

CLAIMS:

1. A method of decreasing shedding of CD28 from a surface of a cell expressing membranal CD28 (mCD28), the method comprising contacting said cell with a matrix metalloprotease-2 (MMP-2) inhibitor, a matrix metalloprotease-13 (MMP-13) inhibitor or both, thereby decreasing shedding of CD28 from a surface of a cell.
2. A method of decreasing soluble CD28 (sCD28) levels in a subject in need thereof, the method comprising administering to said subject an MMP-2 inhibitor, an MMP-13 inhibitor or both, thereby decreasing sCD28 levels in a subject.
3. A method of treating and/or preventing cancer in a subject in need thereof, the method comprising administering to said subject an MMP-2 inhibitor, an MMP-13 inhibitor or both, thereby treating and/or preventing cancer in said subject.
4. A method of improving PD-1 and/or PD-L1 based immunotherapy in a subject in need thereof, the method comprising administering to said subject an MMP-2 inhibitor, an MMP-13 inhibitor or both, thereby improving PD-1 and/or PD-L1 based immunotherapy in a subject.
5. The method of claim 4, wherein said subject in need of immunotherapy suffers from cancer.
6. The method of any one of claims 2 to 5, wherein said subject does not respond or lowly responds to PD-1 and/or PD-L1 based immunotherapy.
7. The method of any one of claims 2 to 6, wherein said subject's blood prior to said decreasing comprises at least 5 ng/mL sCD28.
8. The method of any one of claims 3 to 7, wherein said cancer is selected from melanoma, urothelial carcinoma, head and neck, non-small cell lung cancer, ovarian, kidney, gastric and colorectal.
9. The method of any one of claims 1 to 8, wherein said method increases mCD28 expression on a surface of said cell or in said subject, and/or increases mCD28-mediated immune cell activation in said cell or subject.
10. The method of any one of claims 1 to 9, comprising administering an MMP-2 inhibitor.
11. The method of any one of claims 1 to 10, wherein said inhibitor is specific to MMP-2.
12. The method of any one of claims 1 to 11, wherein said inhibitor is specific to MMP-2 induced cleaving of CD28.

13. The method of any one of claims 1 to 10, comprising administering an MMP-13 inhibitor.
14. The method of any one of claims 1 to 9 and 13, wherein said inhibitor is specific to MMP-13.
15. The method of any one of claims 1 to 9 and 13 to 14, wherein said inhibitor is specific to MMP-13 induced cleaving of CD28.
16. The method of claim 12 or 15, wherein said inhibitor inhibits induced cleavage of mCD28 between P144 and L145 of mCD28.
17. The method of claim 12 or 16, wherein said inhibitor binds mCD28 and inhibits MMP-2 induced shedding of CD28.
18. The method of claim 15 or 16, wherein said inhibitor binds mCD28 and inhibits MMP-13 induced shedding of CD28.
19. The method of claim 17 or 18, wherein said inhibitor binds a stalk region of mCD28, wherein the stalk region,
 - a. comprises the amino acid sequence GKHLCPSPFLPGPSKP (SEQ ID NO: 7), KGKHLCPSPFLPGPS (SEQ ID NO: 8) or KGKHLCPSPFLPGPSKP (SEQ ID NO: 9);
 - b. consists of the amino acid sequence HVKGKHLCPSPFLPGPSKP (SEQ ID NO: 10); or
 - c. both (a) and (b).
20. The method of claim 19, wherein said inhibitor binds, occludes or blocks an MMP-2, MMP-13 or both cleavage site, PX_1X_2/X_3 wherein X_3 is a hydrophobic residue, in said stalk region.
21. The method of claim 20, wherein said cleavage site is PSPL and said MMP-2, MMP-13 or both cleaves between said P and said L.
22. The method of any one of claims 1 to 21, further comprising administering another immunotherapy to said subject.
23. The method of claim 22, wherein said immunotherapy is selected from:
 - a. a checkpoint inhibitor;
 - b. a chimeric antigen receptor (CAR) based therapy; and
 - c. a cancer vaccine.

24. The method of claims 23, wherein said checkpoint inhibitor is a PD-1 and/or PD-L1 based immunotherapy.
25. A method for producing an agent for the performance of any one of claims 1 to 24, 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 MMP-2, MMP-13 or both, and selecting at least one agent that blocks cleavage of mCD28 by MMP-2, MMP-13 or both; 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 MMP-2, MMP-13 or both; and
 - iii. selecting at least one agent that blocks cleavage of mCD28 by MMP-2, MMP-13 or both;
- thereby producing an agent for performance of a method of any one of claims 1 to 24.
26. The method of claim 25, 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.
27. The method of claim 26, wherein said agent that binds specifically to a CD28 stalk domain, binds specifically to an MMP-2, MMP-13 or both cleavage site in said CD28 stalk domain.
28. The method of claim 27, wherein said cleavage site in said CD28 stalk domain is PSPL.
29. The method of any one of claims 25 to 28, 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.
30. The method of any one of claims 25 to 29, comprising testing an ability of said agent to block cleavage of mCD28 by MMP-2, and selecting at least one agent that blocks cleavage of mCD28 by MMP-2, 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 a method comprising testing an ability of said agent to block cleavage of mCD28 by MMP-2, and selecting at least one agent that blocks cleavage of mCD28 by MMP-2.

31. The method of any one of claims 25 to 29, testing an ability of said agent to block cleavage of mCD28 by MMP-13, and selecting at least one agent that blocks cleavage of mCD28 by MMP-13, 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 a method comprising testing an ability of said agent to block cleavage of mCD28 by MMP-13, and selecting at least one agent that blocks cleavage of mCD28 by MMP-13.
32. An agent produced by the method of any one of claims 25 to 31.
33. A pharmaceutical composition comprising an agent of claim 32, and a pharmaceutically acceptable carrier, excipient or adjuvant.