

shedding during T cell activation was described in the past as a regulatory mechanism to counteract persistent activation by the proteolysis of adhesion molecules.

[006] While CTLA-4 limits the amplitude of early T cell responses, another inhibitory receptor, PD-1, suppresses T cell function in periphery. The expression of PD-1 is elevated during the activation of T cells, and its known ligands are the B7 family homologs: B7-H1 (PD-L1) and B7-H2 (PD-L2). These homologs are found on APCs and cancer cells and drive activated T cells into a state of cellular anergy, leading to a dampened immune response. Accordingly, targeted therapies for the CTLA-4 and PD-1/PD-L1 axis have shown clinical activity in a wide variety of cancer types. Recently, studies have shown that the signaling pathway of CD28 is targeted and repressed by PD-1 (Hui, E., Science, 2017) and concomitantly for an effective PD-1 therapy to take place an intact active CD28/B7 axis is essential (Kamphorst, A.O., Science, 2017).

[007] However, not all patients respond to PD-1 based immunotherapy or immunotherapy in general, and those that do often relapse. Methods and molecules that can improve the ability of a patient's immune cells to attack cancer are thus greatly in need.

SUMMARY

[008] The present disclosure provides [m](#)Methods of treating cancer and improving PD-1/PD-L1 based immunotherapy comprising decreasing soluble CD28 levels. Agents that bind membranal CD28 and inhibit proteolytic cleavage of mCD28 and agents that bind soluble CD28 and that are neither CD28 agonists nor antagonists are also provided, as are methods of producing these agents.

[009] According to a first example, there is provided a method of treating and/or preventing cancer in a subject in need thereof, the method comprising decreasing soluble CD28 (sCD28) levels in the subject.

[009a] According to one aspect, the present disclosure refers to use of at least one of:

- a. an antibody or antigen binding fragment thereof~~agent~~ that binds to soluble CD28 (sCD28), wherein said antibody or antigen binding fragment thereof~~agent~~, upon binding, ~~degrades said sCD28 or~~ targets said sCD28 for degradation;
 - ~~b. 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);~~
 - ~~eb.~~ b. an antibody or antigen binding fragment thereof~~agent~~ that binds membranal CD28 (mCD28) and inhibits proteolytic cleavage of said mCD28; or
 - ~~dc.~~ d. a dimeric peptide comprising a stalk region of human CD28;~~or~~
 - ~~e. an agent that inhibits a protease capable of cleaving mCD28; —~~
- in the manufacture of a medicament for treating ~~and/or preventing~~ cancer in a subject in need thereof, wherein soluble CD28 (sCD28) levels in said subject is decreased.

[010] According to another example, there is provided 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 the subject.

[010a] According to another aspect, the present disclosure refers to use of at least one of:

- a. an antibody or antigen binding fragment thereof~~agent~~ that binds to sCD28, wherein said antibody or antigen binding fragment thereof~~agent~~, upon binding, ~~degrades said sCD28 or~~ targets said sCD28 for degradation;
 - ~~b. 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); —~~
 - ~~be.~~ b. an antibody or antigen binding fragment thereof~~agent~~ that binds mCD28 and inhibits proteolytic cleavage of said mCD28; or
 - ~~dc.~~ d. a dimeric peptide comprising a stalk region of human CD28;~~or~~
 - ~~e. an agent that inhibits a protease capable of cleaving mCD28; —~~
- in the manufacture of a medicament for improving PD-1 and/or PD-L1 based immunotherapy in a subject in need thereof, wherein sCD28 levels in said subject is decreased.

[011] According to another ~~aspect~~example, the present disclosure refers to an agent that binds soluble CD28 (sCD28) and is neither a CD28 agonist nor antagonist.

[011a] According to another aspect, the present disclosure refers to an antibody or antigen binding fragment thereof that binds soluble CD28 (sCD28) and is neither a CD28 agonist nor antagonist.

[012] According to some examples, the subject in need of immunotherapy suffers from cancer. According to some examples, the subject does not respond or lowly responds to PD-1 and/or PD-L1 based immunotherapy.

[013] According to some examples, the decreasing occurs in the blood, peripheral blood, or tumor microenvironment of the subject. According to some examples, the decreasing comprises at least one of:

- a. administering to the subject an agent that binds to sCD28 and wherein the agent, upon binding, degrades the sCD28 or targets the sCD28 for degradation;
- b. administering to the subject an inhibitory nucleic acid molecule, wherein the molecule binds to an mRNA coding for sCD28 and does not bind to an mRNA coding for membranal CD28 (mCD28);
- c. administering to the subject an agent that binds mCD28 and inhibits proteolytic cleavage of the mCD28;
- d. administering to the subject a dimeric peptide comprising a stalk region of human CD28;
- e. administering to the subject an agent that inhibits a protease capable of cleaving mCD28 and
- f. withdrawing blood from the subject, decreasing the amount of sCD28 in the blood and returning the blood to the subject.

[014] According to some examples, the stalk region,

- a. comprises the amino acid sequence GKHLCPSPFLPGPSKP (SEQ ID NO: 9) or KGKHLCPSPFLPGPS (SEQ ID NO: 36);
- b. consists of the amino acid sequence HVKGKHLCPSPFLPGPSKP (SEQ ID NO: 10); or
- c. both (a) and (b).

[015] According to some examples, the method does not degrade mCD28 or decrease mCD28-mediated immune cell activation.

[016] According to some examples, the subject's blood prior to the decreasing comprises at least 5 ng/ml sCD28.

[017] According to some examples, the cancer is selected from melanoma, head and neck, non-small cell lung cancer, ovarian, kidney, gastric and colorectal. According to some examples, the cancer is selected from melanoma, head and neck, non-small cell lung cancer, ovarian, and colorectal.

[018] According to some examples, the method of the disclosure further comprises administering another immunotherapy to the subject. According to some examples, the immunotherapy is selected from:

- a. a checkpoint inhibitor;
- b. a chimeric antigen receptor (CAR) based therapy; and
- c. a cancer vaccine.

[019] According to some examples, the checkpoint inhibitor is a PD-1 and/or PD-L1 based immunotherapy.

[020] According to another [aspectexample](#), the present disclosure refers to an agent that binds membranal CD28 (mCD28) and inhibits proteolytic cleavage of said mCD28.

[\[020a\] According to another aspect, the present disclosure refers to an antibody or antigen binding fragment thereof that binds membranal CD28 \(mCD28\) and inhibits proteolytic cleavage of said mCD28.](#)

[021] According to some examples, the agent is neither a CD28 agonist or antagonist. According to some examples, the agent neither degrades the mCD28 nor inhibits mCD28-mediated immune cell activation.

[022] According to some examples, the 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.

[023] According to some examples, the agent binds within the stalk region of CD28. According to some examples, the stalk region

- a. comprises the amino acid sequence GKHLCPSPFLFPGPSKP (SEQ ID NO: 9) or KGKHLCPSPFLFPGPS (SEQ ID NO: 36);
- b. consists of the amino acid sequence HVKGKHLCPSPFLFPGPSKP (SEQ ID NO: 10); or
- c. both (a) and (b).

[024] According to some examples, the agent inhibits proteolytic cleavage by at least one protease. According to some examples, the at least one protease is at least one metalloprotease. According to some examples, the at least one metalloprotease is selected from ADAM10 and ADAM17.

[025] According to some examples, the 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 (IHWPYFYFDS), 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).

[026] According to some examples, the agent of the disclosure comprises 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.

[027] According to some examples, the agent does not bind membranal CD28 (mCD28).

[028] According to some examples, the 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.

[029] According to some examples, binding of the agent to sCD28 in an organism results in at least one of:

- a. degradation of the bound sCD28;
- b. removal of sCD28 from blood; and
- c. transport of the bound sCD28 to a lysosome, endosome, proteasome or a combination thereof.

[030] According to some examples, the agent does not inhibit binding of the sCD28 to a ligand. According to some examples, the agent binds dimeric sCD28, monomeric sCD28 or both. According to some examples, the agent binds outside the IgV domain of sCD28.

[031] According to some examples, the 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 (LQYDEFPT);

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 (SSPYYSNHFY), 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).

[032] According to some examples, the agent of the disclosure comprises 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.

[033] According to some examples, the antigen binding fragment is selected from the group consisting of a Fv, Fab, F(ab')₂, scFV or a scFV2 fragment.

[034] According to some examples, the agent is humanized.

[035] According to some examples, the agent does not induce antibody-dependent cell-mediated cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC).

[036] According to another [aspectexample](#), the present disclosure refers to a method for producing an agent as disclosed herein, the method comprising:

obtaining an agent that binds to a CD28 extracellular domain or fragment thereof, testing an ability of the agent to block cleavage of mCD28 by a protease, and selecting at least one agent that blocks cleavage of mCD28 by the 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 the agent to block cleavage of mCD28 by a protease; and
- iii. selecting at least one agent that blocks cleavage of mCD28 by the protease;

thereby producing an agent as disclosed herein.

[036a] According to another aspect, the present disclosure refers to a method for producing an antibody or antigen binding fragment thereof as disclosed herein, the method comprising: obtaining an antibody or antigen binding fragment thereof that binds to a CD28 extracellular domain or fragment thereof, testing an ability of said antibody or antigen binding fragment thereof to block cleavage of mCD28 by a protease, and selecting at least one antibody or antigen binding fragment thereof 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 antibody or antigen binding fragment thereof, wherein the nucleic acid sequence is that of an antibody or antigen binding fragment thereof that was selected by:

- i. obtaining an antibody or antigen binding fragment thereof that binds to a CD28 extracellular domain or fragment thereof;
 - ii. testing an ability of said antibody or antigen binding fragment thereof to block cleavage of mCD28 by a protease; and
 - iii. selecting at least one antibody or antigen binding fragment thereof that blocks cleavage of mCD28 by said protease;
- thereby producing an antibody or antigen binding fragment thereof as disclosed herein.

[037] According to another aspect, the present disclosure refers to a method for producing an agent as disclosed herein, the method comprising:

obtaining an agent that binds to a CD28 extracellular domain or fragment thereof, assaying mCD28 downstream signaling in the presence of the 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 the obtained agent; and
- iii. selecting at least one agent that neither substantially agonizes nor substantially antagonizes mCD28 signaling;

thereby producing an agent as disclosed herein.

[037a] According to another aspect, the present disclosure refers to a method for producing an antibody or antigen binding fragment thereof as disclosed herein, the method comprising: obtaining an antibody or antigen binding fragment thereof that binds to a CD28 extracellular domain or fragment thereof, assaying mCD28 downstream signaling in the presence of said obtained antibody or antigen binding fragment thereof, and selecting at least one antibody or antigen binding fragment thereof 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 antibody or antigen binding fragment thereof, wherein the nucleic acid sequence is that of an antibody or antigen binding fragment thereof that was selected by:

- i. obtaining an antibody or antigen binding fragment thereof that binds to a CD28 extracellular domain or fragment thereof;
 - ii. assaying mCD28 downstream signaling in the presence of said obtained antibody or antigen binding fragment thereof; and
 - iii. selecting at least one antibody or antigen binding fragment thereof that neither substantially agonizes nor substantially antagonizes mCD28 signaling;
- thereby producing an antibody or antigen binding fragment thereof as disclosed herein.

[038] According to some examples, the protease is selected from, ADAM10 and ADAM17.

[039] According to some examples, 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.

[040] According to some examples, the method of the disclosure further comprises assaying mCD28 downstream signaling in the presence of the obtained agent and selecting at least one agent that neither substantially agonizes nor substantially antagonizes mCD28 signaling.

[041] According to some examples, the method of the disclosure further comprises at least one of:

- a. testing binding of the obtained agent to mCD28 and selecting at least one agent that does not bind mCD28; and
- b. testing binding of the obtained agent to sCD28 from a cancer patient and selecting at least one agent that binds the sCD28 from a cancer patient.

[042] According to some examples, the obtaining the agent comprises at least one of:

- a. immunizing an organism with the CD28 extracellular domain or fragment thereof and collecting antibodies from the immunized organism;
- b. screening a library of agents for binding to a CD28 extracellular domain or fragment thereof and selecting an agent that binds.

[043] According to some examples, the CD28 extracellular domain or fragment thereof is dimeric or monomeric

[044] According to some examples, the organism is selected from a rabbit, a mouse, a rat, a shark, a camelid, a chicken, a goat and a phage.

[045] According to some examples, the collecting antibodies comprises:

- a. extracting B cells from a spleen of the immunized organism;
- b. fusing the extracted B cells with myeloma cells to produce a hybridoma; and
- c. collecting antibodies from the hybridoma.

[046] According to some examples, the selecting an agent that binds comprises sequencing the selected agent and producing a recombinant form of the agent from the sequence.

[047] According to another [example aspect](#), the present disclosure refers to an agent produced by the method as disclosed herein.

[048] According to another [example aspect](#), the present disclosure refers to a pharmaceutical composition comprising an agent as disclosed herein, and a pharmaceutically acceptable carrier, excipient or adjuvant.

[\[048a\] According to another aspect, the present disclosure refers to a pharmaceutical composition comprising an antibody or antigen binding fragment thereof as disclosed herein, and a pharmaceutically acceptable carrier, excipient or adjuvant.](#)

[049] According to another example, there is provided 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 the disclosure.

[049a] According to another aspect, the present disclosure refers to use of a pharmaceutical composition as disclosed herein in the manufacture of a medicament for treating ~~and/or preventing~~ cancer or improving PD-1 and/or PD-L1 based immunotherapy in a subject in need thereof.

[050] According to another example, there is provided a method of determining suitability of a subject to be treated by a method of the disclosure, comprising obtaining a sample from the 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 the disclosure.

[050a] According to another aspect, the present disclosure refers to a method of determining suitability of a subject for the use as disclosed herein, comprising determining sCD28 levels in a sample that has been obtained from said subject, wherein a sCD28 level above 5 ng/ml indicates the subject is suitable for the use as disclosed herein.

[051] According to another aspect, the present disclosure refers to a kit comprising at least one agent as disclosed herein.

[\[051a\] According to another aspect, the present disclosure refers to a kit comprising at least one antibody or antigen binding fragment thereof as disclosed herein.](#)

[052] According to some examples, the kit of the disclosure further comprises at least one of:

- a. an anti-PD-1 and/or PD-L1 immunotherapy;
- b. a label stating the agent of the disclosure is for use with a PD-1 and/or PD-L1 based immunotherapy; and
- c. a secondary detection molecule for detecting the at least one agent as disclosed herein.

[053] Further examples and the full scope of applicability of the present disclosure will become apparent from the detailed description given hereinafter. However, it should be understood that the detailed description and specific examples, while indicating preferred examples of the disclosure, are 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 from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[054] **Figure 1.** Soluble CD28 is generated during stimulation of PBMCs and counteracted by addition of protease inhibitors (PI). Bar charts of the amount of soluble CD28 in the culture of PBMCs stimulated with SEB (0.5 ng/mL, left side) or CMV peptides (0.5 µg/mL, right side) as quantified by human CD28 ELISA (top panel). A cocktail of protease inhibitors was added at the indicated concentrations. The overall health and effector activity were examined by the secretion of interferon gamma (low panel).

[055] **Figure 2.** Soluble CD28 is generated during stimulation of T cells by PHA and counteracted by addition of protease inhibitors. Bar charts of Jurkat cells (upper left) or isolated human CD4 T cells (upper right) stimulated with increasing concentrations of PHA (1-4 µg/mL, upper charts) in the presence of a protease inhibitor cocktail at a fixed concentration (2 µM). In another setup the PHA concentration was fixed to stimulate Jurkat T cells (1 µg/mL PHA, lower left) or human CD4 T cells (2 µg/mL PHA, lower right) and the concentration of the protease inhibitor cocktail was titrated (0.5-2 µM). The concentration of human CD28 in the supernatant was quantified with a standardized sandwich ELISA.

[056] **Figures 3A-B.** Specific ADAM-10 and ADAM-17 inhibitors eliminate the accumulation of soluble CD28 during human PBMCs activation by SEB while not hampering their viability. (3A-B) Bar charts of human PBMCs stimulated with SEB (1 ng/mL) in the presence of (3A)

CLAIMS:

1. Use of at least one of:
 - a. an antibody or antigen binding fragment thereofagent that binds to soluble CD28 (sCD28), wherein said antibody or antigen binding fragment thereofagent, upon binding, ~~degrades said sCD28 or~~ targets said sCD28 for degradation;
 - ~~b. an inhibitory nucleic acid molecule, wherein said molecule binds to an mRNA coding for sCD28 and does not bind to an mRNA coding for membran CD28 (mCD28);~~
 - ~~eb.~~ an antibody or antigen binding fragment thereofagent that binds membran CD28 (mCD28) and inhibits proteolytic cleavage of said mCD28; or
 - ~~dc.~~ a dimeric peptide comprising a stalk region of human CD28; ~~or~~
 - ~~e. an agent that inhibits a protease capable of cleaving mCD28;~~in the manufacture of a medicament for treating ~~and/or preventing~~ cancer in a subject in need thereof, wherein soluble CD28 (sCD28) levels in said subject is decreased.
2. Use of at least one of:
 - a. an antibody or antigen binding fragment thereofagent that binds to sCD28, wherein said antibody or antigen binding fragment thereofagent, upon binding, ~~degrades said sCD28 or~~ targets said sCD28 for degradation;
 - ~~b. an inhibitory nucleic acid molecule, wherein said molecule binds to an mRNA coding for sCD28 and does not bind to an mRNA coding for membran CD28 (mCD28);~~
 - ~~be.~~ an antibody or antigen binding fragment thereofagent that binds mCD28 and inhibits proteolytic cleavage of said mCD28; or
 - ~~dc.~~ a dimeric peptide comprising a stalk region of human CD28; ~~or~~
 - ~~e. an agent that inhibits a protease capable of cleaving mCD28;~~in the manufacture of a medicament for improving PD-1 and/or PD-L1 based immunotherapy in a subject in need thereof, wherein sCD28 levels in said subject is decreased.
3. The use of claim 2, wherein said subject in need of immunotherapy suffers from cancer.
4. The use of any one of claims 1 to 3, wherein said subject is one that does not respond or lowly responds to PD-1 and/or PD-L1 based immunotherapy.
5. The use 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 use of any one of claims 1 to 5, wherein
the amount of sCD28 in blood that has been withdrawn from said subject is decreased, and wherein the blood is to be returned to said subject.
7. The use 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 use of any one of claims 1 to 7, wherein said use does not degrade mCD28 or decrease mCD28-mediated immune cell activation.
9. The use 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 use 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 use of claim 10, wherein said cancer is selected from melanoma, head and neck, non-small cell lung cancer, ovarian, and colorectal.
12. The use of any one of claims 1 to 11, wherein another immunotherapy is to be further administered to said subject.
13. The use 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 use of claims 13, wherein said checkpoint inhibitor is a PD-1 and/or PD-L1 based immunotherapy.
15. An antibody or antigen binding fragment thereof~~agent~~ that binds membranal CD28 (mCD28) and inhibits proteolytic cleavage of said mCD28.
16. The antibody or antigen binding fragment thereof~~agent~~ of claim 15, wherein said antibody or antigen binding fragment thereof~~agent~~ is neither a CD28 agonist nor antagonist.

17. The antibody or antigen binding fragment thereof of claim 15 or 16, wherein said antibody or antigen binding fragment thereof neither degrades said mCD28 nor inhibits mCD28-mediated immune cell activation.
18. The antibody or antigen binding fragment thereof of any one of claims 15 to 17, wherein said antibody or antigen binding fragment thereof
- 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 antibody or antigen binding fragment thereof of any one of claims 15 to 18, wherein said antibody or antigen binding fragment thereof binds within the stalk region of CD28.
20. The antibody or antigen binding fragment thereof of claim 19, 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).
21. The antibody or antigen binding fragment thereof of any one of claims 15 to 20, wherein said antibody or antigen binding fragment thereof inhibits proteolytic cleavage by at least one protease.
22. The antibody or antigen binding fragment thereof of claim 21, wherein said at least one protease is at least one metalloprotease.

23. The antibody or antigen binding fragment thereof~~agent~~ of claim 22, wherein said at least one metalloprotease is selected from ADAM10 and ADAM17.
24. The antibody or antigen binding fragment thereof~~agent~~ of any one of claims 15 to 23, ~~wherein said agent is an antibody or antigen binding fragment thereof and~~ comprising 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 antibody or antigen binding fragment thereof~~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 antibody or antigen binding fragment thereof~~agent~~ that binds soluble CD28 (sCD28) and is neither a CD28 agonist nor antagonist.
27. The antibody or antigen binding fragment thereof~~agent~~ of claim 26, wherein said antibody or antigen binding fragment thereof~~agent~~ does not bind membranal CD28 (mCD28).
28. The antibody or antigen binding fragment thereof~~agent~~ of claim 26 or 27, wherein said antibody or antigen binding fragment thereof~~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 antibody or antigen binding fragment thereof~~agent~~ of any one of claims 26 to 28, wherein binding of said antibody or antigen binding fragment thereof~~agent~~ to sCD28 in an organism results in at least one of:
- a. degradation of said bound sCD28;
 - b. removal of sCD28 from blood; and

- c. transport of said bound sCD28 to a lysosome, endosome, proteasome or a combination thereof.
30. The antibody or antigen binding fragment thereof~~agent~~ of any one of claims 26 to 29, wherein said antibody or antigen binding fragment thereof~~agent~~ does not inhibit binding of said sCD28 to a ligand.
31. The antibody or antigen binding fragment thereof~~agent~~ of any one of claims 26 to 30, wherein said antibody or antigen binding fragment thereof~~agent~~ binds dimeric sCD28, monomeric sCD28 or both.
32. The antibody or antigen binding fragment thereof~~agent~~ of any one of claims 26 to 31, wherein said antibody or antigen binding fragment thereof~~agent~~ binds outside the IgV domain of sCD28.
33. The antibody or antigen binding fragment thereof~~agent~~ of any one of claims 26 to 32, ~~wherein said agent is an antibody or antigen binding fragment thereof and comprising~~ing 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 (LQYDEFPT);

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

(SSPYYDSNHFDY), 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 antibody or antigen binding fragment thereof of claim 33, comprising at least one of
- a heavy chain comprising an amino acid sequence selected from SEQ ID NO: 41, 45 or 49; and
 - a light chain comprising an amino acid sequence selected from SEQ ID NO: 43, 47 or 51.
35. The antibody or antigen binding fragment thereof 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 antibody or antigen binding fragment thereof of any one of claims 33 to 35, wherein said antibody or antigen binding fragment thereof is humanized.
37. The antibody or antigen binding fragment thereof of any one of claims 33 to 36, wherein said antibody or antigen binding fragment thereof does not induce antibody-dependent cell-mediated cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC).
38. A method for producing an antibody or antigen binding fragment thereof of any one of claims 15 to 25, the method comprising:
- obtaining an antibody or antigen binding fragment thereof that binds to a CD28 extracellular domain or fragment thereof, testing an ability of said antibody or antigen binding fragment thereof to block cleavage of mCD28 by a protease, and selecting at least one antibody or antigen binding fragment thereof 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 antibody or antigen binding fragment thereof, wherein

the nucleic acid sequence is that of an antibody or antigen binding fragment thereof that was selected by:

- i. obtaining an antibody or antigen binding fragment thereof that binds to a CD28 extracellular domain or fragment thereof;
- ii. testing an ability of said antibody or antigen binding fragment thereof to block cleavage of mCD28 by a protease; and
- iii. selecting at least one antibody or antigen binding fragment thereof that blocks cleavage of mCD28 by said protease;

thereby producing an antibody or antigen binding fragment thereof 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 antibody or antigen binding fragment thereof that binds specifically to CD28 extracellular domain or a fragment thereof is obtaining an antibody or antigen binding fragment thereof 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 antibody or antigen binding fragment thereof and selecting at least one antibody or antigen binding fragment thereof that neither substantially agonizes nor substantially antagonizes mCD28 signaling.

42. A method for producing an antibody or antigen binding fragment thereof of any one of claims 26 to 37, the method comprising:

obtaining an antibody or antigen binding fragment thereof that binds to a CD28 extracellular domain or fragment thereof, assaying mCD28 downstream signaling in the presence of said obtained antibody or antigen binding fragment thereof, and selecting at least one antibody or antigen binding fragment thereof 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 antibody or antigen binding fragment thereof, wherein

the nucleic acid sequence is that of an antibody or antigen binding fragment thereof~~agent~~ that was selected by:

- i. obtaining an antibody or antigen binding fragment thereof~~agent~~ that binds to a CD28 extracellular domain or fragment thereof;
- ii. assaying mCD28 downstream signaling in the presence of said obtained antibody or antigen binding fragment thereof~~agent~~; and
- iii. selecting at least one antibody or antigen binding fragment thereof~~agent~~ that neither substantially agonizes nor substantially antagonizes mCD28 signaling;

thereby producing an antibody or antigen binding fragment thereof~~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 antibody or antigen binding fragment thereof~~agent~~ to mCD28 and selecting at least one antibody or antigen binding fragment thereof~~agent~~ that does not bind mCD28; and
- b. testing binding of said obtained antibody or antigen binding fragment thereof~~agent~~ to sCD28 from a cancer patient and selecting at least one antibody or antigen binding fragment thereof~~agent~~ that binds said sCD28 from a cancer patient.

44. The method of claim 38 or 40, wherein said obtaining the antibody or antigen binding fragment thereof~~agent~~ comprises at least one of:

- a. providing antibodies that have been collected from an organism that has been immunized with said CD28 extracellular domain or fragment thereof;
- b. screening a library of antibodies or antigen binding fragments thereof~~agents~~ for binding to a CD28 extracellular domain or fragment thereof and selecting an antibody or antigen binding fragment thereof~~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:

- a. fusing B cells that have been extracted from a spleen of said immunized organism with myeloma cells to produce a hybridoma; and
- b. collecting antibodies from said hybridoma.

48. The method of claim 44 or 45, wherein said selecting an antibody or antigen binding fragment thereof~~agent~~ that binds comprises sequencing said selected antibody or antigen binding fragment thereof~~agent~~ and producing a recombinant form of said antibody or antigen binding fragment thereof~~agent~~ from said sequence.

~~49. An agent produced by the method of any one of claims 38 to 48.~~

~~50.~~49. A pharmaceutical composition comprising an antibody or antigen binding fragment thereof~~agent~~ of any one of claims 15 to 37 ~~and 49~~, and a pharmaceutically acceptable carrier, excipient or adjuvant.

~~51.~~50. Use of a pharmaceutical composition of claim ~~49~~50 in the manufacture of a medicament for treating ~~and/or preventing~~ cancer or improving PD-1 and/or PD-L1 based immunotherapy in a subject in need thereof.

~~52.~~51. A method of determining suitability of a subject for the use of any one of claims 1 to 12, comprising determining sCD28 levels in a sample that has been obtained from said subject, wherein a sCD28 level above 5 ng/ml indicates the subject is suitable for the use of any one of claims 1 to 12.

~~53.~~52. A kit comprising at least one antibody or antigen binding fragment thereof~~agent~~ of any one of claims 15 to 37 ~~and 49~~.

~~54.~~53. The kit of claim ~~53~~52, further comprising at least one of:

- a. an anti-PD-1 and/or PD-L1 immunotherapy;
- b. a label stating the antibody or antigen binding fragment thereof~~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 antibody or antigen binding fragment thereof
- ~~e.~~ ~~agent~~ of any one of claims 15 to 37 ~~and 49~~.

54. The use of any one of claims 1 to 14, wherein said antibody or antigen binding fragment thereof that binds to sCD28 is neither an agonist nor an antagonist of CD28 or does not bind mCD28.

55. The use of any one of claims 1 to 14, wherein said antibody or antigen binding fragment thereof that binds to sCD28 is an antibody or antigen binding fragment thereof of any one of claims 33 to 37, or wherein said antibody or antigen binding fragment thereof that binds mCD28 is an antibody or antigen binding fragment thereof of claim 24 or 25.