Kinetic Exclusion Assay. The Kinetic Exclusion Assay is a general-purpose immunoassay platform that is capable of measuring equilibrium dissociation constants, and association and dissociation rate constants for antigen/anti-body interactions.

[0096] “ALS-TDI” is an abbreviation for ALS Therapy Development Institute. “hSOD1G93A preclinical mouse model”, “hSOD1G93A mouse model”, “G93A preclinical mouse model” and “G93A mouse model” have the same meaning as used herein. “hSOD1G93A mouse” and “G93A mouse” have the same meaning as used herein.

[0097] TMB is an abbreviation of 3,3',5,5'-Tetramethylbenzidine.

[0098] “CDR domain” as used herein means an antibody complementary determining region with or without flanking sequences.

[0099] “Effector functions” refer to those biological activities attributable to the Fc region of an antibody, which vary with the antibody isotype. Examples of antibody effector functions include: C1q binding and complement dependent cytotoxicity (CDC); Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (e.g., B cell receptor); and B cell activation. One effector function is ability of the Fc or constant region of an antibody to bind proteins including, but not limited to, an Fc receptor (FcR) (e.g., high affinity Fc region of IgG receptor Ia (FCγRIa) (CD64) (SEQ ID NO: 34), low affinity immunoglobulin gamma Fc region acceptor IIa (FCγRIIa) (CD32) (SEQ ID NO: 35), low affinity immunoglobulin gamma Fc region receptor IIIa (FCγRIIIa) (CD16a) (SEQ ID NO: 36), low affinity immunoglobulin gamma Fc region receptor IIIb (FCγRIIIb) (CD16b)) (SEQ ID NO: 37). In some embodiments, where the antibodies and antigen binding fragments thereof, have an Fc domain, the Fc domain has been engineered to reduce or eliminate one or more Fc effector function. In a preferred embodiment the Fc domain has been engineered to reduce or eliminate platelet activation and/or platelet aggregation and the concomitant risk of thromboembolism.

In vivo evaluation of compounds

[0100] Compounds used in the methods of the present disclosure may be evaluated for their efficacy using the G93A mouse model , [see Tu PH et al. Proc Natl Acad Sci USA 1996; 93:3155-60 and Gurney ME. et al. J Neurol Sci 1997;152(Suppl 1):S67-73]. This model was constructed by inserting 23 copies of the human *SOD1* gene, which contains a glycine to alanine mutation at position 93, into the mouse genome. These mice accurately recapitulate most major points of pathology of the human sporadic and familial forms of the disease making this model the best currently available option for testing disease altering interventions.

[0101] There is no discernable phenotypic expression of the abnormality in the G93A mouse at birth. Visible disease signs do not express until about 90 days of age, whereupon it experiences a progressive loss of hindlimb function resulting in complete paralysis and death around 134 days of age. While muscle wasting is caused by the death or dysfunction of motor neurons, the death of these cells is associated with and partially caused by interactions with surrounding cells including microglia and astrocytes. A pronounced astrocytosis first appears around 80 days of age while a neuroinflammation, mediated mainly by microglia, appears around 100 days of age and expands until death.

[0102] While the human disease may begin in any motor region, the mouse disease reliably affects the cervical and lumbar regions first. In the G93A mouse, motor neuron numbers have declined significantly by the time of visible symptom onset, at approximately day 85, and reach upwards of 50% loss at death. Abnormalities in the cytoskeleton, neurofilaments, axonal transport, Golgi, endoplasmic reticulum, mitochondria, apoptotic machinery, proteasome, and cytosolic protein handling are observed in neurons during the course of the disease.

[0103] While the human disease may begin in any motor region, the mouse disease reliably affects the lumbar and sacral regions first. In the G93A mouse, motor neuron numbers have declined significantly by the time of visible symptom onset, at approximately day 85, and reach upwards of 50% loss at death. Abnormalities in the cytoskeleton, neurofilaments, axonal transport, Golgi, endoplasmic reticulum, mitochondria, apoptotic machinery, proteasome, and cytosolic protein handling are observed in neurons during the course of the disease.

[0104] To date, there have been at least 50 publications describing therapeutic agents that extend the lifespan of this mouse. However, no therapeutic agent other than riluzole has shown corresponding clinical efficacy. The ALS Therapy Development Institute has described optimized therapeutic drug screening in the G93A mouse model which controls for noise variables [Scott S. et al. *Amyotrophic Lateral Sclerosis* 2008; 9: 4-15 which is hereby incorporated by reference]. Scott et al. describe a minimum study design for the G93A mouse model which addresses and manages the noise caused by the inherent confounding biological variables. In validating this study design, nine compounds that had previously been reported to be efficacious on in the preclinical model and subsequently failed in human clinical trials, were evaluated, most at varying doses. Several of these molecules are anti-inflammatory molecules that inhibit tumor necrosis factor signaling (TNF) and microglial activation including Celebrex[®], minocycline, thalidomide, and creatine. Celebrex[®] was reported to improve lifespan in the G93A model by 19% (24 days) yet a high-powered study failed to detect any change in survival (1.8 days, 0.52%), (Scott et al.). Similar results were obtained by for minocycline (previously reported; 15.8% improvement; high powered study, -0.60%), creatine (previously reported 17.8%; high powered study 0.67%), and thalidomide (previously reported 16% improvement in survival; high powered study -1.9%).

Identification of candidate compounds

[0105] Genome wide expression profiling analysis was performed for wildtype mice and G93A mice at various time points during disease progression. Genes identified as differentially expressed between the two groups were analyzed and the resulting data were used to focus the selection of drugs to be screened. Among the genes that were differentially expressed include genes involved in the immune response and cell adhesion including CD86, CD44, ICAM, ITGAM, ITGA ITGAX, ITGB2, H2-K1 (MHC II), H2-AB1(MHC II), H2-D1 (MHCII), and H2-Eb1 (MHC II). These data show that inflammatory signatures increase during disease progression and are consistent with the involvement of co-stimulatory pathway. The co-stimulatory pathway involves interactions between cell types via CD28/CD80 or CD28/CD86 or CD40/CD40L interactions some of which were identified in the gene expression analysis.

[0106] In order to identify molecular pathways amenable to therapeutic development, changes in gene expression patterns were characterized during disease progression in the G93A mouse model. Whole genome transcriptional profiling was studied using Affymetrix GeneChip® Mouse Expression set 430vII MOE430vII gene chips. A longitudinal study design was employed comparing G93A skeletal muscle and spinal cord to non-transgenic littermates. In the G93A mouse model symptom onset is first seen as tail paralysis starting at approximately day 75 with progressive paralysis in the hindlimbs, then forelimbs, and finally diaphragm. The mean survival of the G93A animal colony is 134 days. The longitudinal study design collected calf muscle (gastrocnemius muscle) and spinal cord from G93A animals and wild type litter mates at days 30, 50, 60, 80, 90, 100, 110, and 120 (day 0 is date of birth). For each time point, tissues were collected from 5 wild type and 5 G93A animals and processed independently for a total of 160 tissues.

[0107] Animals were euthanized according to IACUC protocols at the appropriate time points described above. Tissues were immediately harvested and snap frozen in liquid nitrogen. Frozen tissues were stored at -80 degrees Celsius. Total RNA was isolated simultaneously from the tissues using the Qiagen RNeasy kit as described by the manufacturer. The isolated total RNA was amplified using standard T7 linear amplification incorporating a biotinylated nucleotide using the Ambion Message AMPH T7 in vitro transcription kit. Labeled probe was fragmented and hybridized to Affymetrix GeneChip® Mouse Expression set 430vII according to the manufacturer's protocol. Genechips were washed in Affymetrix GeneChip® Fluids Station 450 to remove non-hybridized probe. Genechips were scanned in an Affymetrix GeneChip® 3000 7G scanner.

[0108] All computational processing and modeling was performed using the R development language version 2.6 from Bioconductor. The spinal cord and gastrocnemius data sets were analyzed independently. Affymetrix CEL files were used for all data preprocessing. All the CEL files in each data set were quality controlled using the Bioconductor vignettes SIMPLE AFFY, AFFY and AFFY PLM. The statistical changes in gene expression between G93A and wild type tissues at a given time point were assessed using the LIMMA package. The bayes model was used to determine the significance of expression changes between groups.

[0109] These data show that inflammatory signatures increase during disease progression. These gene expression changes reflect the activation of antigen presenting cells such as dendritic cells, macrophages, and B cells. Blocking the interaction may ameliorate immune responses that exacerbate disease progression in ALS. The co-stimulatory pathway can be inhibited by blocking CD-28/CD80 or CD-28/CD86 or CD40/CD40L interactions.

[0110] The co-stimulatory pathway involves, along with other interactions, the binding of CD40 on B cells to CD40L (also known as CD154, gp39, T-BAM, 5c8 antigen, CD40CR and TRAP) on T cells. Human CD40 is expressed on mature B cells, as well as macrophages, dendritic cells, fibroblasts and activated endothelial cells. It is thought that blockade of the CD40:CD40L binding promotes the development of Type I T-helper cell responses.

[0111] Using compounds that block these interactions and inhibit the co-stimulatory signals, a significant body of work has demonstrated the immunomodulatory effects of blocking one or more of CD40L, CD80 or CD86 in preclinical models of transplantation and autoimmunity. Blocking CD40L function with blocking antibodies or adenoviral expression of CD40L-Ig improves allograft transplant by 30 to 90 days. Similar studies blocking CD80/CD86 on APCs with CTLA4-Ig or adenoviral expression of CTLA4-Ig transiently improves allograft transplant survival. Transplant rejection in these models is transient and graft rejection ensues over time. Longer term repression of transplant rejection can be accomplished by blocking both the co-stimulatory pathway with CTLA4-Ig and blocking CD40L activation of APCs with anti-CD40L antibodies.

[0112] Blocking antibodies to CD40L or genetic deletion of CD40L in mice has demonstrated that CD40L ameliorates disease progression, survival, and surrogate markers of disease in preclinical models of experimental allergic encephalomyelitis (EAE) a model

of multiple sclerosis, collagen induced arthritis, and systemic lupus. Blockade of CD40:CD40L binding appears to reduce the ability of macrophages to produce nitric oxide, which mediates many of the macrophages' pro-inflammatory activities.

[0113] It appears from such studies that blocking CD40:CD40L interactions and/or blocking of CD28:CD80 or CD28:CD86 interactions can modulate immune responses.

[0114] Immunohistochemical data was shown to correlate well with the gene expression data and these data identify macrophages as an antigen presenting cell infiltrating skeletal muscle during disease progression in the G93A mouse.

[0115] In order to determine the presence and localization of potential antigen presenting cells in gastrocnemius muscle from G93A and wild type animals, immunohistochemistry was performed on gastrocnemius tissues that were harvested from G93A and wildtype mice, at day 110. Immediately after harvesting, the tissues were embedded in OCT. Frozen sections were H&E stained and hybridized with antibodies to myelin (anti-S100b antibody) or antibodies to hematopoietic cell lineages including T cells (anti-CD3 antibody), B cells (CD45R pan B cell antibody), and macrophages (anti-CD11b antibody). At day 110 there was infiltration of CD11b positive macrophages and the macrophages appear to be localized to the axons of nerves innervating the skeletal muscle. The localization of macrophages is not dispersed across the entire muscle suggesting that the inflammation is not due to muscle atrophy or muscle fiber remodeling.

[0116] In order to confirm that identity of the monocyte lineage cells in the skeletal muscle at day 110 and to clarify the relevance of these cells compared to non-transgenic animals, additional immunohistochemistry was performed with a panel of antibodies specific for the macrophage lineage. An anti-S100b antibody was utilized to label the myelin associated axons innervating the skeletal muscle. All of the macrophage specific antibodies (anti-CD11b antibody, anti-CD86 antibody and anti-MAC1 antibody) localized macrophages to the axons of nerves innervating the skeletal muscle of G93A mice with no macrophages present on the axons of wildtype animals. The localization of macrophages was specific for nerves innervating the muscle with no macrophages present on myofibers in the muscle.

[0117] The gene expression data suggest that the genes associated with the co-stimulatory pathway are temporally increasing during disease progression both in spinal cord as well as in skeletal muscle (FIGs. 1A-1B). In order to characterize the timing of macrophage infiltration into skeletal muscle immunohistochemistry was performed on

gastrocnemius sections from days 60, 80, and 100 in G93A mice. There was no evidence of macrophage infiltration and localization to axons at day 60. Macrophages were evident at day 80 and localized to the axons innervating the muscle as described previously for skeletal muscle at day 110. The number of macrophages increased between day 80 and day 100 and accumulation of macrophages was specific to the axons innervating the skeletal muscle.

[0118] In order to quantitate the increase in macrophage infiltration, representative sections from 5 G93A and 5 wild type animals were hybridized with anti-CD86 antibody and the number of macrophages per 10,000 square microns were counted. The number of macrophages in the wild type animals was the same at the 60-, 80- and 110-day time points. As can be seen in FIG. 2 macrophages are accumulating in skeletal muscle temporally between days 80 and 100 and there are very few macrophages present in wild type skeletal muscle.

[0119] In summary, the immunohistochemical data correlates very well with the gene expression data and identify macrophages as the antigen presenting cell infiltrating skeletal muscle during disease progression in the G93A mouse model. An unexpected finding is that the macrophage infiltration appears to be specifically targeted to the axons innervating the skeletal muscle as localized by labeling with antibodies to myelin and macrophages.

Pharmacokinetic analysis of MR1 in G93A tissues

[0120] Tissue levels of MR1 directed against murine CD40L were determined using a matrix matched, non-competitive enzyme linked immunosorbent assay (ELISA) in the sandwich format. Seven-point standard curves were included on each plate. Standards were prepared using purified MR1 spiked into PBS diluent solution. PBS diluent solution was matrix matched with the normal mouse tissue at the equivalent dilution of the unknown samples to correct for any non-specific effects resulting from tissue lysates.

[0121] 84 plasma samples were taken for pharmacokinetic analysis over a period of two weeks after dosing (10 mg/kg, IP) in both female and male G93A mice.

[0122] The elimination half-life was similar in females (23 d) and males (22 d) and similar to the half-life for a typical mouse IgG2-based antibody in the mouse. No signs of anti-hamster antibody response were seen.

[0123] Females show a somewhat smaller volume of distribution for MR1 than males, and thus show higher plasma levels when given the same 10 mg/kg dose. Males

show a faster clearance and higher volume of distribution. Thus, to attain similar plasma levels, males would require a higher dose. FIG. 1A shows MR1 concentration over time using a linear concentration (Y) axis. FIG. 1B shows MR1 concentration over time using a log concentration (Y) axis.

[0124] 36 female G93A mice were litter matched and randomized into two study groups. 18 G93A mice were placed into the MRI-treatment group and the other 18 G93A mice were placed into the control group. Study days are based on days from birth.

[0125] A single injection of 56 µg of MR1 was administered intraperitoneally (IP) on day 50. Subsequent to the bolus injection a weekly maintenance injection of 18 µg of MR1 was administered by IP injection. Doses were prepared in vehicle (phosphate buffered saline (PBS, pH 7.3)), to a total volume 200 µl. Control animals were administered 200 µl PBS. Beginning at day 54, animals were monitored daily throughout the course of the study and daily body weight measurements as well as neurological score were measured.

[0126] Neurological scores for both hind legs were assessed daily for each mouse from 50 days of age. The neurological score employed a scale of 0 to 4. (Scott et al., ALS Journal Jan 2008). Briefly animals assigned a score of 0 had full extension of hind legs away from lateral midline when mouse is suspended by its tail, and mouse can hold this for 2 seconds, suspended 2-3 times. Animals score a 1 when they display collapse or partial collapse of leg extension towards lateral midline (weakness) or trembling of hind legs during tail suspension. Animals score a 2 when the toes curl under at least twice during walking of 12 inches, or any part of foot is dragging along cage bottom/table. Animals score a 3 when they have rigid paralysis or minimal joint movement, or a foot not being used for forward motion. Animals score a 4 when they cannot right itself within 30 seconds from either side. If one hind leg is scored as 2, food pellets are left on bedding. If both hind legs are scored as 2, Nutra-Gel® (Bio-Serve #S4798) is provided as food in addition to food pellets on bedding and a long sipper tube is placed on the water bottle.

[0127] Date and cause of death were recorded for each mouse. For humane reasons, animals are closely monitored and sacrificed as moribund prior to actual death using criteria for severe moribundity. To determine duration of survival reliably and humanely, the moribund state, defined as the inability of mice to right themselves 30 seconds after being placed on a side (a neurological score of 4) was used. The moribund mice were scored as "dead," and were euthanized using carbon dioxide.

[0128] Standard procedure is to remove non-ALS related deaths in both treatment and control groups prior to statistical analysis. In this case all animal deaths in either the control or treatment groups were attributed to ALS. Thus, no animals were censored due to non-ALS related deaths.

[0129] Mutant *SOD1* transgenic animals display normal body weight (BW) characteristics as neonates and gain weight normally compared to non-transgenic animals into adulthood. Depending on the nature of the genetic mutation in the transgene and the number of copies of mutant transgene weight loss becomes apparent in adult animals and continues until death. Analysis of weight loss in treatment and control groups can provide insight into putative treatment effects on disease onset and rate of progression. In order to assess the impact of MR1 treatment on body weight, two summary parameters are examined (1) Changes in BW from initiation of study to the attainment of peak body weight which may reflect an impact on disease onset (2) The changes in BW from peak body weight until death which may reflect an impact on disease progression.

[0130] Comparative MR1-treated and control group time-to-event curves for the time from day 40 to the attainment of peak body weight are shown in FIG. 2. The median time to peak body weight for the control group was 50 days compared to the MR1 treated group which was 51 days. This difference was not significant when analyzed by Kaplan Meier using the log rank and Wilcoxon statistical models, Cox proportional hazard, or parametric statistical tests.

[0131] Comparative MR1-treated and control group time-to-event curves for the time from peak body weight to death are shown in FIG. 3. Time from peak body weight to death was statistically significantly later by 15 days in MRI treated animals. Control animals have a 2.4 to 4.7-fold greater risk of dying sooner after attaining peak body weight than did MR1-treated animals. The median time from peak body weight to death in the control group was 26 days whereas in the MR1 treated group it was 41 days. Significance for each of the analyses in this example was calculated in several ways in order to better evaluate significance. The delay is statistically significant when analyzed using several approaches. (Kaplan Meier, log rank $p=0.0110$ and Wilcoxon, $p=0.0069$; Cox proportional Hazard model $p=0.05151$; parametric statistical model, $p=0.0122$). Based on the body weight data MR1 appears to have less of an impact on the onset of disease in the G93A mouse model but has a dramatic effect in slowing down the rate of body weight loss from peak body weight until death.

[0132] The timing of disease onset was also characterized by analyzing the daily neurological scores of the saline treated and MR1 treated groups. At the start of the study (day 50), all animals have a neurological score of 0 with no observable symptoms or paralysis. Disease onset can be characterized by examining the progression in neurological score from a neurological score of 0 to a neurological score of 2 when animals are clearly dragging a hind limb. The time-to-event plot for the age at which MR1 and control group animals progressed to a neurological score of 2 and the number of days at a neurological score of 2 is shown in FIG. 4. The median time at a neurological score of 2 for each group is plotted in FIG. 5. The time to attain a score of 2 in the control group is 115 days and for the MR1 treated group is 122 days. Based on the neurological score data MR1 delays disease onset in the G93A mouse model by approximately 7 days and the delay is statistically significant when analyzed using several approaches. (Kaplan Meier, log rank ($p = 0.0378$) and Wilcoxon, ($p = 0.0591$); Cox proportional hazard model, ($p = 0.0521$); parametric analysis, ($p = 0.0582$)). Days spent at each neurological score level is plotted against the median age at that score level in FIG. 5.

[0133] Treated animals survival time was later by 13 days than control animals. Comparative MR1-treated and control group time-to-event curves for the time from peak body weight to death are shown in FIG. 6. The median survival time for the control group was 128 days and the median survival for the MR1 treated group was 141 days. Control animals had a 2.8 to 3.2-fold greater risk of dying sooner than did MR1 treated animals. The delay is statistically significant when analyzed using several approaches. (Kaplan Meier, log rank ($p = 0.0040$) and Wilcoxon test ($p = 0.0109$); Cox proportional hazard model ($p = 0.0060$); parametric analysis, ($p = 0.0049$)).

[0134] 60 female and 36 male G93A mice were litter matched and randomly assigned to treatment or control groups. 30 of the female and 18 of the male mice were treated with MR1 starting at day 50. Study days are based on birth.

[0135] A single bolus injection of 5.22 mg/kg or 6.75 mg/kg of MR1 was administered intraperitoneally at day 50 in females or males respectively. Subsequent to the bolus injection, females received weekly injections of 1mg/kg of MR1, and males received weekly injections of 1.34 mg/kg of MR1 via IP injection. Doses were prepared in vehicle (phosphate buffered saline (PBS, pH 7.3)), to a total volume of 200 μ l. Control animals were administered 200 μ l PBS. Animals were monitored for body weight, neurological score, non-related ALS deaths, and criteria for euthanization as previously described.

[0136] Comparative MR1-treated and control group time-to-event curves for the time from day 40 to the attainment of peak body weight are shown in FIG. 7A. The median time to peak body weight for the control group was 49 days compared to the MR1 treated group which was 53 days. This difference was not significant when analyzed by Kaplan Meier using the log rank and Wilcoxon statistical models, Cox proportional hazard, or parametric statistical tests.

[0137] Comparative MR1-treated and control group time-to-event curves for the time from peak body weight to death are shown in FIG. 7B. Time from peak body weight to death was statistically significantly later by 6 days in MR1 treated animals. The median time from peak body weight to death in the control group was 29 days whereas in the MR1 treated group it was 35 days. Significance for each of the analyses in this example was calculated in several ways in order to better evaluate significance. The delay is statistically significant when analyzed using several statistical models: Kaplan Meier, log rank ($p=0.0413$) and Wilcoxon, ($p=0.0732$); and the Cox proportional Hazard model ($p=0.0460$). Based on the body weight data, MR1 appears to have less of an impact on the onset of disease in the G93A mouse model but has a dramatic effect in slowing down the rate of body weight loss from peak body weight until death.

[0138] The time-to-event plots for the age at which MR1 and control group animals progressed to a neurological score of 2 and the number of days at a neurological score of 2 is shown in FIG. 7C. The time to attain a score of 2 in the control group was 113 days and for the MR1 treated group is 121 days. Based on the neurological score data, MR1 delays disease onset in the G93A mouse model by approximately 8 days and the delay is statistically significant when analyzed using several statistical models: Kaplan Meier, log rank ($p = 0.0038$) and Wilcoxon; ($p = 0.0017$); and the Cox proportional hazard model, ($p = 0.0010$).

[0139] Treated animals survival time was later by 9 days than control animals. The median survival time for the control group was 124 days and the median survival for the MR1 treated group was 133 days, as shown in FIG. 7D. The delay is statistically significant when analyzed using several statistical models: Kaplan Meier, log rank ($p = 0.0043$) and Wilcoxon test ($p = 0.0040$); and the Cox proportional hazard model ($p = 0.0030$).

[0140] It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will

be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims.

[0141] Nucleic acid sequences encoding the heavy chain and the light chain of the desired antibody were designed to be suitable for expression in mammalian cells such as Chinese Hamster Ovary (CHO) cells. The nucleic acids were then artificially synthesized and ligated into the antibody expression vector BPJPuro using standard molecular biology techniques. BPJPuro is a dual gene mammalian expression vector optimized for selectable and stable expression of immunoglobulins in Chinese Hamster Ovary (CHO) cells. The vector was then transfected into CHO cells and stable transfectants selected.

[0142] Similarly, a nucleic acid (SEQ ID NO: 10) encoding a heavy chain having the amino acid sequence of SEQ ID NO: 9, and a nucleic acid (SEQ ID NO: 8) encoding a light chain having the amino acid sequence of SEQ ID NO: 7, were synthesized and ligated into the antibody expression vector BPJPuro.

[0143] The resulting expression vector encoding the heavy and light chains was transfected into the CHO line (CHO SA, Collectis SA, Paris, France) using liposome mediated transfection. Stable transfectants were isolated by puromycin selection and subcloned to provide clonal cell lines. Candidate cell lines were adapted to serum free suspension culture and screened for IgG production and robust growth. One of the cell lines was selected and named JB5, the cell line was cultured in a pilot scale bioreactor and the antibody JB5 was purified from conditioned medium by sequential concentration, Protein A/G affinity chromatography, and size exclusion chromatography.

[0144] A three-part sandwich ELISA assay was used to determine binding kinetics of the JB5 antibody relative to the parental antibody hu5c8. All washes were performed using 3 washes of 250 µl of PBS. A 96-well polystyrene plate was coated with 100 µl/well of JB5 or hu5c8 antibody (2 µg/ml) for 16 hours at 4°C. The plate was washed and then blocked with 2% bovine serum albumin/PBS for 1 hour at room temperature. The plate was washed and recombinant human CD40L protein (Santa Cruz Biotechnology, Santa Cruz, California, USA) was added to the plate titrated out by 2-fold dilution starting at 2000 ng/ml. After binding and washing, the bound CD40L protein was detected using 100 µl a biotinylated goat anti-human CD40L polyclonal antibody (200 ng/ml) and 100 µl a streptavidin-horseradish peroxidase conjugate at 100 ng/ml. Colorimetric detection was performed with the chromagen TMB (3,3',5,5'-tetramethylbenzidine) and spectrophotometric analysis of absorption at 450 nm. The resulting binding curves (FIG. 8) show that JB5 (circle) has highly similar CD40L binding relative to the parental antibody

hu5c8 (square). The control protein CTLA4-IgG1 (triangle), having the same Fc domain as JB5 showed no significant binding. The calculated EC₅₀ for hu5c8 and JB5 is 114 and 137 nM, respectively. JB5-R28K and JB5-K74R showed binding similar to that of JB5.

[0145] In order to make a comparison of CD40L binding of antibodies from all 16 clones versus 5c8 or AT-1501 a binding assay was run using 2 clones with 5c8 and AT-1501 run on the same 96-well assay plate. A three-part sandwich ELISA assay was used to determine the level of binding of the antibodies of the present disclosure in comparison to reference antibodies 5c8-19 and AT1501. 96-well polystyrene plates were coated with recombinant human CD40L (BioLegend Cat. # 591706) using 2 µg/ml in PBS and 50 µl/well was added to Costar 96-well ½ area high binding assay plates (Corning 3690) and incubated overnight at 4°C. Plates were blocked with (1X) PBS/1.0%BSA (140 µl/well) for 1 hour at room temperature to prevent background binding. Binding curves of 5C8 or AT1501 (from 2 µg/ml out serial 2-fold dilutions) were added (50 µl/well) and incubated for 1 hour at room temperature. Plates are washed and incubated with HRP-(Fab2) donkey anti-human IgG (Fc specific) (Jackson Immuno. 709-036-098) at a 1:10,000 dilution (50 µl/well) for 1 hour at room temperature. Plates were washed and TMB substrate (Surmodics BioFX TMBW-1000-01) was added (50 µl/well). Color development is stopped after 5 minutes at room temperature with 25 µl 2NH₂SO₄. Plates are read on Molecular Devices SpectraMax M5 plate reader using SoftMax Pro 6.2.2 program to determine absorbance at 450 nm.

[0146] Relative CD40L binding potency was calculated as follows: [IC₅₀ (clone) / IC₅₀ (5c8 or AT-1501) X 100%]. The ranked potency of the 16 clones versus 5c8 is shown in FIG. 15A and the ranked potency versus AT-1501 is shown in FIG. 15B. The resulting binding curves are shown in FIGs. 16A-16Q (comparison of 16 clones versus 5c8, FIG. 16H shows the comparison between AT-1501 and 5c8) and FIGs. 17A-17Q (comparison of the 16 clones versus AT-1501, FIG. 17H shows the comparison between AT-1501 and 5c8). The IC₅₀, LCL, UCL and Relative Potency for each clone are shown in **Table 1** (comparison with 5c8) and **Table 2** (comparison with AT-1501).

Table 1:

Fig. No.	Clone	IC ₅₀	LCL	UCL	Rel. Pot.
16A	16-3	0.022	0.017	0.026	46
	5c8	0.047	0.040	0.054	

Fig. No.	Clone	IC50	LCL	UCL	Rel. Pot.
16B	8-3	0.036	0.028	0.043	51
	5c8	0.070	0.062	0.078	
16C	12-4	0.020	0.013	0.027	47
	5c8	0.042	0.038	0.046	
16D	8-4	0.036	0.030	0.041	51
	5c8	0.070	0.062	0.078	
16E	13-2	0.020	0.013	0.027	48
	5c8	0.041	0.038	0.045	
16F	4-4	0.037	0.029	0.045	51
	5c8	0.072	0.064	0.081	
16G	17-1	0.028	0.023	0.033	63
	5c8	0.044	0.036	0.053	
16H	AT-1501	0.049	0.043	0.056	95
	5c8	0.052	0.046	0.058	
16I	5-3	0.055	0.047	0.063	76
	5c8	0.072	0.064	0.081	
16J	6-6	0.065	0.059	0.070	106
	5c8	0.061	0.054	0.068	
16K	18-2	0.041	0.036	0.046	92
	5c8	0.044	0.036	0.053	
16L	10-4	0.075	0.065	0.086	113
	5c8	0.067	0.058	0.076	
16M	10-1	0.079	0.070	0.089	119
	5c8	0.067	0.058	0.076	
16N	7-5	0.102	0.094	0.111	168
	5c8	0.061	0.054	0.068	
16O	15.4	0.063	0.056	0.069	133
	5c8	0.047	0.040	0.054	
16P	11-5	0.092	0.071	0.112	177
	5c8	0.052	0.046	0.058	
16Q	15-1	0.081	0.073	0.089	163

Fig. No.	Clone	IC50	LCL	UCL	Rel. Pot.
	5c8	0.050	0.046	0.053	

Table 2:

Fig. No.	Clone	IC50	LCL	UCL	Rel. Pot.
17A	12-4	0.020	0.013	0.027	45
	AT-1501	0.044	0.039	0.049	
17B	8-3	0.036	0.028	0.043	59
	AT-1501	0.060	0.053	0.068	
17C	13-2	0.020	0.013	0.027	45
	AT-1501	0.044	0.038	0.049	
17D	8-4	0.036	0.030	0.041	59
	AT-1501	0.060	0.053	0.068	
17E	16-3	0.022	0.017	0.026	48
	AT-1501	0.045	0.039	0.051	
17F	4-4	0.037	0.029	0.045	60
	AT-1501	0.062	0.054	0.070	
17G	17-1	0.028	0.023	0.033	66
	AT-1501	0.042	0.035	0.050	
17H	5c8	0.052	0.046	0.058	105
	AT-1501	0.049	0.043	0.056	
17I	5-3	0.055	0.047	0.063	89
	AT-1501	0.062	0.054	0.070	
17J	10-4	0.075	0.065	0.086	115
	AT-1501	0.066	0.056	0.076	
17K	18-2	0.041	0.036	0.046	96
	AT-1501	0.042	0.035	0.050	
17L	10-1	0.079	0.070	0.089	121
	AT-1501	0.066	0.056	0.076	
17M	6-6	0.065	0.059	0.070	132

Fig. No.	Clone	IC50	LCL	UCL	Rel. Pot.
	AT-1501	0.049	0.047	0.051	
17N	11-5	0.092	0.071	0.112	174
	AT-1501	0.053	0.050	0.056	
17O	15-4	0.063	0.056	0.069	139
	AT-1501	0.045	0.039	0.051	
17P	7-5	0.102	0.094	0.111	210
	AT-1501	0.049	0.047	0.051	
17Q	15-1	0.081	0.073	0.089	159
	AT-1501	0.051	0.046	0.055	

[0147] The 16 VH/VL antibody clones constructed with an IgG1 Fc having two mutations P238S and N297G (SEQ ID NO: 21). These antibody clones were assayed for Fc effector function for binding to human FcγRI, FcγRIIa and FcγRIIIa.

[0148] Anti-CD40L antibodies (Abatacept included as negative control) are diluted to 2 μg/ml in (1X) PBS and 50 μl/well was added to Costar 96-well ½ area high binding assay plates (Corning 3690) for overnight incubation at 4°C. Plates were blocked with (1X) PBS/1.0% BSA (140 μl/well) for 1 hour at room temperature to prevent background binding. Binding curves of recombinant human FcγRI, IIa, IIIa and IIIb (from 5 μg/ml out serial 2-fold dilutions) were added (50 μl/well) and incubated for 1 hour at room temperature. Plates were washed and incubated with mouse anti-human CD16 (anti-FcγRIII); CD32 (anti-FcγRIIa) or CD64 (anti-FcγRI) (eBioSciences/Invitrogen 14-0168-82; 16-0329-81; 14-0649-82) at 2 μg/ml (50 μl/well) for 1 hour at room temperature. Plates were washed and incubated with HRP-(Fab₂) goat anti-mouse IgG (Fc specific) (Jackson Immuno. 116-036-071) at a 1:10,000 dilution (50 μl/well) for 1 hour at room temperature. Plates were washed and TMB substrate (Surmodics BioFX TMBW-1000-01) was added (50 μl/well). Color development was stopped after 5 mins at room temperature with (25 μl/well) 2NH₂SO₄. Plates were read on Molecular Devices SpectraMax M5 plate reader using SoftMax Pro 6.2.2 program to determine absorbance at 450nm.

[0149] As can be seen in the figures, the clones disclosed in this application were negative for binding to the three Fc receptors while 5c8 bound to FcγRI, FcγRIIa but not to FcγRIIIa or FcγRIIIb. Each of FIGs. 18A and 18B show the binding to FcγRIa for eight antibody clones compared with the binding curves of 5c8, AT-1501 and Abatacept.

Each of FIGs. 19A and 19B show the binding to FcγRIIa for eight antibody clones compared with the binding curve of 5c8, AT-1501 and Abatacept. Each of FIGs. 20A and 20B show the binding to FcγRIIIa for eight antibody clones compared with the binding curves of 5c8, AT-1501 and Abatacept. Each of FIGs. 20C and 20D show the binding to FcγRIIIb for eight antibody clones compared with the binding curves of 5c8, AT-1501 and Abatacept. (FIGs. 18A, 19A, 20A and 20B show results from antibody clones 4-4, 5-3, 6-6, 7-5, 8-3, 8-4, 10-1 and 10-4; FIGs. 18B, 19B, 20C and 20D show results from antibody clones 11-5, 12-4, 13-2, 15-1, 15-4, 16-3, 17-1 and 18-2).

[0150] Anti-CD40L antibodies (Abatacept included as negative control) were diluted to 2 µg/ml in (1X) PBS and 50 µl/well was added to Costar 96-well ½ area high binding assay plates (Corning 3690) for overnight incubation at 4°C. Plates were blocked with (1X) PBS/1.0% BSA (140 µl/well) for 1 hour at room temperature to prevent background binding. Binding curves of natural human C1q protein (Abcam ab96363) from 10 µg/ml out serial 2-fold dilutions were added (50 µl/well) for 1 hour at room temperature. Plates were washed and HRP-sheep anti-human C1q (Abcam ab46191) is added at a 1:400 dilution (50 µl/well) and incubated for 1 hour at room temperature. Plates were washed and TMB substrate (Surmodics BioFX TMBW-1000-01) is added (50 µl/well). Color development was stopped after 5 minutes at room temperature with (25 µl/well) 2NH₂SO₄. Plates were read on Molecular Devices SpectraMax M5 plate reader using SoftMax Pro 6.2.2 program to determine absorbance at 450nm.

[0151] As can be seen in the figures, the antibody from all sixteen antibody clones disclosed in this application were negative for binding to C1q while 5c8 showed significant binding. Each of FIGs. 21A and 21B show the binding to C1q for eight antibody clones compared with the binding curves of 5c8, AT-1501 and Abatacept. FIG. 21A shows results from antibody clones 4-4, 5-3, 6-6, 7-5, 8-3, 8-4, 10-1 and 10-4; FIG. 21B shows results from antibody clones 11-5, 12-4, 13-2, 15-1, 15-4, 16-3, 17-1 and 18-2.

[0152] Because the genetic expression data indicated the involvement of the co-stimulator pathway, the efficacy of MRI was evaluated in the G93A model. MR1 binds to CD40L thus blocking its interaction of CD40 which participates in the co-stimulatory pathway involved in an immune response. It has been reported in the literature that MR1 is efficacious for the treatment of rheumatoid arthritis and graft versus host disease both of which have a strong immunologic component. Rheumatoid arthritis is an autoimmune disease and graft versus host disease arises when the host's body mounts a vigorous immune response against the graft tissue.

[0153] Although ALS has an immune component mediated by activation of microglia and astrocytes it is not considered to be an autoimmune disorder. Several anti-inflammatory drugs failed to show efficacy in preclinical or clinical testing including TNF α -inhibitors, Celebrex[®], minocycline, and thalidomide. It was thus unexpected to find that MR1, showed efficacy in the G93A ALS model.

[0154] Methods according to the present disclosure include methods of treating a patient with a neurodegenerative and/or a neuromuscular disorder by administering to the patient a compound that blocks the interaction of CD40L and CD40 and/or blocks the interaction of CD28 and CD80 and/or blocks the interaction of CD28 and CD86. One embodiment is a method of treating a patient having Alzheimer's Disease, Parkinson's Disease, Amyotrophic Lateral Sclerosis, Myasthenia Gravis, Multifocal Motor Neuropathy, Primary Lateral Sclerosis, Spinal Muscular Atrophy, Kennedy's Disease, and Spinocerebellar Ataxia by administering to the patient a compound that blocks the interaction of CD40L and CD40. In another embodiment the method of treating a patient is by administering an anti-CD40L antibody.

[0155] In some embodiments, therapeutic compounds include any compound that blocks the interaction of CD40 with CD40L. For example, a number of animal studies describe agents capable of interrupting CD40:CD40L binding (see for example US2005158314 and US7173046 which are hereby incorporated by reference.) And for example, numerous anti-CD40L antibodies have been produced and characterized. (See, e.g., U.S. Pat. No. 5,876,950) to Bristol-Myers Squibb, which is hereby incorporated by reference). Anti-CD40L antibodies useful in the methods of the present disclosure include, but are not limited to, MR1, a hamster monoclonal antibody available from Taconic (Hudson, NY) and BD Biosciences (San Jose, CA); 5c8, a humanized antibody described in U.S. Pat. No. 5,474,771 (which is hereby incorporated by reference); a hamster human chimeric antibody, IDEC 131/E6040 is a humanized monoclonal antibody comprising human gamma-1 heavy chains and human kappa-light chains with CDRs of murine monoclonal antibody clone 24-31, commercially available from Ancell (catalog X 353-020, Bayport, Minn.); ABI 793;, Sgn-40; ImxM90 (Immunex); ImxM91 (Immunex); ImxM92 (Immunex); and an anti-CD40L mAb commercially available from Genzyme (Cambridge, Mass., catalog No. 80-3703-01). Also commercially available is an anti-CD40L mAb from PharMingen (San Diego, Catalog #33580D). Embodiments according to the disclosure include methods of treating a patient with a neurodegenerative or

neuromuscular disorder, comprises administering a therapeutically effective amount of an anti-CD40L antibody. One embodiment is a method of treating a patient with a neurodegenerative or neuromuscular disorder, comprising administering a therapeutically effective amount of an anti-CD40L antibody selected from MR1, 5c8, IDEC 131/E6040, clone 24-31, ABI 793, ImxM90, ImxM91, ImxM92, or Sgn-40. In one embodiment the antibody is 5c8. In another embodiment the antibody is MR1.

[0156] In some embodiments the neurodegenerative or neuromuscular disorder is Alzheimer's Disease, Parkinson's Disease, Amyotrophic Lateral Sclerosis, Myasthenia Gravis, Multifocal Motor Neuropathy, Primary Lateral Sclerosis, Spinal Muscular Atrophy, Kennedy's Disease, or Spinocerebellar Ataxia. One embodiment is a method of treating a patient with Amyotrophic Lateral Sclerosis comprising administering a therapeutically effective amount of an anti-CD40L antibody. In a particular embodiment the anti-CD40L antibody is MR1. In another particular embodiment the anti-CD40L antibody is 5c8.

[0157] In another embodiment the method of treatment comprises administering a therapeutically effective amount of an anti-CD40 antibody. In some embodiments the anti-CD40L compounds are Fab fragments, F(ab')₂, F(ab'), single chain antibodies, polypeptides, fusion constructs of polypeptides and the like. In some embodiments the compounds are small molecule compounds that are capable of blocking the CD40:CD40L interaction. In other embodiments these compounds include BIO3417, or any of the compounds disclosed in United States Patent Number 7,173,046, having the ability to block the CD40:CD40L interaction.

[0158] The compounds that block the CD40:CD40L interaction may be administered in combination with other compounds. Thus, another embodiment is a method of treating a patient with a neurodegenerative or neuromuscular disorder comprising administering a compound that blocks the interaction of CD40L and CD40 in combination with a compound that blocks the interaction between CD80 and CD28. Another embodiment is a method of treating a patient by administering a compound that blocks the interaction of CD40L and CD40 in combination with a compound that blocks the interaction between CD86 and CD28. In one embodiment, the compound that blocks the interaction of CD80 and CD28 is galiximab, or H1f1 & h3d1, or 16C10, or 7C10. In one embodiment the compound that blocks the interaction between CD86 and CD28 is a CTLA4-Ig protein conjugate such as abetacept or belatacept. Embodiments according to the present disclosure also include a method of treating a patient with a neurodegenerative

or neuromuscular disorder comprising administering a compound that blocks the interaction of CD40L and CD40 in combination with a compound that blocks the interaction between CD80 and CD28 or administering a therapeutically effective amount of a compound that blocks the interaction of CD40L and CD40 in combination with a compound that blocks the interaction between CD86 and CD28, wherein the neurodegenerative or neuromuscular disorder is Alzheimer's Disease, Parkinson's Disease, Amyotrophic Lateral Sclerosis, Myasthenia Gravis, Multifocal Motor Neuropathy, Primary Lateral Sclerosis, Spinal Muscular Atrophy, Kennedy's Disease, or Spinocerebellar Ataxia. In certain embodiments the compound that blocks the interaction of CD40L and CD40 is MR1 and the compound that blocks the interaction between CD28 and CD86 or between CD28 and CD80 is abatacept, galiximab or belatacept. In other embodiments, the compound that blocks the interaction of CD40L and CD40 is 5c8. Another embodiment is a method of treating a patient with Amyotrophic Lateral Sclerosis comprising administering a therapeutically effective amount of MR1 in combination with abatacept or belatacept. Another embodiment is a method of treating a patient with Amyotrophic Lateral Sclerosis comprising administering a therapeutically effective amount of 5c8 in combination with abatacept or belatacept.

Pharmaceutical compositions and methods of administration

[0159] To treat any of the foregoing disorders, pharmaceutical compositions for use in accordance with the methods of the present disclosure may be formulated in a conventional manner using one or more physiologically acceptable carriers. Pharmaceutically acceptable carriers are determined in part by the particular composition being administered, as well as by the particular method used to administer the composition. Accordingly, there are a wide variety of suitable formulations of the compounds useful in the methods of the present disclosure (see, e.g., Remington: The Science and Practice of Pharmacy, 20th ed., Gennaro et al. Eds., Lippincott Williams and Wilkins, 2000).

[0160] Formulations suitable for oral administration include, for example, solid, semi-solid and liquid systems such as tablets; soft or hard capsules containing multi- or nano-particulates, liquids, or powders; lozenges (including liquid-filled); chews; gels; fast dispersing dosage forms; films; ovules; sprays; and buccal/mucoadhesive patches.

[0161] Formulations suitable for parenteral administration include aqueous and non-aqueous, isotonic sterile injection solutions, which can contain antioxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended

recipient, and aqueous and nonaqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives.

[0162] According to the present disclosure the compounds can be administered by any suitable means, which can vary, depending on the type of disorder being treated and on the nature of the compound itself. For example, the compounds may be administered orally, parenterally, or topically. For proteins such as antibodies, administration routes preferably include parenteral, e.g., intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous. Preferably, the parenteral dosing is given by injection, most preferably intravenous, intramuscular, or subcutaneous injection. The amount to be administered will depend on a variety of factors such as the clinical symptoms, weight of the individual, and whether other drugs are administered. It should be appreciated that determination of proper dosage forms, dosage amounts, and routes of administration is within the level of ordinary skill in the pharmaceutical and medical arts, and is described below.

[0163] In one exemplary pharmaceutical composition containing the anti-CD40L antibody or antigen-binding fragment thereof, the composition is formulated as a sterile, preservative-free solution of the anti-CD40L antibody or antigen-binding fragment thereof for intravenous or subcutaneous administration. The formulation may be supplied as either a single-use, prefilled pen, as a single-use, for example containing about 1 mL prefilled glass syringe, or as a single-use institutional use vial. Preferably, the pharmaceutical composition containing the anti-CD40L antibody or antigen-binding fragment thereof is clear and colorless, with a pH of about 5.0 to about 6.9, preferably a pH of about 5.0 to about 6.5, and even more preferably a pH ranging from about 5.0 to about 6.0. In various embodiments, the formulations comprising the pharmaceutical compositions may contain from about 500 mg to about 1 mg, or from about 400 mg to about 10 mg, or from about 300 mg to about 30 mg or from about 200 mg to about 50 mg of the anti-CD40L antibody or antigen-binding fragment thereof per mL of solution when reconstituted and administered to the subject.

[0164] Selecting an administration regimen for a therapeutic depends on several factors, including the serum or tissue turnover rate of the entity, the level of symptoms, the immunogenicity of the entity, and the accessibility of the target cells in the biological matrix, general health of the patient, the prior medical history of the patient, and the like. Preferably, an administration regimen maximizes the amount of therapeutic delivered to the patient consistent with an acceptable level of side effects. Accordingly, the amount of biologic delivered depends in part on the particular entity and the severity of the condition

being treated. It should be appreciated that determination of proper dosage forms, dosage amounts, and routes of administration is within the level of ordinary skill in the pharmaceutical and medical arts.

Doses and Exposure

[0165] The pharmaceutical formulations of the present disclosure may contain from about 0.001 to about 200 mg/kg of an anti-CD40L antibody or antigen binding fragment thereof, for example, from about 0.001 mg/kg to about 100 mg/kg, or from about 0.001 mg/kg to about 50 mg/kg, or from about 0.001 mg/kg to about 10 mg/kg intravenous injection of the anti- CD40L antibody, or antigen-binding fragment thereof, may be given as a bolus, and the rest of the antibody dose may be administered by intravenous injection. A predetermined dose of the anti- CD40L antibody, or antigen-binding fragment thereof, may be administered, for example, over a period of an hour to two hours to five hours.

[0166] In a further embodiment, part of the dose is administered by a subcutaneous injection and/or infusion in the form of a bolus and the rest by infusion of the antibody formulation. In some exemplary doses, the antibody formulation may be administered subcutaneously in a dose ranging from about 0.001 to about 200 mg/kg, for example, from about 0.001 mg/kg to about 100 mg/kg, or from about 0.001 mg/kg to about 50 mg/kg, or from about 0.001 mg/kg to about 10 mg/kg intravenous injection of the anti-CD40L antibody, or antigen-binding fragment thereof. In some embodiments the dose may be given as a bolus, and the rest of the antibody dose may be administered by subcutaneous or intravenous injection. A predetermined dose of the anti- CD40L antibody, or antigen-binding fragment thereof, may be administered, for example, over a period of an hour, or a period of two hours, or a period of three hours, or a period of four hours or a period of five hours or longer.

[0167] In some embodiments, the dose is administered such at a constant rate such as to maintain a steady state in the plasma. In some embodiments, the dose is administered every two weeks. In some embodiments, the compound is administered such that the plasma of the subject maintains a concentration of the compound at a dose that is between 1 µg/mL and 1 mg/mL. In some the compound is administered such that the plasma of the subject maintains a concentration of the compound at a dose that is between 1 µg/mL and 500 µg/mL. In some embodiments, the compound is administered such that the plasma of the subject maintains a concentration of the compound at a dose that is between 1 µg/mL and 250 µg/mL. In some embodiments, the compound is administered such that the plasma

of the subject maintains a concentration of the compound at a dose that is between 5 µg/mL and 100 µg/mL. In some embodiments, the compound is administered such that the plasma of the subject maintains a concentration of the compound at a dose that is between 30 µg/mL and 60 µg/mL.

Biomarkers

[0168] Some embodiments of the present application relate to modifying biomarkers in a subject with treatment of a compound targeting CD40/CD40L. In some embodiments, the biomarker is a protein involved in CD40/CD40L activity. In some embodiments, the biomarker is CD40. In some embodiments, the biomarker is CD40L. In some embodiments, the biomarker is NFL. In some embodiments, the biomarker is a pro-inflammatory marker, or linked to inflammation. In some embodiments, the biomarker is linked to ALS. In some embodiments, the biomarker is linked to neurodegeneration or a neuromuscular disease or disorder. In some embodiments, the biomarker is a cytokine and/or an interleukin. In some embodiments, the biomarker is one or more of NFL, CXCL13, CD40L, CXCL9, TNF-alpha, En-Raged, TNFR2, IgM, IgA, IL2r, CXCL10, CD40, B2M, VCAM, IL-18, IL-16, SAP, MIP1beta, MDC, C3, CRP, Fibrogen, IgE, ICAM, FactorVIII, Ena-78, Pal1, Rantes, TIMP1, vWF, IL-6, MCP1, MMP3, MMP9, MCP1, MIG, vWF, IL-8, IP10, and MMP2.

[0169] In some embodiments, treatment with a compound targeting CD40/CD40L increases the concentration of one or more biomarkers. In some embodiments, treatment with a compound targeting CD40/CD40L decreases the concentration of one or more biomarkers. In some embodiments, treatment with a compound targeting CD40/CD40L increases at least one biomarker concentration, and decreases at least one other biomarker concentration. In some embodiments, the baseline levels of the at least one biomarker serve as a predictor for the response of a subject with a disease, disorder, or condition to treatment with a compound targeting CD40/CD40L.

Combination Therapies

[0170] The antibodies or antibody fragments thereof described herein can be administered alone (monotherapy) or in combination, i.e., combined with other agents. For example, in one embodiment the combination therapy may include one or more additional therapeutic agents. In another embodiment the combination therapy includes standard of

care treatment that may, or may not, include additional therapeutic agents (consists essentially of the antibody or antibody fragment thereof).

[0171] Adjunctive or combined administration (co-administration) includes simultaneous administration of any of the antibodies or antigen binding fragments thereof, described herein and one or more agents in the same or different dosage form, or separate administration of the polypeptide and one or more agents (e.g., sequential administration). Such concurrent or sequential administration preferably results in both the polypeptide and the one or more agents being simultaneously present in treated patients.

Kits and Articles of Manufacture

[0172] Further provided are kits containing the antibody or antigen binding fragments thereof described herein and instructions for use. Kits typically include a packaged combination of reagents in predetermined amounts with instructions and a label indicating the intended use of the contents of the kit. The term label or instruction includes any writing, or recorded material supplied on or with the kit, or which otherwise accompanies the kit at any time during its manufacture, transport, sale, or use. It can be in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of the manufacture, use or sale for administration to a human or for veterinary use. The label or instruction can also encompass advertising leaflets and brochures, packaging materials, and audio or video instructions.

Anti-CD40L Antigen Binding Molecules

[0173] Some embodiments provided herein relate to antibodies that are modified versions of the anti-CD40L antibody hu5c8 that comprise a human IgG1 consensus framework having the variable light chain and the variable heavy chain CDR sequences of hu5c8 with an Fc domain modified to prevent platelet activation.

[0174] **Table 3** provides a description of the SEQ ID NOs: referenced in the application.

Table 3:

SEQ ID NO:	Description of Sequence
1	Light chain variable region amino acid sequence (hu5c8 and JB5)

2	Heavy chain variable region amino acid sequence (hu5c8 and JB5)
3	Fc region amino acid sequence (hu5c8)
4	JB5 Fc region amino acid sequence
5	JB5-R28K light chain variable region amino acid sequence
6	JB5-K74R heavy chain variable region amino acid sequence
7	JB5 light chain amino acid sequence
8	JB5 light chain nucleic acid sequence
9	JB5 heavy chain amino acid sequence
10	JB5 heavy chain nucleic acid sequence
11	JB5-R28K light chain amino acid sequence
12	JB5-R28K light chain synthetic gene nucleic acid sequence
13	JB5-K74R heavy chain amino acid sequence
14	JB5-K74R heavy chain synthetic gene nucleic acid sequence
15	CDR-1 of the JB5 Variable Light Chain amino acid sequence
16	CDR-2 of the JB5 Variable Light Chain amino acid sequence
17	CDR-3 of the JB5 Variable Light Chain amino acid sequence
18	CDR-1 of the JB5 Variable Heavy Chain amino acid sequence
19	CDR-2 of the JB5 Variable Heavy Chain amino acid sequence
20	CDR-3 of the JB5 Variable Heavy Chain amino acid sequence
21	Hu5c8 Heavy Chain amino acid sequence

[0175] One embodiment (embodiment A) is an isolated antibody that binds to CD40L and that comprises a light chain and a heavy chain, wherein the light chain comprises a light chain variable region comprising an amino acid sequence having at least 90%, or at least 91%, or at least 92%, or at least 93%, or at least 94%, or at least 95%, or at least 96% or at least 97%, or at least 98% or at least 99% sequence identity with SEQ ID NO: 1 and the heavy chain comprises a variable heavy chain region and an Fc region, wherein the heavy chain variable region comprises an amino acid sequence having at least 90%, or at least 91%, or at least 92%, or at least 93%, or at least 94%, or at least 95%, or at least 96%, or at least 97%, or at least 98%, or at least 99% sequence identity with SEQ ID NO: 2 and the Fc region comprises an amino acid sequence having at least at least 90%, or at least 91%, or at least 92%, or at least 93%, or at least 94%, or at least 95%, or at least

96%, or at least 97%, or at least 98%, or at least 99% sequence identity with SEQ ID NO: 3 wherein the Fc region comprises one or a combination of substitutions selected from the group consisting of C11S, C14S, and P23S.

[0176] Another embodiment (embodiment B) is an isolated antibody according to embodiment A, wherein the Fc region further comprises the amino acid substitution C5S.

[0177] In variations of the embodiments A and B the antibody comprises a light chain variable region that does not comprise any of the substitutions T33W, S26D, and Q27E.

[0178] In other variations of embodiments A and B, the light chain variable region comprises the substitution R28K.

[0179] In some variations of the embodiments of A and B, the CDRs of the heavy and light chain have the sequences listed in **Table 4**.

Table 4:

CDR1 light chain	ISCRASQRVSSSTYSYMH (SEQ ID NO: 15)
CDR2 light chain	YASNLES (SEQ ID NO: 16)
CDR3 light chain	QHSWEIPPT (SEQ ID NO: 17)
CDR1 heavy chain	SYMY (SEQ ID NO: 18)
CDR2 heavy chain	EINPSNGDTNFNEKFKS (SEQ ID NO: 19)
CDR3 heavy chain	SDGRNDMDS (SEQ ID NO: 20)

[0180] In yet other variation of embodiments A and B, the light chain variable region comprises the amino acid sequence ICRRASQRVSSSTYSYMH (SEQ ID NO: 15). In still other embodiments, the light chain variable region comprises the amino acid sequence ICRRASQRVSSSTYSYMH (SEQ ID NO: 15) and one or both of the amino acid sequences YASNLES (SEQ ID NO: 16) and QHSWEIPPT (SEQ ID NO: 17).

[0181] In some variations of embodiments A and B, the light chain variable region comprises the amino acid sequence of SEQ ID NO: 1. In yet other embodiments the light chain variable region consists of the amino acid of SEQ ID NO: 1. In some embodiments, the light chain consists essentially of the amino acid sequence of SEQ ID NO: 7. In other embodiments, the light chain consists of the amino acid sequence of SEQ ID NO: 7. In still other embodiments, the light chain comprises the amino acid sequence of SEQ ID NO: 11. In yet other embodiments, the light chain consists essentially of the amino

acid sequence of SEQ ID NO: 11. In still other embodiments, the light chain consists of the amino acid sequence of SEQ ID NO: 11.

[0182] In other variations of the embodiments A and B, the antibody comprises a heavy chain variable region that does not comprise any of the substitutions T30H, Y33W, or S54N. In some embodiments of the antibodies of embodiments A and B, the light chain variable region does not comprise any of the substitutions T33W, S26D, and Q27E. In other variations of embodiments A and B, the light chain variable region does not comprise any of the substitutions T33W, S26D, and Q27E and the heavy chain variable region does not comprise any of the substitutions T30H, Y33W, or S54N.

[0183] In yet other variations of the embodiments A and B, the heavy chain variable region comprises the substitution K74R. In one embodiment the heavy chain variable region comprises one or any combination of the amino acid sequences SYMY (SEQ ID NO: 18), EINPSNGDTNFNEKFKS (SEQ ID NO: 19), and SDGRNDMDS (SEQ ID NO: 20).

[0184] In another embodiment, the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 2. In yet another embodiment the heavy chain variable region consists essentially of the amino acid sequence of SEQ ID NO: 2. In still another embodiment the heavy chain variable region consists of the amino acid sequence of SEQ ID NO: 2. In some embodiments, the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 6. In yet other embodiments the heavy chain variable region consists essentially of the amino acid sequence of SEQ ID NO: 6. In still other embodiments the heavy chain variable region consists of the amino acid sequence of SEQ ID NO: 6.

[0185] Some embodiments provided herein relate to an isolated antibody, wherein the light chain comprises the amino acid sequence of SEQ ID NO: 1 and the heavy chain consists of the amino acid sequence of SEQ ID NO: 9.

[0186] Some embodiments provided herein relate to an isolated antibody, wherein the light chain consists of the amino acid sequence of SEQ ID NO: 7 and the heavy chain consists of the amino acid sequence of SEQ ID NO: 9.

[0187] Some embodiments provided herein relate to an isolated antibody wherein the light chain variable region comprises the amino acid sequence of SEQ ID NO: 5 and the heavy chain consists of the amino acid sequence of SEQ ID NO: 9.

[0188] Still another embodiment is an isolated antibody wherein the light chain consists of the amino acid sequence of SEQ ID NO: 11 and the heavy chain consists of the amino acid sequence of SEQ ID NO: 9.

[0189] Yet another embodiment, is an isolated antibody wherein the light chain consists of the amino acid sequence of SEQ ID NO: 7 and the heavy chain consists of the amino acid sequence of SEQ ID NO: 13.

[0190] Another embodiment is an isolated antibody wherein the light chain consists of the amino acid sequence of SEQ ID NO: 11 and the heavy chain consists of the amino acid sequence of SEQ ID NO: 13.

[0191] Some embodiments provided herein relate to the following enumerated alternatives.

[0192] A method of predicting the response of a subject with a disease or disorder to treatment with a compound against CD40L or CD40, the method comprising: collecting a sample from the subject; measuring a concentration of at least one biomarker present in the sample, wherein the at least one biomarker is NFL, MMP9, MMP3, IL6, IL18, IgA, TNFR2, TNFa, IL2ra, FactorVIII, CRP, SAP, MCP1, En-Raged, MIG, vWF, IgE, IL8, IP10, C3, and MMP2; wherein the concentration of the at least one biomarker is predictive that the subject is likely to be responsive to treatment against the disease or disorder with a compound against CD40L or CD40.

[0193] The method of alternative 1, wherein the disease or disorder is inflammation, a neurodegenerative disease, or neuromuscular disease.

[0194] The method of alternative 2, wherein the neurodegenerative disease or neuromuscular disease is Amyotrophic Lateral Sclerosis, Alzheimer's Disease, Parkinson's Disease, Myasthenia Gravis, Multifocal Motor Neuropathy, Primary Lateral Sclerosis, Spinal Muscular Atrophy, Kennedy's Disease, or Spinocerebellar Ataxia.

[0195] The method of alternative 3, wherein the neurodegenerative disease or neuromuscular disease is Amyotrophic Lateral Sclerosis (ALS).

[0196] The method of any one of alternatives 1-4, wherein the subject is mammalian and/or human.

[0197] The method of any one of alternatives 1-5, wherein the compound blocks the interaction of CD40 and CD40L.

[0198] The method of any one of alternatives 1-6, wherein the compound is an anti-CD40L antibody or an anti-CD40 antibody.

[0199] The method of any one of alternatives 1-7, wherein the compound is tegoprunbart.

[0200] The method of any one of alternatives 1-8, wherein the compound is MR1 or 5c8.

[0201] A method treating a subject with a disease or disorder, the method comprising: administering a therapeutically effective dose of a compound against CD40L or CD40, wherein administering the therapeutically effective dose maintains the compound in plasma of the subject at a concentration between 5 µg/mL and 100 µg/mL; preferably at a dose that is between 30 µg/mL and 60 µg/mL.

[0202] The method of alternative 10, wherein the disease or disorder is inflammation, a neurodegenerative disease, or neuromuscular disease.

[0203] The method of alternative 11, wherein the neurodegenerative disease or neuromuscular disease is Amyotrophic Lateral Sclerosis, Alzheimer's Disease Parkinson's Disease, Myasthenia Gravis, Multifocal Motor Neuropathy, Primary Lateral Sclerosis, Spinal Muscular Atrophy, Kennedy's Disease, or Spinocerebellar Ataxia.

[0204] The method of alternative 12, wherein the neurodegenerative disease or neuromuscular disease is Amyotrophic Lateral Sclerosis (ALS).

[0205] The method of any one of alternatives 10-13, wherein the subject is mammalian and/or human.

[0206] The method of any one of alternatives 10-14, wherein the compound blocks the interaction of CD40 and CD40L.

[0207] The method of any one of alternatives 10-15, wherein the compound is an anti-CD40L antibody or an anti-CD40 antibody.

[0208] The method of any one of alternatives 10-16, wherein the compound is tegoprunbart.

[0209] The method of any one of alternatives 10-17, wherein the compound is MR1 or 5c8.

[0210] The method of any one of alternatives 10-18, wherein the compound is administered at least once every two weeks.

[0211] The method of any one of alternatives 10-19, wherein the compound is administered for a period of at least 12 weeks.

[0212] The method of any one of alternatives 10-20, wherein the compound is administered at a dose of between 200 mg/kg and 100 mg/kg; preferably at a dose between 1 mg/kg and 8 mg/kg; most preferably at a dose between 2 mg/kg and 4 mg/kg.

[0213] The method of any one of alternatives 10-21, the method further comprising administering a second pharmaceutically effective compound.

[0214] The method of alternative 22, wherein the second compound blocks the interaction between CD28 and CD86 or between CD28 and CD80.

[0215] The method of alternative 22, wherein the second compound targets at least one of the biomarkers selected from the group consisting of: interleukins, cytokines, and proinflammatory markers.

[0216] The method of alternative 24, wherein the second compound targets at least one of the biomarkers selected from the group consisting of: NFL, CXCL13, CD40L, CXCL9, TNF-alpha, En-Raged, TNFR2, IgM, IgA, Il2r, CXCL10, CD40, B2M, VCAM, Il-18, Il-16, SAP, MIP1beta, MDC, C3, CRP, Fibrogen, IgE, ICAM, FactorVIII, Ena-78, Pal1, Rantes, TIMP1, vWF, Il-6, MCP1, MMP3, MMP9, and MMP2.

[0217] The method of alternative 22, wherein the second compound is a CTLA4-Ig fusion protein, an abatacept, a belatacept, or a galiximab.

[0218] The method of any one of alternatives 10-26, wherein the compound is administered orally, parenterally, or topically.

[0219] The method of alternative 27, wherein the compound is administered parenterally.

[0220] The method of alternative 28, wherein the compound is administered by injection, most preferably intravenous, intramuscular, or subcutaneous injection.

[0221] The method of any one of alternatives 1-29, wherein the disease or disorder is an inflammatory or immune disease or disorder selected from the group consisting of colitis, drug induced lupus nephritis, graft versus host disease, immune graft response, transplant rejection and atherosclerosis.

[0222] The method of any one of alternatives 1-30, wherein the disease or disorder is an autoimmune disease, selected from the group consisting of systemic lupus erythematosus, type-1 diabetes, myasthenia gravis, inflammatory bowel disease, immune thrombocytopenic purpura, rheumatoid arthritis, psoriasis, Addison's disease, Crohn's disease, uveitis, multiple sclerosis, hemolytic anemia, and Graves' disease.

[0223] The method of any one of alternatives 1-31, wherein the compound that targets CD40L or CD40 is an antibody that comprises: (a) a heavy chain variable region (V_H) comprising: i) a CDRH1 domain comprising the sequence set forth in SEQ ID NO: 9; ii) a CDRH2 domain comprising the sequence set forth in SEQ ID NO: 10, 11, 12, 13, or 14; and iii) a CDRH3 domain comprising the sequence set forth in SEQ ID NO: 15; and

(b) a light chain variable region (V_L) comprising: i) a CDRL1 domain comprising the sequence set forth in SEQ ID NO: 16 or 17; ii) a CDRL2 domain comprising the sequence set forth in SEQ ID NO: 18 or 19; and iii) a CDRL3 domain comprising the sequence set forth in SEQ ID NO: 20.

[0224] The method of any one of alternatives 1-32, wherein the compound that targets CD40L or CD40 is an antibody that comprises: (a) a heavy chain variable region (V_H) having the amino acid sequence as set forth in SEQ ID NOs: 1, 2, 3, or 4; and (b) a light chain variable region (V_L) having the amino acid sequence as set forth in SEQ ID NOs: 5, 6, 7, or 8.

[0225] A method of modulating a concentration of at least one biomarker in a subject, the method comprising: administering to the subject a therapeutically effective amount of a compound against CD40L or CD40, wherein the at least one biomarker is NFL, CXCL13, CD40L, CXCL9, TNF-alpha, En-Raged, TNFR2, IgM, IgA, IL2r, CXCL10, CD40, B2M, VCAM, IL-18, IL-16, SAP, MIP1beta, MDC, C3, CRP, Fibrogen, IgE, ICAM, FactorVIII, Ena-78, Pal1, Rantes, TIMP1, vWF, IL-6, MCP1, MMP3, MMP9, MCP1, MIG, vWF, IL-8, IP10, or MMP2.

[0226] The method of alternative 34, wherein the concentration of the at least one biomarker increases following administration of the compound.

[0227] The method of alternative 34, wherein the concentration of the at least one biomarker decreases following administration of the compound.

[0228] The method of alternative 34, wherein the concentration of the at least one biomarker increases, and the concentration of at least one other biomarker decreases, following administration of the compound.

[0229] The method of any one of alternatives 34-37, wherein the subject has a disease or disorder.

[0230] The method of alternative 38, wherein the disease or disorder is inflammation, a neurodegenerative disease, or neuromuscular disease.

[0231] The method of alternative 39, wherein the neurodegenerative disease or neuromuscular disease is Amyotrophic Lateral Sclerosis, Alzheimer's Disease Parkinson's Disease, Myasthenia Gravis, Multifocal Motor Neuropathy, Primary Lateral Sclerosis, Spinal Muscular Atrophy, Kennedy's Disease, or Spinocerebellar Ataxia.

[0232] The method of alternative 40, wherein the neurodegenerative disease or neuromuscular disease is Amyotrophic Lateral Sclerosis (ALS).

[0233] The method of any one alternatives 34-41, wherein the subject is mammalian and/or human.

[0234] The method of any one of alternatives 34-42, wherein the compound blocks the interaction of CD40 and CD40L.

[0235] The method of any one of alternatives 34-43, wherein the compound is an anti-CD40L antibody or an anti-CD40 antibody.

[0236] The method of any one of alternatives 34-44, wherein the compound is tegoprunart.

[0237] The method of any one of alternatives 34-45, wherein the compound is MR1 or 5c8.

[0238] The method of any one of alternatives 34-46, wherein administering the therapeutically effective dose maintains the compound in plasma of the subject at a concentration between 5 µg/mL and 100 µg/mL; preferably at a dose that is between 30 µg/mL and 60 µg/mL.

[0239] The method of any one of alternatives 34-47, wherein the compound is administered at least once every two weeks.

[0240] The method of any one of alternatives 34-48, wherein the compound is administered for a period of at least 12 weeks.

[0241] The method of any one of alternatives 34-49, wherein the compound is administered at a dose of between 200 mg/kg and 100 mg/kg; preferably at a dose between 1 mg/kg and 8 mg/kg; most preferably at a dose between 2 mg/kg and 4 mg/kg.

[0242] The method of any one of alternatives 34-50, wherein the compound is administered orally, parenterally, or topically.

[0243] The method of alternative 51, wherein the compound is administered parenterally.

[0244] The method of alternative 52, wherein the compound is administered by injection, most preferably intravenous, intramuscular, or subcutaneous injection.

[0245] The method of any one of alternatives 34-53, wherein the disease or disorder is an inflammatory or immune disease or disorder selected from the group consisting of colitis, drug induced lupus nephritis, graft versus host disease, immune graft response, transplant rejection and atherosclerosis.

[0246] The method of any one of alternatives 34-54, wherein the disease or disorder is an autoimmune disease, selected from the group consisting of systemic lupus erythematosus, type-1 diabetes, myasthenia gravis, inflammatory bowel disease, immune

thrombocytopenic purpura, rheumatoid arthritis, psoriasis, Addison's disease, Crohn's disease, uveitis, multiple sclerosis, hemolytic anemia, and Graves' disease.

[0247] The method of any one of alternatives 34-55, wherein the compound that targets CD40L or CD40 is an antibody that comprises: (a) a heavy chain variable region (V_H) comprising: i) a CDRH1 domain comprising the sequence set forth in SEQ ID NO: 9; ii) a CDRH2 domain comprising the sequence set forth in SEQ ID NO: 10, 11, 12, 13, or 14; and iii) a CDRH3 domain comprising the sequence set forth in SEQ ID NO: 15; and (b) a light chain variable region (V_L) comprising: i) a CDRL1 domain comprising the sequence set forth in SEQ ID NO: 16 or 17; ii) a CDRL2 domain comprising the sequence set forth in SEQ ID NO: 18 or 19; and iii) a CDRL3 domain comprising the sequence set forth in SEQ ID NO: 20.

[0248] The method of any one of alternatives 34-56, wherein the compound that targets CD40L or CD40 is an antibody that comprises: (a) a heavy chain variable region (V_H) having the amino acid sequence as set forth in SEQ ID NOs: 1, 2, 3, or 4; and (b) a light chain variable region (V_L) having the amino acid sequence as set forth in SEQ ID NOs: 5, 6, 7, or 8.

[0249] A method of maintaining or improving the ALS-FRS scores of a subject with ALS, the method comprising: administering a therapeutically effective dose of a compound against CD40L or CD40 wherein administering the therapeutically effective dose maintains the compound in plasma of the subject at a concentration between 5 $\mu\text{g/mL}$ and 100 $\mu\text{g/mL}$; preferably at a dose that is between 30 $\mu\text{g/mL}$ and 60 $\mu\text{g/mL}$.

[0250] A method of pre-screening and treating a subject with a disease or disorder with a compound against CD40L or CD40, the method comprising: collecting a sample from the subject; detecting a concentration of at least one biomarker present in the fluid, wherein the at least one biomarker is NFL, MMP9, MMP3, IL6, IL18, IgA, TNFR2, TNFa, IL2ra, FactorVIII, CRP, SAP, MCP1, En-Raged, MIG, vWF, IgE, IL8, IP10, C3, or MMP2; screening the concentration of the at least one biomarker to the concentration of the at least one biomarker in a subject that does not have that disease or disorder; and administering a therapeutically effective dose of a compound against CD40L or CD40 such that the plasma of the subject maintains a concentration of the compound at a dose that is between 5 $\mu\text{g/mL}$ and 100 $\mu\text{g/mL}$; preferably at a dose that is between 30 $\mu\text{g/mL}$ and 60 $\mu\text{g/mL}$.

[0251] The method of alternative 59, wherein the compound is administered to the subject if the concentration of the at least one biomarker in the subject is significantly

different than the concentration of the at least one biomarker in a subject that does not have the disease or disorder.

EXAMPLES

[0252] Embodiments provided herein are further defined in the following Examples. It should be understood that these Examples are given by way of illustration only. From the above discussion and these Examples, one skilled in the art can ascertain the essential characteristics of this disclosure, and without departing from the spirit and scope thereof, can make various changes and modifications of the embodiments of the disclosure to adapt it to various usages and conditions. Thus, various modifications of the embodiments, in addition to those shown and described herein, will be apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims. The disclosure of each reference set forth herein is incorporated herein by reference in its entirety, and for the disclosure referenced herein.

EXAMPLE 1

Clinical study design

[0253] The effectiveness of a compound targeting CD40L or CD40 was assessed in clinical trials. A total of 54 human subjects were involved; these subjects were selected based on either a positive ALS diagnosis, or a diagnosis of possible, probable, or definite through the laboratory-supported revised El Escorial criteria. Each subject also had an ALSFRS-R aggregate score of 35 or greater, and had no more than 24 months since their initial diagnoses. Furthermore, each subject selected for the trial either had a tracheostomy, or used a permanent assistive ventilation system. The demographics of the subjects chosen are as shown in **Table 5**.

Table 5:

	1mg/kg (N=9)	2mg/kg (N=9)	4mg/kg (N=18)	8mg/kg (N=18)	All Subjects (N=54)
Age (years)	61.2	59.0	56.4	60.3	59.0
Mean					

Age < 65 yo	7 (77.8%)	6 (66.7%)	15 (83.3%)	11 (61.1%)	39 (72.2%)
Sex – Male	6 (66.7%)	7 (77.8%)	12 (66.7%)	10 (55.6%)	35 (64.8%)
Ethnicity – Hispanic / Latino	0	0	0	3 (16.7%)	3 (5.6%)
Race - white	9 (100%)	8 (88.9%)	17 (94.4%)	18 (100%)	52 (96.3%)
Race - Asian	0	1 (11.1%)	1 (5.6%)	0	2 (3.7%)
BMI Mean	26.2	27.6	25.1	26.0	26.0
ALSFRS-R (screening) Mean (Min, Max)	41.1 (38, 46)	40.2 (38, 43)	39.7 (35, 47)	39.2 (35, 45)	39.9 (35, 47)
ALSFRS-R (Baseline) Mean (Min, Max) ALSFRS-R <=34*	40.4 (38, 46) 0 (0%)	40.0 (37, 43) 0 (0%)	39.6 (34, 47) 1 (3%)	38.6 (29, 47) 3 (17%)	39.5 (29, 47) 4 (7%)
ALS Bulbar domain<=4 (Baseline)	0 (0%)	0 (0%)	2 (11%)	4 (22%)	6 (11%)

[0254] There were four dosing cohorts: 1 mg/kg (n=9), 2 mg/kg (n=9), 4 mg/kg (n=18), 8 mg/kg (n=18). As outlined schematically in FIG. 22, each subject had two biosamples of blood collected from them as baseline data at either -28 or -4 days before the first dose with compound. On day 0, two more subsequent biosamples were collected. These biosamples consisted of 1 plasma sample used for the analysis of pK/ADA, and 1 plasma sample for analysis of biomarker levels.

[0255] Following biosample collections, each subject immediately received an IV infusion of the compound AT-1501 (also called tegoprubart) at either 1 mg/kg, 2 mg/kg, 4 mg/kg, or 8 mg/kg. This pattern of collecting a biosample of plasma, followed by

administration of AT-1501 at a set dose was repeated every 14 days for a total of 101 days. Infusions were given at Day 0, 15, 29, 43, 57, and 71. Two hours post-transfusion, another two biosamples were collected.

[0256] After the dosage trail was completed, the endpoints analyzed included: safety and tolerability, pharmacokinetics, and anti-drug antibody levels, CD40L target engagement, changes in pro-inflammatory markers, and changes in ALSFRS-R, respiratory function, and neurofilament light chain (NFL) levels.

Safety

[0257] Over 90% of subjects completed the clinical trial, as summarized in Table 6. One subject, marked under “other” in the table, qualified at screening but did not receive dosage and was instead replaced by another participant.

Table 6:

	1mg/kg (N=9)	2mg/kg (N=9)	4mg/kg (N=18)	8mg/kg (N=18)	All Subjects (N=54)
Screened					70
Enrolled					55
Screen Failures					15
Treated	9	9	18	18	54
Per Protocol	9 (100%)	8 (88.9%)	18 (100%)	17 (94.4%)	52 (96%)
Completed	8 (88.9%)	7 (77.8%)	18 (100%)	16 (88.9%)	49 (90.7%)
Discontinued	1 (11.1%)	2 (22.2%)	0	2 (11.1%)	5 (9.3%)
Reason for D/C					
AE	1 (11.1%)	1 (11.1%)	0	0	2 (3.7%)
Withdrawal of Consent	0	0	0	2 (11.1%)	2 (3.7%)
Other	0	1 (11.1%)	0	0	1 (1.9%)

[0258] The safety of AT-1501 was assessed through counting the number of Treatment Emergent Adverse Events (TEAEs) in subjects over the course of the clinical

trial. The results are as summarized in **Table 7**. As a whole, Tegoprobart was well-tolerated with no treatment-related serious adverse events, nor any significant anti-drug antibody responses.

Table 7:

	1mg/kg (N=9)	2mg/kg (N=9)	4mg/kg (N=18)	8mg/kg (N=18)	All Subjects (N=54)
Number of TEAEs	30	66	54	67	217
Number of Subjects with at Least One TEAE	8 (88.9%)	8 (88.9%)	10 (55.6%)	16 (88.9%)	42 (77.8%)
Number of Serious TEAEs	0	1	0	0	1
Number of Subjects with at Least One Serious TEAE	0	1 (11.1%)	0	0	1 (1.9%)
Number of Subjects with Related TEAEs	3 (33.3%)	5 (55.6%)	4 (22.2%)	7 (38.9%)	19 (35.2%)
Number of Subjects with Serious Related TEAEs	0	0	0	0	0

[0259] The particular symptoms of adverse effects, and the percent of those that self-reported those symptoms, are as summarized in **Table 8**.

Table 8:

Symptoms Reported	No. of Subjects (%)
Fatigue	25.9

Fall	22.2
Headache	20
Muscle spasms	11.1
Arthralgias	9.3
Dizziness	9.3
Dyspnea	9.3
Muscular weakness	9.3
Contusion	7.4
Depression	7.4
Nausea	7.4
Blood pressure increased	5.6
Hypoesthesia	5.6
Muscle stiffness	5.6
Nasopharyngitis	5.6
Neck pain	5.6

EXAMPLE 2

Pharmacokinetics and Anti-Drug Antibody Concentrations

[0260] As discussed in Example 1, plasma biosamples were collected from the subjects at before dosing, and two hours post-dosing through infusion. Infusion was conducted on days 0, 15, 29, 43, 57, and 71 of the clinical trial. These biosamples were used in part to assess concentration of AT-1501 drug levels present in the blood. Shown in FIG. 23 is an overlay of each subject's levels of AT-1501 in plasma over time. From this analysis, the half-life of AT-1501 was determined to be over 14 days. As a consequence, exposure levels increased in subjects over time, so a steady state was not reached.

[0261] Furthermore, there was no evidence of neutralizing ADAs. There were nine ADAs out of 215 samples in 7 subjects (FIGs. 24A-24I). Four of those nine ADAs were detected at week 14, when the subjects were off the test article. One subject (102201) had pre-existing ADA titers prior to the trial. The subjects that were ADA-positive and the time of occurrence was as shown in **Table 9**. All nine ADAs were of very low titer, and were at the cut-off point of detection in the assay. These ADAs were non-neutralizing, and did not affect levels of the drug tegoprobart (AT-1501) that were given to those subjects.

Table 9:

Subject ID	Dose (mg/kg)	Week	Serial Dilution
103202	1	14	2
108204	1	14	2
104202	1	14	4
104203	2	6	2
110202	4	14	4
110204	8	4	4
102201	2	2	1
102201	2	4	1
102201	2	6	1

EXAMPLE 3**General Biomarker Analysis**

[0262] Each biosample collected from subjects during the clinical trial was also assessed for changes in molecular biomarkers. These markers included those used as a measurement for CD40L target engagement (CXCL13, IgA, and CD40L), and as a measurement inflammation (TNF-alpha, IL-6, En-Raged MCP-1, IL-1, IL1-beta, and CRP). Two method of analysis was employed to examine biomarker changes: (1) to calculate regression models and plot all analytes for all subjects, and (2) to normalize the data, then calculate fold-changes of biomarkers over time and correct for false discovery rate.

[0263] Before analysis, data pre-processing was conducted to remove outliers such as subjects with a score of less than 4 in bulbar subdomain, and with an ALSFRS less than 35 at day 0. The subjects that were removed as outliers were as summarized in **Table 10**.

Table 10:

Subject:	109202	111203	103204	103205	107202	114203	114205
Dose:	4 mg/kg	4 mg/kg	8 mg/kg	8 mg/kg	8 mg/kg	8 mg/kg	8 mg/kg
Baseline Scores:	Bulbar <=4	Bulbar <=4 and	Bulbar <=4 and	Total <=34	Bulbar <=4	Bulbar <=4 and	Bulbar <=4

		Total <=34	Total <=34			Total <=34	
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[0264] Furthermore, proteins with sparse positive signals were filtered. These proteins included: AFP, CA-125, CA-19-9, Eotaxin-1, EPO, G-CSF, GH, GM-CSF, hCG, INFg, IL1-a, IL1-b, IL10, IL12-p40, IL12-p70, IL-13, IL-17, IL1-ra, IL-2, IL-3, IL-4, IL-5, IL-6 (Luminex), IL-7, MCP-2, MICA, MIP1-A, PSA-F, SCF, and TNFa (Luminex).

[0265] Upon analysis, it was found that a significant number of proteins were down-regulated following tegoprubart treatment (FIG. 25). The degree of increased or downregulation of proteins was dose dependent.

Biomarkers for Target Engagement

[0266] Engagement of CD40L and CD40 by AT-1501 was assessed by monitoring levels of CXCL13, CD40L, and CD40 in subjects. AT-1501 exposure decreased all three protein levels in a dose-dependent fashion (FIGs. 26A, 26B, 27A, 27B, 28A, and 28B). The reductions were statistically significant at 4 mg/kg and 8 mg/kg doses. The maximum reduction was achieved by the second day of dosing subjects with AT-1501. This demonstrates that the target of CD40L/CD40 was significantly engaged upon treatment.

Pro-Inflammatory Cytokine Biomarkers

[0267] The levels of pro-inflammatory markers and other related stress proteins were also assessed for changes in concentrations after treatment of subjects with AT-1501. Of those assessed, CD40L, CXCL13, CXCL9, TNF-alpha, En-Raged, TNFR2, IgM, IgA, IL2r, CXCL10, CD40, and B2M had the highest reduction in protein concentration following AT-1501 treatment (FIGs. 29A-29L). A significant reduction in concentration was also detected for proteins VCAM, IL-18, IL-16, SAP, MIP1-beta, MDC, C3, CRP, Fibrinogen, IgE, ICAM, and MCP1 (FIGs. 30A-30L). A few proteins increased in concentration upon subject exposure to AT-1501; these included IL-6, ENA-78, RANTES, MMP3, and MMP9 (FIGs. 31A-31E). As a whole, treatment of subjects with AT-1501 decreased the expression of more than 50% of the proteins examined that were associated with inflammation and costimulatory signaling.

[0268] The concentration of neurofilament light chain (NFL) levels was also assessed at week 12 (FIG. 32). There was no statistically significant change in NFL

concentration at any dose. However, the NFL levels at enrollment did correlate with the progression rate.

EXAMPLE 4

ALS Functional Rating Scale

[0269] Every two weeks during the clinical trial, subjects were assessed for changes to their ALS Functional Rating Scale (ALS-FRS) by clinicians. The change in ALS-FRS score was plotted for all subjects over time; as a whole, each cohort worsened at a rate comparable to the general rate of untreated patients, as supplied by the database Pro-ACT (FIG. 33A).

[0270] Next, the data was modified to exclude patients that no longer met the initial enrollment criteria at a time of twelve weeks (FIG. 33B). While this reduced the overall error for each group, the overall trend of ALS-FRS scores correlated strongly with the rate of untreated patients.

[0271] The data was further modified to exclude patients with treatment-related effects (TE) (FIG. 33C). This mostly reduced the decline in the FRS for subjects given low levels of AT-1501, but did not have a significant effect on the higher dosage cohorts.

[0272] Finally, the data was modified to only include those patients who seemed to respond the best to treatment with AT-1501 (termed hereafter as “responders”) (FIG. 33D). This shifted the data most significantly, in which treated subjects, especially those in the 4 mg/kg dosage cohort, had a reduced decline in ALS-FRS score compared to untreated.

[0273] The overall slope for the monthly change in ALS-FRS of human subjects by dosage group is as shown in **Table 11**. The columns are: “All” (no patients are excluded), “EC” (those who did not meet enrollment criteria were excluded), “TE” (those who had treatment-related adverse effects were excluded), and “Res” (only those who responded positively to drug treatment were included). The “Pro-Act” row represents ALS-FRS data for untreated patients, taken from the Pro-ACT database.

Table 11:

Group	All	EC	EC/TE	Res
1 mg/kg	-0.83	-0.83	-0.63	-0.63

2 mg/kg	-0.94	-0.94	-0.75	-0.75
4 mg/kg	-0.5	-0.48	-0.48	-0.36
8 mg/kg	-1.79	-0.93	-0.93	-0.88
ProAct	-0.83	-0.83	-0.83	-0.83

Responders versus Non-Responders

[0274] Why did some patients seemingly respond well to AT-1501 (dubbed “Responders”), while others did not (dubbed “non-Responders”)? In order to answer this question, the ALS-FRS scores were plotted over time for each individual. The ALS-FRS score values for 16 of those individuals (8 Responders, and 8 Non-Responders) is as seen in **Table 12**, and is plotted in graph form in FIG. 34.

Table 12:

Dose	Subject	Screening Value	Baseline Value	Week 3	Week 5	Week 7	Week 9	Week 11	Total change
Responders									
1.0 mg/kg	105206	46	46	46	47	46	46	46	0
2.0 mg/kg	105209	41	41	41	41	41	41	41	0
4.0 mg/kg	104205	47	47	47	47	47	47	47	0
4.0 mg/kg	106201	43	43	43	43	43	43	43	0
4.0 mg/kg	109203	45	46	46	45	45	45	45	0
4.0 mg/kg	111204	41	45	40	44	44	42	41	0
4.0 mg/kg	115205	35	36	38	37	37	36	35	0
8.0 mg/kg	104211	41	41	41	41	40	41	41	0
Non-Responders									
1.0 mg/kg	108202	41	41	37	37	37	37	34	-7
2.0 mg/kg	102201	42	42	37	32	32	30	34	-8
4.0 mg/kg	105211	35	35	32	31	31	29	29	-6
4.0 mg/kg	112201	38	38	37	40	37	24	31	-7
4.0 mg/kg	115203	39	39	36	34	31	32	33	-6
8.0 mg/kg	103204	35	29	28	28	24	24	18	-17
8.0 mg/kg	107202	37	35	34	31	26	27	20	-17
8.0 mg/kg	114203	35	34	34	34	29	28	26	-9

[0275] As can be seen in FIG. 34 and the above table, the subgroup “Responders” had no significant change in ALS-FRS from baseline to week 12. Meanwhile, the “Non-Responders” declined at a rate consistent with untreated patients. This indicates that the group “non-Responders” gained no detectable benefit from AT-1501, while the “Responders” gained significant resistance to loss of ALS-FRS score upon treatment with AT-1501.

[0276] Next, the concentration of proteins in each subject before treatment (the baseline value) with AT-1501 was compared through multiple linear regression analyses. As shown in FIG. 35 and in **Tables 13-14**, there were several proteins that were present at significantly higher concentrations in the plasma of subjects who would later have a positive response to treatment with AT-1501. **Table 13** shows the F statistic and p-value for the differences in average protein concentration for each protein at baseline plasma levels, as normalized for intensity. The differences in each protein at baseline was also analyzed using linear regression as a predictor of the slope for ALS-FRS changes; these values were as shown in **Table 14**. Among those, the most significant was the initial concentrations of NFL (not shown in FIG. 35 due to differences in scale), which had an almost 50-point F statistic. Also, among the most significant initial protein concentrations were MMP9, MMP3, IL2ra, TNF-alpha, and IL-18.

Table 13:

Protein	F Statistic	pValue
MMP3	90.02	2.2E-16
TNFa	35.11	7.206E-09
IL18	34.64	8.961E-09
SAP	33.28	1.70E-07
NFL	26.18	5.04E-07
MCP1	14.38	0.0001751
Enraged	14	0.0002119
MIG	8.559	0.003652
vWF	8.065	0.004765
IgE	7.634	0.006016
MMP9	5.981	0.01493

IL8	5.969	0.01503
IP10	5.926	0.0154
C3	5.65	0.0197
MMP2	5.002	0.02591

Table 14:

Protein	F Statistic	pValue
NFL	44.08	1.41E-10
MMP9	10.77	0.00113
MMP3	10.76	0.001138
IL6	9.978	0.001716
IL18	9.746	0.00194
IgA	9.537	0.002167
TNFR2	7.211	0.007575
TNFa	7.077	0.01629
IL2ra	5.651	0.01796
FactorVIII	5.302	0.02186
CRP	4.108	0.0434

[0277] As shown in FIG. 35 and in **Tables 12-13**, there are statistically significant differences in protein marker expression between Non-Responders and Responders before any treatment. None of these protein levels correlated significantly with co-variables such as ethnicity, gender, age, or time of treatment.

[0278] This finding is incredibly significant, as early assessments for the concentrations of these proteins in the plasma of ALS patients may serve as a predictor for responsiveness of those patients to AT-1501 treatment. That is, if an individual has biomarkers at concentrations corresponding with the Responders cohort, they are likely to benefit significantly from treatment with a compound targeting CD40L/CD40.

Summary

[0279] As a whole, Tegoprubart demonstrates a good safety profile with linear pK and dose proportionality with a low incidence of ADA. Furthermore, Tegoprubart demonstrated target engagement with dose dependent reduction for the T cell marker

CD40L and the B cell markers CXCL13, IgA and IgE as well as increased patient response with dose escalation. Tegoprubart also decreased 3 of the 4 prospectively selected pro-inflammatory markers TNFa, MCP1, and En-Raged in a dose-dependent manner with increased patient response. IL1b was below the limits of detection in the multiplex ELISA and IL6 was not down regulated by tegoprubart.

[0280] Tegoprubart decreased the expression of 23 of 32 pro-inflammatory proteins assayed clearly demonstrating downstream modulation of pro-inflammatory signaling that has been described for inhibition of CD40L in multiple preclinical models of autoimmunity and transplant. A decrease in pro-inflammatory signaling trended towards improvement in disease progression as measured by ALS FRS. Linear regression identified several proteins that correlated with changes in ALSFRS over time.

[0281] Again, the findings reported in the present application indicate that the as early quantification of select proteins in the plasma of ALS patients may serve as a predictor for responsiveness of those patients to treatment with a compound targeting CD40L/CD40.

[0282] As used herein, the section headings are for organizational purposes only and are not to be construed as limiting the described subject matter in any way. All literature and similar materials cited in this application, including but not limited to, patents, patent applications, articles, books, treatises, and internet web pages are expressly incorporated by reference in their entirety for any purpose, including the disclosures specifically referenced herein. When definitions of terms in incorporated references appear to differ from the definitions provided in the present teachings, the definition provided in the present teachings shall control. It will be appreciated that there is an implied “about” prior to the temperatures, concentrations, times, etc. discussed in the present teachings, such that slight and insubstantial deviations are within the scope of the present teachings herein.

[0283] Although this disclosure has been disclosed in the context of certain embodiments and examples, those skilled in the art will understand that the present disclosure extends beyond the specifically disclosed embodiments to other alternative embodiments and/or uses of the embodiments and obvious modifications and equivalents thereof. In addition, while several variations of the disclosure have been shown and described in detail, other modifications, which are within the scope of this disclosure, will be readily apparent to those of skill in the art based upon this disclosure. It is also contemplated that various combinations or sub-combinations of the specific features and

aspects of the embodiments may be made and still fall within the scope of the embodiments. It should be understood that various features and aspects of the disclosed embodiments can be combined with, or substituted for, one another in order to form varying modes or embodiments. Thus, it is intended that the scope of the present disclosure should not be limited by the particular disclosed embodiments described above.

[0284] It should be understood, however, that this detailed description, while indicating preferred embodiments, is given by way of illustration only, since various changes and modifications within the spirit and scope of the disclosure will become apparent to those skilled in the art.

[0285] The terminology used in the description presented herein is not intended to be interpreted in any limited or restrictive manner. Rather, the terminology is simply being utilized in conjunction with a detailed description of embodiments of the systems, methods, and related components. Furthermore, embodiments may comprise several novel features, no single one of which is solely responsible for its desirable attributes or is believed to be essential to practicing the embodiments.

WHAT IS CLAIMED IS:

1. A method of predicting the response of a subject with a disease or disorder to treatment with a compound against CD40L or CD40, the method comprising:
 - collecting a sample from the subject;
 - measuring a concentration of at least one biomarker present in the sample, wherein the at least one biomarker is NFL, MMP9, MMP3, IL6, IL18, IgA, TNFR2, TNFa, IL2ra, FactorVIII, CRP, SAP, MCP1, En-Raged, MIG, vWF, IgE, IL8, IP10, C3, and MMP2;
 - wherein the concentration of the at least one biomarker is predictive that the subject is likely to be responsive to treatment against the disease or disorder with a compound against CD40L or CD40.
2. The method of claim 1, wherein the disease or disorder is inflammation, a neurodegenerative disease, or neuromuscular disease.
3. The method of claim 2, wherein the neurodegenerative disease or neuromuscular disease is Amyotrophic Lateral Sclerosis, Alzheimer's Disease, Parkinson's Disease, Myasthenia Gravis, Multifocal Motor Neuropathy, Primary Lateral Sclerosis, Spinal Muscular Atrophy, Kennedy's Disease, or Spinocerebellar Ataxia.
4. The method of claim 3, wherein the neurodegenerative disease or neuromuscular disease is Amyotrophic Lateral Sclerosis (ALS).
5. The method of claim 1, wherein the subject is mammalian and/or human.
6. The method of claim 1, wherein the compound blocks the interaction of CD40 and CD40L.
7. The method of claim 1, wherein the compound is an anti-CD40L antibody or an anti-CD40 antibody.
8. The method of claim 1, wherein the compound is tegoprubart.
9. The method of claim 1, wherein the compound is MR1 or 5c8.
10. A method treating a subject with a disease or disorder, the method comprising:
 - administering a therapeutically effective dose of a compound against CD40L or CD40, wherein administering the therapeutically effective dose maintains the compound in plasma of the subject at a concentration between 5 µg/mL and 100 µg/mL; preferably at a dose that is between 30 µg/mL and 60 µg/mL.
11. The method of claim 10, wherein the disease or disorder is inflammation, a neurodegenerative disease, or neuromuscular disease.

12. The method of claim 11, wherein the neurodegenerative disease or neuromuscular disease is Amyotrophic Lateral Sclerosis, Alzheimer's Disease Parkinson's Disease, Myasthenia Gravis, Multifocal Motor Neuropathy, Primary Lateral Sclerosis, Spinal Muscular Atrophy, Kennedy's Disease, or Spinocerebellar Ataxia.

13. The method of claim 12, wherein the neurodegenerative disease or neuromuscular disease is Amyotrophic Lateral Sclerosis (ALS).

14. The method of claim 10, wherein the subject is mammalian and/or human.

15. The method of claim 10, wherein the compound blocks the interaction of CD40 and CD40L.

16. The method of claim 10, wherein the compound is an anti-CD40L antibody or an anti-CD40 antibody.

17. The method of claim 10, wherein the compound is tegoprubart.

18. The method of claim 10, wherein the compound is MR1 or 5c8.

19. The method of claim 10, wherein the compound is administered at least once every two weeks.

20. The method of claim 10, wherein the compound is administered for a period of at least 12 weeks.

21. The method of claim 10, wherein the compound is administered at a dose of between 200 mg/kg and 100 mg/kg; preferably at a dose between 1 mg/kg and 8 mg/kg; most preferably at a dose between 2 mg/kg and 4 mg/kg.

22. The method of claim 10, the method further comprising administering a second pharmaceutically effective compound.

23. The method of claim 22, wherein the second compound blocks the interaction between CD28 and CD86 or between CD28 and CD80.

24. The method of claim 22, wherein the second compound targets at least one of the biomarkers selected from the group consisting of: interleukins, cytokines, and proinflammatory markers.

25. The method of claim 24, wherein the second compound targets at least one of the biomarkers selected from the group consisting of: NFL, CXCL13, CD40L, CXCL9, TNF-alpha, En-Raged, TNFR2, IgM, IgA, Il2r, CXCL10, CD40, B2M, VCAM, Il-18, Il-16, SAP, MIP1beta, MDC, C3, CRP, Fibrogen, IgE, ICAM, FactorVIII, Ena-78, Pal1, Rantes, TIMP1, vWF, Il-6, MCP1, MMP3, MMP9, and MMP2.

26. The method of claim 22, wherein the second compound is a CTLA4-Ig fusion protein, an abatacept, a belatacept, or a galiximab.

27. The method of claim 10, wherein the compound is administered orally, parenterally, or topically.

28. The method of claim 27, wherein the compound is administered parenterally.

29. The method of claim 28, wherein the compound is administered by injection, most preferably intravenous, intramuscular, or subcutaneous injection.

30. The method of claim 1, wherein the disease or disorder is an inflammatory or immune disease or disorder selected from the group consisting of colitis, drug induced lupus nephritis, graft versus host disease, immune graft response, transplant rejection and atherosclerosis.

31. The method of claim 1, wherein the disease or disorder is an autoimmune disease, selected from the group consisting of systemic lupus erythematosus, type-1 diabetes, myasthenia gravis, inflammatory bowel disease, immune thrombocytopenic purpura, rheumatoid arthritis, psoriasis, Addison's disease, Crohn's disease, uveitis, multiple sclerosis, hemolytic anemia, and Graves' disease.

32. The method of claim 1, wherein the compound that targets CD40L or CD40 is an antibody that comprises:

- (a) a heavy chain variable region (V_H) comprising:
 - i) a CDRH1 domain comprising the sequence set forth in SEQ ID NO: 9;
 - ii) a CDRH2 domain comprising the sequence set forth in SEQ ID NO: 10, 11, 12, 13, or 14; and
 - iii) a CDRH3 domain comprising the sequence set forth in SEQ ID NO: 15; and
- (b) a light chain variable region (V_L) comprising:
 - i) a CDRL1 domain comprising the sequence set forth in SEQ ID NO: 16 or 17;
 - ii) a CDRL2 domain comprising the sequence set forth in SEQ ID NO: 18 or 19; and
 - iii) a CDRL3 domain comprising the sequence set forth in SEQ ID NO: 20.

33. The method of any one of claims 1-32, wherein the compound that targets CD40L or CD40 is an antibody that comprises:

- (a) a heavy chain variable region (V_H) having the amino acid sequence as set forth in SEQ ID NOs: 1, 2, 3, or 4; and
 - (b) a light chain variable region (V_L) having the amino acid sequence as set forth in SEQ ID NOs: 5, 6, 7, or 8.
34. A method of modulating a concentration of at least one biomarker in a subject, the method comprising:
- administering to the subject a therapeutically effective amount of a compound against CD40L or CD40, wherein the at least one biomarker is NFL, CXCL13, CD40L, CXCL9, TNF-alpha, En-Raged, TNFR2, IgM, IgA, IL2r, CXCL10, CD40, B2M, VCAM, IL-18, IL-16, SAP, MIP1beta, MDC, C3, CRP, Fibrogen, IgE, ICAM, FactorVIII, Ena-78, Pal1, Rantes, TIMP1, vWF, IL-6, MCP1, MMP3, MMP9, MCP1, MIG, vWF, IL-8, IP10, or MMP2.
35. The method of claim 34, wherein the concentration of the at least one biomarker increases following administration of the compound.
36. The method of claim 34, wherein the concentration of the at least one biomarker decreases following administration of the compound.
37. The method of claim 34, wherein the concentration of the at least one biomarker increases, and the concentration of at least one other biomarker decreases, following administration of the compound.
38. The method of claim 34, wherein the subject has a disease or disorder.
39. The method of claim 38, wherein the disease or disorder is inflammation, a neurodegenerative disease, or neuromuscular disease.
40. The method of claim 39, wherein the neurodegenerative disease or neuromuscular disease is Amyotrophic Lateral Sclerosis, Alzheimer's Disease Parkinson's Disease, Myasthenia Gravis, Multifocal Motor Neuropathy, Primary Lateral Sclerosis, Spinal Muscular Atrophy, Kennedy's Disease, or Spinocerebellar Ataxia.
41. The method of claim 40, wherein the neurodegenerative disease or neuromuscular disease is Amyotrophic Lateral Sclerosis (ALS).
42. The method of claim 34, wherein the subject is mammalian and/or human.
43. The method of claim 34, wherein the compound blocks the interaction of CD40 and CD40L.
44. The method of claim 34, wherein the compound is an anti-CD40L antibody or an anti-CD40 antibody.
45. The method of claim 34, wherein the compound is tegoprubart.

46. The method of claim 34, wherein the compound is MR1 or 5c8.
47. The method of claim 34, wherein administering the therapeutically effective dose maintains the compound in plasma of the subject at a concentration between 5 µg/mL and 100 µg/mL; preferably at a dose that is between 30 µg/mL and 60 µg/mL.
48. The method of claim 34, wherein the compound is administered at least once every two weeks.
49. The method of claim 34, wherein the compound is administered for a period of at least 12 weeks.
50. The method of claim 34, wherein the compound is administered at a dose of between 200 mg/kg and 100 mg/kg; preferably at a dose between 1 mg/kg and 8 mg/kg; most preferably at a dose between 2 mg/kg and 4 mg/kg.
51. The method of claim 34, wherein the compound is administered orally, parenterally, or topically.
52. The method of claim 51, wherein the compound is administered parenterally.
53. The method of claim 52, wherein the compound is administered by injection, most preferably intravenous, intramuscular, or subcutaneous injection.
54. The method of claim 34, wherein the disease or disorder is an inflammatory or immune disease or disorder selected from the group consisting of colitis, drug induced lupus nephritis, graft versus host disease, immune graft response, transplant rejection and atherosclerosis.
55. The method of claim 34, wherein the disease or disorder is an autoimmune disease, selected from the group consisting of systemic lupus erythematosus, type-1 diabetes, myasthenia gravis, inflammatory bowel disease, immune thrombocytopenic purpura, rheumatoid arthritis, psoriasis, Addison's disease, Crohn's disease, uveitis, multiple sclerosis, hemolytic anemia, and Graves' disease.
56. The method of claim 34, wherein the compound that targets CD40L or CD40 is an antibody that comprises:
- (a) a heavy chain variable region (V_H) comprising;
 - i) a CDRH1 domain comprising the sequence set forth in SEQ ID NO: 9;
 - ii) a CDRH2 domain comprising the sequence set forth in SEQ ID NO: 10, 11, 12, 13, or 14; and

- iii) a CDRH3 domain comprising the sequence set forth in SEQ ID NO: 15; and
- (b) a light chain variable region (V_L) comprising:
 - i) a CDRL1 domain comprising the sequence set forth in SEQ ID NO: 16 or 17;
 - ii) a CDRL2 domain comprising the sequence set forth in SEQ ID NO: 18 or 19; and
 - iii) a CDRL3 domain comprising the sequence set forth in SEQ ID NO: 20.

57. The method of claim 34, wherein the compound that targets CD40L or CD40 is an antibody that comprises:

- (a) a heavy chain variable region (V_H) having the amino acid sequence as set forth in SEQ ID NOs: 1, 2, 3, or 4; and
- (b) a light chain variable region (V_L) having the amino acid sequence as set forth in SEQ ID NOs: 5, 6, 7, or 8.

58. A method of maintaining or improving the ALS-FRS scores of a subject with ALS, the method comprising:

administering a therapeutically effective dose of a compound against CD40L or CD40 wherein administering the therapeutically effective dose maintains the compound in plasma of the subject at a concentration between 5 µg/mL and 100 µg/mL; preferably at a dose that is between 30 µg/mL and 60 µg/mL.

59. A method of pre-screening and treating a subject with a disease or disorder with a compound against CD40L or CD40, the method comprising:

collecting a sample from the subject;

detecting a concentration of at least one biomarker present in the fluid, wherein the at least one biomarker is NFL, MMP9, MMP3, IL6, IL18, IgA, TNFR2, TNFa, IL2ra, FactorVIII, CRP, SAP, MCP1, En-Raged, MIG, vWF, IgE, IL8, IP10, C3, or MMP2;

screening the concentration of the at least one biomarker to the concentration of the at least one biomarker in a subject that does not have that disease or disorder; and

administering a therapeutically effective dose of a compound against CD40L or CD40 such that the plasma of the subject maintains a concentration of the compound at a dose that is between 5 µg/mL and 100 µg/mL; preferably at a dose that is between 30 µg/mL and 60 µg/mL.

60. The method of claim 59, wherein the compound is administered to the subject if the concentration of the at least one biomarker in the subject is significantly different than the concentration of the at least one biomarker in a subject that does not have the disease or disorder.