

CLAIMS:

1. A method of treating and/or preventing cancer in a subject in need thereof, the method comprising decreasing soluble CD28 (sCD28) levels in said subject.
2. A method of improving PD-1 and/or PD-L1 based immunotherapy in a subject in need thereof, the method comprising decreasing sCD28 levels in said subject.
3. The method of claim 2, wherein said subject in need of immunotherapy suffers from cancer.
4. The method of any one of claims 1 to 3, wherein said subject does not respond or lowly responds to PD-1 and/or PD-L1 based immunotherapy.
5. The method of any one of claims 1 to 4, wherein said decreasing occurs in the blood, peripheral blood, or tumor microenvironment of said subject.
6. The method of any one of claims 1 to 5, wherein said decreasing comprises at least one of:
 - a. administering to said subject an agent that binds to sCD28 and wherein said agent, upon binding, degrades said sCD28 or targets said sCD28 for degradation;
 - b. administering to said subject an inhibitory nucleic acid molecule, wherein said molecule binds to an mRNA coding for sCD28 and does not bind to an mRNA coding for membranal CD28 (mCD28);
 - c. administering to said subject an agent that binds mCD28 and inhibits proteolytic cleavage of said mCD28;
 - d. administering to said subject a dimeric peptide comprising a stalk region of human CD28;
 - e. administering to said subject an agent that inhibits a protease capable of cleaving mCD28 and
 - f. withdrawing blood from said subject, decreasing the amount of sCD28 in said blood and returning said blood to said subject.
7. The method of claim 6, wherein the stalk region,
 - a. comprises the amino acid sequence GKHLCPSPFPGPSKP (SEQ ID NO: 9) or KGKHLCPSPFPGPS (SEQ ID NO: 36);
 - b. consists of the amino acid sequence HVKGKHLCPSPFPGPSKP (SEQ ID NO: 10); or
 - c. both (a) and (b).

8. The method of any one of claims 1 to 7, wherein said method does not degrade mCD28 or decrease mCD28-mediated immune cell activation.
9. The method of any one of claims 1 to 8, wherein said subject's blood prior to said decreasing comprises at least 5 ng/ml sCD28.
10. The method of any one of claims 1 to 9, wherein said cancer is selected from melanoma, head and neck, non-small cell lung cancer, ovarian, kidney, gastric and colorectal.
11. The method of claim 10, wherein said cancer is selected from melanoma, head and neck, non-small cell lung cancer, ovarian, and colorectal.
12. The method of any one of claims 1 to 11, further comprising administering another immunotherapy to said subject.
13. The method of claim 12, wherein said immunotherapy is selected from:
 - a. a checkpoint inhibitor;
 - b. a chimeric antigen receptor (CAR) based therapy; and
 - c. a cancer vaccine.
14. The method of claims 13, wherein said checkpoint inhibitor is a PD-1 and/or PD-L1 based immunotherapy.
15. An agent that binds membranal CD28 (mCD28) and inhibits proteolytic cleavage of said mCD28.
16. The agent of claim 15, wherein said agent is neither a CD28 agonist or antagonist.
17. The agent of claim 15 or 16, wherein said agent neither degrades said mCD28 nor inhibits mCD28-mediated immune cell activation.
18. The agent of any one of claims 15 to 17, wherein said agent
 - a. does not induce antibody-dependent cell-mediated cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC);
 - b. comprises IgG2 or IgG4;
 - c. comprises an Fc domain engineered to decrease CDC, ADCC or both;
 - d. lacks an Fc domain;
 - e. is a Fab fragment;
 - f. is a single chain antibody;
 - g. is a single-domain antibody;
 - h. is a small molecule;

- i. is a peptide with specific binding to mCD28; or
 - j. a combination thereof.
19. The agent of any one of claims 15 to 18, wherein said agent binds within the stalk region of CD28.
20. The agent of claim 19, wherein the stalk region
- a. comprises the amino acid sequence GKHLCPSPLFPGPSKP (SEQ ID NO: 9) or KGKHLCPSPLFPGPS (SEQ ID NO: 36);
 - b. consists of the amino acid sequence HVKGKHLCPSPLFPGPSKP (SEQ ID NO: 10); or
 - c. both (a) and (b).
21. The agent of any one of claims 15 to 20, wherein said agent inhibits proteolytic cleavage by at least one protease.
22. The agent of claim 21, wherein said at least one protease is at least one metalloprotease.
23. The agent of claim 22, wherein said at least one metalloprotease is selected from ADAM10 and ADAM17.
24. The agent of any one of claims 15 to 23, wherein said agent is an antibody or antigen binding fragment thereof and comprises three heavy chain CDRs (CDR-H) and three light chain CDRs (CDR-L), wherein:
- CDR-H1 comprises the amino acid sequence set forth in SEQ ID NO: 30 (GFTFSSYYMS), CDR-H2 comprises the amino acid sequence as set forth in SEQ ID NO: 31 (TISDGGDNTYYAGTVTG), CDR-H3 comprises the amino acid sequence as set forth in SEQ ID NO: 32 (IHWPYYFDS), CDR-L1 comprises the amino acid sequence as set forth in SEQ ID NO: 33 (RASSSVSYMN), CDR-L2 comprises the amino acid sequence as set forth in SEQ ID NO: 34 (ATSDLAS), and CDR-L3 comprises the amino acid sequence as set forth in SEQ ID NO: 35 (QQWSSHPPT).
25. The agent of claim 24, comprising at least one of
- a. a heavy chain comprising the amino acid sequence of SEQ ID NO: 53; and
 - b. a light chain comprising the amino acid sequence of SEQ ID NO: 55.
26. An agent that binds soluble CD28 (sCD28) and is neither a CD28 agonist nor antagonist.
27. The agent of claim 26, wherein said agent does not bind membranal CD28 (mCD28).

28. The agent of claim 26 or 27, wherein said agent is selected from an antibody or antigen binding fragment thereof, a Fab fragment, a single chain antibody, a single domain antibody, a small molecule, and a peptide with specific binding to sCD28.
29. The agent of any one of claims 26 to 28, wherein binding of said agent to sCD28 in an organism results in at least one of:
- degradation of said bound sCD28;
 - removal of sCD28 from blood; and
 - transport of said bound sCD28 to a lysosome, endosome, proteasome or a combination thereof.
30. The agent of any one of claims 26 to 29, wherein said agent does not inhibit binding of said sCD28 to a ligand.
31. The agent of any one of claims 26 to 30, wherein said agent binds dimeric sCD28, monomeric sCD28 or both.
32. The agent of any one of claims 26 to 31, wherein said agent binds outside the IgV domain of sCD28.
33. The agent of any one of claims 26 to 32, wherein said agent is an antibody or antigen binding fragment thereof and comprises three heavy chain CDRs (CDR-H) and three light chain CDRs (CDR-L), wherein:
- CDR-H1 comprises the amino acid sequence set forth in SEQ ID NO: 12 (GYTLTNY), CDR-H2 comprises the amino acid sequence as set forth in SEQ ID NO: 13 (NTYTGK), CDR-H3 comprises the amino acid sequence as set forth in SEQ ID NO: 14 (GDANQQFAY), CDR-L1 comprises the amino acid sequence as set forth in SEQ ID NO: 15 (KASQDINSYLS), CDR-L2 comprises the amino acid sequence as set forth in SEQ ID NO: 16 (RANRLVD), and CDR-L3 comprises the amino acid sequence as set forth in SEQ ID NO: 17 (LQYDEFPPPT);
- CDR-H1 comprises the amino acid sequence set forth in SEQ ID NO: 18 (GYTFTSY), CDR-H2 comprises the amino acid sequence as set forth in SEQ ID NO: 19 (YPGDGD), CDR-H3 comprises the amino acid sequence as set forth in SEQ ID NO: 20 (NYRYSSFGY), CDR-L1 comprises the amino acid sequence as set forth in SEQ ID NO: 21 (KSSQSLLNSGNQKNYLT), CDR-L2 comprises the amino acid sequence as set forth

in SEQ ID NO: 22 (WASTRES), and CDR-L3 comprises the amino acid sequence as set forth in SEQ ID NO: 23 (QSDYSYPLT); or

CDR-H1 comprises the amino acid sequence set forth in SEQ ID NO: 24 (GYTFTDY), CDR-H2 comprises the amino acid sequence as set forth in SEQ ID NO: 25 (NPNYDS), CDR-H3 comprises the amino acid sequence as set forth in SEQ ID NO: 26 (SSPYYSNHFYD), CDR-L1 comprises the amino acid sequence as set forth in SEQ ID NO: 27 (SARSSINYM), CDR-L2 comprises the amino acid sequence as set forth in SEQ ID NO: 28 (DTSKLAS), and CDR-L3 comprises the amino acid sequence as set forth in SEQ ID NO: 29 (HQRNSYPFT).

34. The agent of claim 33, comprising at least one of
- a. a heavy chain comprising an amino acid sequence selected from SEQ ID NO: 41, 45 or 49; and
 - b. a light chain comprising an amino acid sequence selected from SEQ ID NO: 43, 47 or 51.
35. The agent of claim 33 or 34, wherein the antigen binding fragment is selected from the group consisting of a Fv, Fab, F(ab')₂, scFV or a scFV₂ fragment.
36. The agent of any one of claims 33 to 35, wherein said agent is humanized.
37. The agent of any one of claims 33 to 36, wherein said agent does not induce antibody-dependent cell-mediated cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC).
38. A method for producing an agent of any one of claims 15 to 25, the method comprising:
- obtaining an agent that binds to a CD28 extracellular domain or fragment thereof,
 - testing an ability of said agent to block cleavage of mCD28 by a protease, and
 - selecting at least one agent that blocks cleavage of mCD28 by said protease; or
 - culturing a host cell comprising one or more vectors comprising a nucleic acid sequence encoding an agent, wherein the nucleic acid sequence is that of an agent that was selected by:
 - i. obtaining an agent that binds to a CD28 extracellular domain or fragment thereof;

- ii. testing an ability of said agent to block cleavage of mCD28 by a protease;
and
 - iii. selecting at least one agent that blocks cleavage of mCD28 by said protease;
thereby producing an agent of any one of claims 15 to 25.
- 39. The method of claim 38, wherein said protease is selected from, ADAM10 and ADAM17.
- 40. The method of claim 38 or 39, wherein obtaining an agent that binds specifically to CD28 extracellular domain or a fragment thereof is obtaining an agent that binds specifically to a CD28 stalk domain.
- 41. The method of any one of claims 38 to 40, further comprising assaying mCD28 downstream signaling in the presence of said obtained agent and selecting at least one agent that neither substantially agonizes nor substantially antagonizes mCD28 signaling.
- 42. A method for producing an agent of any one of claims 26 to 37, the method comprising:
 - obtaining an agent that binds to a CD28 extracellular domain or fragment thereof,
 - assaying mCD28 downstream signaling in the presence of said obtained agent, and
 - selecting at least one agent that neither substantially agonizes nor substantially antagonizes mCD28 signaling; or
 - culturing a host cell comprising one or more vectors comprising a nucleic acid sequence encoding an agent, wherein the nucleic acid sequence is that of an agent that was selected by:
 - i. obtaining an agent that binds to a CD28 extracellular domain or fragment thereof;
 - ii. assaying mCD28 downstream signaling in the presence of said obtained agent; and
 - iii. selecting at least one agent that neither substantially agonizes nor substantially antagonizes mCD28 signaling;thereby producing an agent of any one of claims 26 to 37.
- 43. The method of claim 42, further comprising at least one of:
 - a. testing binding of said obtained agent to mCD28 and selecting at least one agent that does not bind mCD28; and
 - b. testing binding of said obtained agent to sCD28 from a cancer patient and selecting at least one agent that binds said sCD28 from a cancer patient.

44. The method of claim 38 or 40, wherein said obtaining the agent comprises at least one of:
- immunizing an organism with said CD28 extracellular domain or fragment thereof and collecting antibodies from said immunized organism;
 - screening a library of agents for binding to a CD28 extracellular domain or fragment thereof and selecting an agent that binds.
45. The method of claim 44, wherein said CD28 extracellular domain or fragment thereof is dimeric or monomeric
46. The method of claim 44 or 45, wherein said organism is selected from a rabbit, a mouse, a rat, a shark, a camelid, a chicken, a goat and a phage.
47. The method of any one of claims 44 to 46, wherein said collecting antibodies comprises:
- extracting B cells from a spleen of said immunized organism;
 - fusing said extracted B cells with myeloma cells to produce a hybridoma; and
 - collecting antibodies from said hybridoma.
48. The method of claim 44 or 45, wherein said selecting an agent that binds comprises sequencing said selected agent and producing a recombinant form of said agent from said sequence.
49. An agent produced by the method of any one of claims 38 to 48.
50. A pharmaceutical composition comprising an agent of any one of claims 15 to 37 and 49, and a pharmaceutically acceptable carrier, excipient or adjuvant.
51. A method of treating and/or preventing cancer or improving PD-1 and/or PD-L1 based immunotherapy in a subject in need thereof, the method comprising administering to the subject a pharmaceutical composition of claim 50.
52. A method of determining suitability of a subject to be treated by a method of any one of claims 1 to 12, comprising obtaining a sample from said subject and determining sCD28 levels in the sample, wherein a sCD28 level above 5 ng/ml indicates the subject is suitable for a method of treatment of any one of claims 1 to 12.
53. A kit comprising at least one agent of any one of claims 15 to 37 and 49.
54. The kit of claim 53, further comprising at least one of:
- an anti-PD-1 and/or PD-L1 immunotherapy;
 - a label stating the agent of the invention is for use with a PD-1 and/or PD-L1 based immunotherapy; and

- c. a secondary detection molecule for detecting said at least one agent of any one of claims 15 to 37 and 49.