Claims

1) An isolated antagonistic antibody or an antigen binding portion thereof specifically binding human CD154 of SEQ ID NO: 1, comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 37 (RASQSISSYLN), a LCDR2 of SEQ ID NO: 44 (YANSLQS), a LCDR3 of SEQ ID NO: 52 (QQSDSIPWT), a heavy chain complementarity determining region (HCDR) 1 of SEQ ID NO: 17 (SYGIS), a HCDR2 of SEQ ID NO: 23 (WISPIFGNTNYAQKFQG), and a HCDR3 of SEQ ID NO: 30 (SRYYGDLDY), wherein optionally:
   a) the LCDR1 residue Q4 is mutated to A, C, D, E, F, G, H, I, K, L, M, N, R, S, T, V, W or Y;
   b) the LCDR1 residue S5 is mutated to A, C, D, E, F, G, H, I, K, L, M, N, Q, R, T, V, W or Y;
   c) the LCDR1 residue S7 is mutated to A, C, D, E, F, G, H, I, K, L, M, N, Q, R, T, V, W or Y;
   d) the LCDR1 residue S8 is mutated to A, C, D, E, F, G, H, I, K, L, M, N, Q, R, T, V, W or Y;
   e) the LCDR2 residue A2 is mutated to S;
   f) the LCDR2 residue N3 is mutated to A, C, D, E, F, G, H, I, K, L, M, Q, R, S, T, V, W or Y;
   g) the LCDR2 residue S4 is mutated to A, C, D, E, F, G, H, I, K, L, M, N, Q, R, T, V, W or Y;
   h) the LCDR2 residue L5 is mutated to A, C, D, E, F, G, H, I, K, M, N, Q, R, S, T, V, W or Y;
   i) the LCDR2 residue Q6 is mutated to E, D or N;
   j) the LCDR2 residue S7 is mutated to A, C, D, E, F, G, H, I, K, L, M, N, Q, R, T, V, W or Y;
   k) the LCDR3 residue S3 is mutated to A;
   l) the LCDR3 residue D4 is mutated to N;
   m) the LCDR3 residue S5 is mutated to A, C, D, E, F, G, H, I, K, L, M, N, Q, R, T, V, W or Y;
   n) the LCDR3 residue I6 is mutated to A, C, D, E, G, K, L, M, N, Q, R, S, T or V;

o) the HCDR1 residue S1 is mutated to A, C, D, E, G, I, K, L, M, N, Q, R, T or V;
p) the HCDR1 residue I4 is mutated to M, L or V;
q) the HCDR1 residue S5 is mutated to A;
r) the HCDR2 residue S3 is mutated to A, T or V;
s) the HCDR2 residue P4 is mutated to V, T, L Q or E;
t) the HCDR2 residue N8 is mutated to A, C, D, E, F, G, H, I, K, L, M, Q, R, S, T, V, W or Y;
u) the HCDR2 residue T9 is mutated to A, C, D, E, F, G, H, I, K, L, M, Q, R, S, T, V, W or Y;
v) the HCDR2 residue N10 is mutated to A, C, D, E, F, G, H, I, K, L, M, Q, R, S, T, V, W or Y;
w) the HCDR3 residue S1 is mutated to A or M;
x) the HCDR3 residue R2 is mutated to A, S, Q or K; or
y) the HCDR3 residue L7 is mutated to M.

2) An isolated antagonistic antibody or an antigen binding portion thereof specifically binding human CD154 of SEQ ID NO: 1, comprising a heavy chain complementarity determining region (HCDR) 1 of SEQ ID NO: 17 (SYGIS), a HCDR2 of SEQ ID NO: 23 (WISPIFGNTNYAQKFQG) and a HCDR3 of SEQ ID NO: 30 (SRYYGDLDY), wherein optionally
a) the HCDR1 residue S1 is mutated to A, C, D, E, G, I, K, L, M, N, Q, R, T or V;
b) the HCDR1 residue I4 is mutated to M, L or V;
c) the HCDR1 residue S5 is mutated to A;
d) the HCDR2 residue S3 is mutated to A, T or V;
e) the HCDR2 residue P4 is mutated to V, T, L Q or E;
f) the HCDR2 residue N8 is mutated to A, C, D, E, F, G, H, I, K, L, M, Q, R, S, T, V, W or Y;
g) the HCDR2 residue T9 is mutated to A, C, D, E, F, G, H, I, K, L, M, Q, R, S, T, V, W or Y;
h) the HCDR2 residue N10 is mutated to A, C, D, E, F, G, H, I, K, L, M, Q, R, S, T, V, W or Y;
i) the HCDR3 residue S1 is mutated to A or M;

j) the HCDR3 residue R2 is mutated to A, S, Q or K;

k) the HCDR3 residue L7 is mutated to M; and

a light chain complementarity determining region (LCDR) 1, a LCDR2 and a LCDR3 of:

a) SEQ ID NOs: 36, 43 and 51, respectively;

b) SEQ ID NOs: 37, 44 and 52, respectively;

c) SEQ ID NOs: 38, 45 and 53, respectively;

d) SEQ ID NOs: 39, 46 and 54, respectively;

e) SEQ ID NOs: 40, 47 and 55, respectively;

f) SEQ ID NOs: 41, 47 and 56, respectively;

g) SEQ ID NOs: 42, 48 and 57, respectively;

h) SEQ ID NOs: 37, 49 and 52, respectively; or

i) SEQ ID NOs: 37, 50 and 52, respectively.

3) The antibody or antigen binding portion thereof of claim 1, comprising the HCDR1 of SEQ ID NO: 17 (SYGIS), the HCDR2 of SEQ ID NO: 23 (WISPIFGNTNYAQKFQG), the HCDR3 of SEQ ID NO: 30 (SRYYGDLDY), the LCDR1 of SEQ ID NO: 37 (RASQSISSYLN), the LCDR2 of SEQ ID NO: 44 (YANSLQS) and the LCDR3 of SEQ ID NO: 52 (QQSDSIPWT).

4) The antibody or antigen binding portion thereof of claim 1, wherein the antibody has at least one of the following properties:

a) an immune complex of the antibody and soluble human CD154 (shCD154) does not activate platelets, wherein platelet activation is measured by P-selectin surface expression on platelets;

b) binds to CD154 with a dissociation constant (K_D) of about $5x10^{-9}$ M or less, when the K_D is measured using ProteOn XPR36 system at 25°C in Dulbecco's phosphate buffered saline containing 0.03% polysorbate P20 and 100 µg/ml bovine serum albumin;

c) inhibits CD154-mediated human B cell proliferation with an IC_{50} value of about $2.7x10^{-9}$ M or less; or

d) inhibits CD154-mediated expression of secreted embryonic alkaline phosphatase (SEAP) under NF-κB-inducible interferon-β (IFN-β) minimal promoter in HEK293 cells stably expressing SEAP and human CD40 with an IC_{50} value of about $2.1x10^{-8}$ M or less.

5) The antibody or antigen binding portion thereof of claim 1, wherein the human CD154 is a homotrimer and the antibody binds a first CD154 monomer in the homotrimer within amino acid residues 182-207 of CD154 and a second CD154 monomer in the homotrimer within amino acid residues 176-253 of CD154, wherein residue numbering is according to SEQ ID NO: 1.
6) The antibody or antigen binding portion thereof of claim 5, wherein the antibody binds residues E182, S185, Q186, A187, P188, S214, A215 and R207 in the first CD154 monomer, wherein residue numbering is according to SEQ ID NO: 1.
7) The antibody or antigen binding portion thereof of claim 5, wherein the antibody binds residues T176, F177, C178, Q220, S248, H249, G250 and F353 in the second CD154 monomer, wherein residue numbering is according to SEQ ID NO: 1.
8) The antibody or antigen binding portion thereof of claim 2, comprising a heavy chain variable region (VH) of SEQ ID NO: 59.
9) The antibody or antigen binding portion thereof of claim 8, comprising a light chain variable region (VL) of SEQ ID NOs: 65, 66, 67, 68, 69, 70, 71, 72 or 73.
10) The antibody or antigen binding portion thereof of claim 9, comprising the VL of SEQ ID NOs: 66, 72 or 73.
11) The antibody or antigen binding portion thereof of claim 10, comprising the VH of SEQ ID NO: 59 and the VL of SEQ ID NO: 66.
12) The antibody or antigen binding portion thereof of claim 10, comprising the VH of SEQ ID NO: 59 and the VL of SEQ ID NO: 72.
13) The antibody or antigen binding portion thereof of claim 10, comprising the VH of SEQ ID NO: 59 and the VL of SEQ ID NO: 73.
14) The antibody or antigen binding portion thereof of claim 1, wherein the antibody is of IgG1, IgG2, IgG3 or IgG4 isotype.
15) The antibody or antigen binding portion thereof of claim 14 comprising at least one substitution in an Fc region, wherein the at least one substitution in the Fc region is a substitution L234A, L235A, G237A, P238S, M252Y, S254T, T256E, H268A, A330S or P331S, wherein residue numbering is according to the EU Index.

16) The antibody or antigen binding portion thereof of claim 15, comprising substitutions L234A, L235A, G237A, P238S, H268A, A330S or P331S in the Fc region, wherein residue numbering is according to the EU Index.

17) The antibody or antigen binding portion thereof of claim 14 comprising at least one substitution in an Fc region, wherein the at least one substitution in the Fc region is a substitution V234A, G237A, P238S, M252Y, S254T, T256E, H268A, V309L, A330S or P331S, wherein residue numbering is according to the EU Index.

18) The antibody or antigen binding portion thereof of claim 17, comprising substitutions V234A, G237A, P238S, H268A, V309L, A330S and P331S in the Fc region, wherein residue numbering is according to the EU Index.

19) The antibody or antigen binding portion thereof of claim 14, comprising a heavy chain (HC) and a light chain (LC) of SEQ ID NOs:

a) 80 and 81, respectively;

b) 82 and 81, respectively; or

c) 83 and 81, respectively.

20) The antibody or antigen binding portion thereof of claim 1, wherein the antibody is multispecific.

21) An isolated antagonistic antibody or an antigen binding portion thereof specifically binding CD154 of SEQ ID NO: 1, comprising

a) the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 17, 23, 30, 37, 44 and 52, respectively;

b) the VH of SEQ ID NO: 59 and the VL of SEQ ID NO: 66; or

c) the heavy chain of SEQ ID NO: 80 and the light chain of SEQ ID NO: 81.

22) The antibody or antigen binding portion thereof of claim 1, wherein the human CD154 is a homotrimer and the antibody binds a first CD154 monomer in the homotrimer within amino acid residues 182-207 of CD154 and a second CD154 monomer in the homotrimer within amino acid residues 176-253 of CD154, wherein residue numbering is according to SEQ ID NO: 1.

23) An immunoconjugate comprising the antibody or antigen binding portion thereof of claim 1 linked to a therapeutic agent or an imaging agent.

24) A pharmaceutical composition comprising the antibody or antigen binding portion thereof of claim 1 and a pharmaceutically accepted carrier.

25) Use of the pharmaceutical composition of claim 24 in the manufacture of a medicament for inhibiting a biological activity of the human CD154 in a subject in need thereof, wherein the subject is in need of treatment of an autoimmune disease or an immune-mediated inflammatory disease.

26) The use of claim 25, wherein the immune-mediated inflammatory disease or the autoimmune disease is arthritis, systemic lupus erythematosus (SLE), inflammatory bowel disease, transplantation, kidney transplantation, skin transplantation, bone marrow transplantation, graft versus host disease (GVHD), immune thrombocytopenia (ITP), multiple sclerosis, thyroiditis, type I diabetes or atherosclerosis.

27) The use of claim 26 wherein the arthritis is rheumatoid arthritis, juvenile arthritis, psoriatic arthritis, Reiter's syndrome, ankylosing spondylitis, or gouty arthritis.

28) The use of claim 25, wherein the autoimmune disease or the immune-mediated inflammatory disease is lupus.

29) The use of claim 25, wherein the autoimmune disease or the immune-mediated inflammatory disease is transplantation.

30) The use of claim 25, wherein the autoimmune disease or the immune-mediated inflammatory disease is inflammatory bowel disease (IBD).

31) The use of claim 30, wherein the IBD is Crohn's disease or ulcerative colitis.

32) The use of claim 25, wherein the medicament further comprises a second therapeutic agent.

33) The use of claim 32, wherein the second therapeutic agent is selected from the group consisting of nonsteroidal anti-inflammatory drugs (NSAIDs), salicylates, hydroxychloroquine, sulfasalazine, corticosteroids, cytotoxic drugs, immunosuppressive drugs and antibodies.

34) An isolated antagonistic antibody or an antigen binding portion thereof specifically binding CD154 of SEQ ID NO: 1, comprising an HCDR1, an HCDR2, an HCDR3, a LCDR1, a LCDR2, and a LCDR3 of

a) SEQ ID NOs: 16, 22, 29, 36, 43 and 51, respectively;
b) SEQ ID NOs: 17, 23, 30, 37, 44 and 52, respectively;
c) SEQ ID NOs: 16, 24, 31, 38, 45 and 53, respectively;

d) SEQ ID NOs: 18, 25, 32, 39, 46 and 54, respectively;

e) SEQ ID NOs: 19, 26, 33, 40, 47 and 55, respectively;

f) SEQ ID NOs: 20, 27, 34, 41, 47 and 56, respectively;

g) SEQ ID NOs: 21, 28, 35, 42, 48 and 57, respectively;

h) SEQ ID NOs: 17, 23, 30, 37, 49 and 52, respectively; or

i) SEQ ID NOs: 17, 23, 30, 37, 50 and 52, respectively, wherein the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 are defined by Kabat, Chothia or IMGT.

35) The antibody or antigen binding portion thereof of claim 34, comprising

a) the HCDR1, the HCDR2 and the HCDR3 of SEQ ID NOs: 16, 22 and 29, respectively, and the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 36, 43 and 51, respectively; or

b) the VH of SEQ ID NO: 58 and the VL of SEQ ID NO: 65.

36) The antibody or antigen binding portion thereof of claim 34, comprising

a) the HCDR1, the HCDR2 and the HCDR3 of SEQ ID NOs: 17, 23 and 30, respectively, and the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 37, 44 and 52, respectively; or

b) the VH of SEQ ID NO: 59 and the VL of SEQ ID NO: 66.

37) The antibody or antigen binding portion thereof of claim 34, comprising

a) the HCDR1, the HCDR2 and the HCDR3 of SEQ ID NOs: 16, 24 and 31, respectively, and the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 38, 45 and 53, respectively; or

b) the VH of SEQ ID NO: 60 and the VL of SEQ ID NO: 67.

38) The antibody or antigen binding portion thereof of claim 34, comprising

a) the HCDR1, the HCDR2 and the HCDR3 of SEQ ID NOs: 18, 25 and 32, respectively, and the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 39, 46 and 54, respectively; or

b) the VH of SEQ ID NO: 61 and the VL of SEQ ID NO: 68.

39) The antibody or antigen binding portion thereof of claim 34, comprising

a) the HCDR1, the HCDR2 and the HCDR3 of SEQ ID NOs: 19, 26 and 33, respectively, and LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 40, 47 and 55, respectively; or

b) the VH of SEQ ID NO: 62 and the VL of SEQ ID NO: 69.

40) The antibody or antigen binding portion thereof of claim 34, comprising
    a) the HCDR1, the HCDR2 and the HCDR3 of SEQ ID NOs: 20, 27 and 34, respectively, and the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 41, 47 and 56, respectively; or
    b) the VH of SEQ ID NO: 63 and the VL of SEQ ID NO: 70.
41) The antibody or antigen binding portion thereof of claim 34, comprising
    a) the HCDR1, the HCDR2 and the HCDR3 of SEQ ID NOs: 21, 28 and 35, respectively, and the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 42, 48 and 57, respectively; or
    b) the VH of SEQ ID NO: 64 and the VL of SEQ ID NO: 71.
42) The antibody or antigen binding portion thereof of claim 34, comprising
    a) the HCDR1, the HCDR2 and the HCDR3 of SEQ ID NOs: 17, 23 and 30, respectively, and LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 37, 49 and 52, respectively; or
    b) the VH of SEQ ID NO: 59 and the VL of SEQ ID NO: 72.
43) The antibody or antigen binding portion thereof of claim 34, comprising
    a) the HCDR1, the HCDR2 and the HCDR3 of SEQ ID NOs: 17, 23 and 30, respectively, and the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 37, 50 and 52, respectively; or
    b) the VH of SEQ ID NO: 59 and the VL of SEQ ID NO: 73.
44) The antibody or antigen binding portion thereof of claim 34, wherein the antibody is of IgG1, IgG2, IgG3 or IgG4 isotype.
45) The antibody or antigen binding portion thereof of claim 44 comprising at least one substitution in an Fc region, wherein the at least one substitution in the Fc region is a substitution L234A, L235A, G237A, P238S, M252Y, S254T, T256E, H268A, A330S or P331S, wherein residue numbering is according to the EU Index.
46) The antibody or antigen binding portion thereof of claim 45, comprising substitutions L234A, L235A, G237A, P238S, H268A, A330S and P331S in the Fc region, wherein residue numbering is according to the EU Index.
47) The antibody or antigen binding portion thereof of claim 44 comprising at least one substitution in an Fc region, wherein the at least one substitution in the Fc region is a

substitution V234A, G237A, P238S, M252Y, S254T, T256E, H268A, V309L, A330S or P331S, wherein residue numbering is according to the EU Index.

48) The antibody or antigen binding portion thereof of claim 47, comprising substitutions V234A, G237A, P238S, H268A, V309L, A330S and P331S in the Fc region, wherein residue numbering is according to the EU Index.

49) The antibody or antigen binding portion thereof of claim 34, wherein the antibody is multispecific.

50) The antibody or antigen binding portion thereof of claim 34, wherein the antibody has at least one of the following properties:

a) an immune complex of the antibody and soluble human CD154 (shCD154) does not activate platelets, wherein platelet activation is measured by P-selectin surface expression on platelets;

b) binds to CD154 with a dissociation constant (K_D) of about $5x10^{-9}$ M or less, when the K_D is measured using ProteOn XPR36 system at 25°C in Dulbecco's phosphate buffered saline containing 0.03% polysorbate P20 and 100 µg/ml bovine serum albumin;

c) inhibits CD154-mediated human B cell proliferation with an IC_{50} value of about $2.7x10^{-9}$ M or less; or

d) inhibits CD154-mediated expression of secreted embryonic alkaline phosphatase (SEAP) under NF-κB-inducible interferon-β (IFN-β) minimal promoter in HEK293 cells stably expressing SEAP and human CD40 with an IC_{50} value of about $2.1x10^{-8}$ M or less.

51) An immunoconjugate comprising the antibody or antigen binding portion thereof of claim 34 linked to a therapeutic agent or an imaging agent.

52) A pharmaceutical composition comprising the antibody or antigen binding portion thereof of claim 34 and a pharmaceutically accepted carrier.

53) Use of the pharmaceutical composition of claim 52 in the manufacture of a medicament for inhibiting a biological activity of the CD154 in a subject in need thereof wherein the subject is in need of treatment of an autoimmune disease or an immune-mediated inflammatory disease.

54) The use of claim 53, wherein the immune-mediated inflammatory disease or the autoimmune disease is arthritis, systemic lupus erythematosus (SLE), inflammatory bowel disease, transplantation, kidney transplantation, skin transplantation, bone marrow transplantation,

graft versus host disease (GVHD), immune thrombocytopenia (ITP), multiple sclerosis, thyroiditis, type I diabetes or atherosclerosis.

55) The use of claim 54, wherein the arthritis is rheumatoid arthritis.

56) The use of claim 53, wherein the autoimmune disease or the immune-mediated inflammatory disease is lupus.

57) The use of claim 53, wherein the autoimmune disease or the immune-mediated inflammatory disease is transplantation.

58) The use of claim 53, wherein the autoimmune disease or the immune-mediated inflammatory disease is inflammatory bowel disease.

59) The use of claim 58, wherein the inflammatory bowel disease is Crohn's disease or ulcerative colitis.

60) The use of claim 53, wherein the medicament further comprises a second therapeutic agent.

61) The use of claim 60, wherein the second therapeutic agent is selected from the group consisting of nonsteroidal anti-inflammatory drugs (NSAIDs), salicylates, hydroxychloroquine, sulfasalazine, corticosteroids, cytotoxic drugs, immunosuppressive drugs and antibodies.

62) A kit comprising the antibody or antigen binding portion thereof of claim 1.

63) The kit of claim 62, further comprising reagents for detecting the antibody or antigen binding portion thereof.

64) An immunoconjugate comprising the antibody or antigen binding portion thereof of claim 21 linked to a therapeutic agent or an imaging agent.

65) A pharmaceutical composition comprising the antibody or antigen binding portion thereof of claim 21 and a pharmaceutically accepted carrier.

66) Use of the pharmaceutical composition of claim 65 in the manufacture of a medicament for inhibiting a biological activity of the CD154 in a subject in need thereof, wherein the subject is in need of treatment of an autoimmune disease or an immune-mediated inflammatory disease.