

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A method of inhibiting or reducing nuclear localization of a nuclear localizable polypeptide, wherein acetylation of an acetylation site of the nuclear localizable polypeptide increases its nuclear localization in a cell, comprising contacting the cell with a proteinaceous molecule comprising, consisting or consisting essentially of an amino acid sequence corresponding to an acetylation site.
2. The method according to claim 1, wherein the polypeptide is PD-1.
3. The method according to claim 1, wherein the polypeptide is PD-L1.
4. The method according to claim 1, wherein the polypeptide is PD-L2.
5. A method of inhibiting or reducing the nuclear localization of PD-1, PD-L1 or PD-L2 in a PD-1-, PD-L1- or PD-L2-overexpressing cell, comprising contacting the cell with a proteinaceous molecule comprising, consisting or consisting essentially of an amino acid sequence corresponding to an acetylation site.
6. The method according to claim 5, wherein the PD-1-, PD-L1- or PD-L2-overexpressing cell is a cancer stem cell or a non-cancer stem cell tumor cell.
7. The method according to claim 6, wherein the PD-1-, PD-L1- or PD-L2-overexpressing cell is a cancer stem cell tumor cell.
8. A method of altering at least one of (i) formation; (ii) proliferation; (iii) maintenance; (iv) epithelial to mesenchymal cell transition (EMT); (v) mesenchymal to epithelial cell transition (MET); or (vi) viability of a PD-1-, PD-L1- or PD-L2-overexpressing cell, comprising contacting said cell with a formation-, proliferation-, maintenance-, EMT-, MET-, or viability-modulating amount of a proteinaceous molecule comprising, consisting or consisting essentially of an amino acid sequence corresponding to an acetylation site.
9. The method according to claim 8, wherein EMT is inhibited or reduced.
10. The method according to claim 8 or claim 9, wherein the PD-1-, PD-L1- or PD-L2-overexpressing cell is a cancer stem cell or a non-cancer stem cell tumor cell.
11. The method according to claim 10, wherein the PD-1-, PD-L1- or PD-L2-overexpressing cell is a cancer stem cell tumor cell.
12. The method according to claim 11, wherein formation of a cancer stem cell tumor cell is inhibited or reduced.
13. The method according to claim 11, wherein proliferation of a cancer stem cell tumor cell is inhibited or reduced.
14. The method according to claim 11, wherein the viability of a cancer stem cell tumor cell is inhibited or reduced.
15. A method of treating or preventing a cancer in a subject wherein the cancer comprises at least one PD-1-, PD-L1- or PD-L2-overexpressing cell, comprising administering to the subject a proteinaceous molecule comprising, consisting or consisting essentially of an amino acid sequence corresponding to an acetylation site.
16. The method according to claim 15, wherein the PD-1-, PD-L1- or PD-L2-overexpressing cell is a cancer stem cell or a non-cancer stem cell tumor cell.
17. The method according to claim 16, wherein the PD-1-, PD-L1- or PD-L2-overexpressing cell is a cancer stem cell tumor cell.

18. The method according to any one of claims 15-17, wherein the cancer is selected from breast, prostate, lung, bladder, pancreatic, colon, liver or brain cancer, or melanoma or retinoblastoma.

19. The method according to any one of claims 15-18, further comprising
5 administering one or more further cancer therapies.

20. The method according to claim 19, wherein the further cancer therapy is a chemotherapeutic agent.

21. The method according to any one of claims 1-20, wherein the proteinaceous molecule comprises, consists or consists essentially of an amino acid sequence corresponding
10 to residues 255 to 271 of PD-L1.

22. A method of producing a proteinaceous molecule that inhibits or reduces nuclear localization of a nuclear localizable polypeptide wherein acetylation of an acetylation site of the nuclear localizable polypeptide increases its nuclear localization in a cell, the method comprising:

- 15 a) contacting a cell with a proteinaceous molecule comprising, consisting or consisting essentially of an amino acid sequence corresponding to an acetylation site; and
- b) detecting a reduction in or inhibition of the nuclear localization of the nuclear localizable polypeptide in the cell relative to a normal or
20 reference level of nuclear localization in the absence of the proteinaceous molecule.

23. The method according to claim 22, wherein the proteinaceous molecule is a fragment of a nuclear localizable polypeptide.

24. The method according to claim 22 or claim 23, wherein the proteinaceous
25 molecule comprises 50 amino acid residues or less.

25. The method according to any one of claims 22-24, wherein the amino acid sequence corresponding to an acetylation site is an amino acid sequence corresponding to residues 255 to 271 of PD-L1.

26. The method according to claim 25, wherein the proteinaceous molecule is
30 distinguished from PD-L1 by the addition, deletion and/or substitution of at least one (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9 etc.) amino acid in residues 255 to 271 of PD-L1.

27. A method of producing a proteinaceous molecule that inhibits or reduces nuclear localization of a nuclear localizable polypeptide wherein acetylation of an acetylation site of the nuclear localizable polypeptide increases its nuclear localization in a cell, the method
35 comprising:

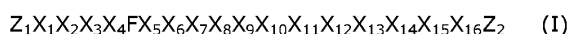
- a) contacting a cell with a proteinaceous molecule comprising, consisting or consisting essentially of an amino acid sequence corresponding to residues 255 to 271 of PD-L1; and
- b) detecting a reduction in or inhibition of the nuclear localization of the nuclear
40 localizable polypeptide in the cell relative to a normal or reference level of nuclear localization in the absence of the proteinaceous molecule.

28. The method according to claim 27, wherein the proteinaceous molecule is distinguished from PD-L1 by the addition, deletion and/or substitution of at least one (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9 etc.) amino acid in residues 255 to 271 of PD-L1.

29. A method of producing a proteinaceous molecule that inhibits or reduces at least one of formation, proliferation, viability or EMT of a cancer stem cell, the method comprising:
- a) contacting a cancer stem cell with a proteinaceous molecule comprising, consisting or consisting essentially of an amino acid sequence corresponding to residues 255 to 271 of PD-L1; and
 - b) detecting a reduction in or inhibition of the formation, proliferation or EMT of the cancer stem cell relative to a normal or reference level of formation, proliferation, viability or EMT of the cell in the absence of the proteinaceous molecule.

30. The method according to claim 29, wherein the proteinaceous molecule is distinguished from PD-L1 by the addition, deletion and/or substitution of at least one (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9 etc.) amino acid in residues 255 to 271 of PD-L1.

31. An isolated or purified proteinaceous molecule represented by Formula I:



wherein:

Z_1 and Z_2 are independently absent or are independently selected from at least one of a proteinaceous moiety comprising from about 1 to about 50 amino acid residues (and all integer residues in between), and a protecting moiety;

X_1 is absent or is selected from small amino acid residues including A, G, S, T and modified forms thereof, and hydrophobic amino acid residues including M, Nle, I, L, V, F, Y, W and modified forms thereof;

X_2 is selected from small amino acid residues including A, G, S, T and modified forms thereof, and charged amino acid residues including K, R, D, E and modified forms thereof;

X_3 is selected from any amino acid residue;

X_4 is selected from charged amino acid residues including K, R, D, E and modified forms thereof, and hydrophobic amino acid residues including M, Nle, I, L, V, F, Y, W and modified forms thereof;

X_5 is selected from any amino acid residue;

X_6 is selected from charged amino acid residues including K, R, D, E and modified forms thereof, and hydrophobic amino acid residues including M, Nle, I, L, V, F, Y, W and modified forms thereof;

X_7 is selected from charged amino acid residues including K, R, D, E and modified forms thereof, and hydrophobic amino acid residues including M, Nle, I, L, V, F, Y, W and modified forms thereof;

X_8 is selected from small amino acid residues including A, G, S, T and modified forms thereof, basic amino acid residues including K, R, Orn and modified forms thereof, and amino

acid residues with an amide-containing side chain including N, Q, Orn(Ac), K(Ac) and modified forms thereof;

X₉ is selected from small amino acid residues including G, S, T and modified forms thereof, charged amino acid residues including K, R, D, E and modified forms thereof, and
5 hydrophobic amino acid residues including M, Nle, I, L, V, F, Y, W and modified forms thereof;

X₁₀ is selected from any amino acid residue;

X₁₁ is selected from any amino acid residue;

X₁₂ is selected from charged amino acid residues including K, R, D, E and modified forms thereof, and hydrophobic amino acid residues including M, Nle, I, L, V, F, Y, W and modified
10 forms thereof;

X₁₃ is selected from any amino acid residue;

X₁₄ is selected from any amino acid residue;

X₁₅ is selected from charged amino acid residues including K, R, D, E and modified forms thereof, and hydrophobic amino acid residues including M, Nle, I, L, V, F, Y, W and modified
15 forms thereof; and

X₁₆ is selected from basic amino acid residues including K, R and modified forms thereof.

32. The proteinaceous molecule according to claim 31, wherein Z₁ is absent.

33. The proteinaceous molecule according to claim 31 or 32, wherein Z₂ is absent.

20 34. The proteinaceous molecule according to any one of claims 31-33, wherein X₁ is selected from L and A.

35. The proteinaceous molecule according to any one of claims 31-34, wherein X₁ is absent.

25 36. The proteinaceous molecule according to any one of claims 31-35, wherein X₂ is selected from small amino acid residues including A, G, S, T and modified forms thereof, and basic amino acid residues including K, R and modified forms thereof.

37. The proteinaceous molecule according to claim 36, wherein X₂ is selected from A and K.

38. The proteinaceous molecule according to claim 37, wherein X₂ is K.

30 39. The proteinaceous molecule according to any one of claims 31-38, wherein X₃ is selected from charged amino acid residues including K, R, D, E and modified forms thereof, and aromatic amino acid residues including F, Y, W and modified forms thereof.

40. The proteinaceous molecule according to claim 39, wherein X₃ is selected from F, E and K.

35 41. The proteinaceous molecule according to claim 40, wherein X₃ is F.

42. The proteinaceous molecule according to any one of claims 31-41, wherein X₄ is selected from acidic amino acid residues including D, E and modified forms thereof, and hydrophobic amino acid residues including I, L, V, M, Nle and modified forms thereof.

40 43. The proteinaceous molecule according to claim 42, wherein X₄ is selected from I, L and E.

44. The proteinaceous molecule according to claim 43, wherein X₄ is I.

45. The proteinaceous molecule according to any one of claims 31-44, wherein X_5 is selected from charged amino acid residues including K, R, D, E and modified forms thereof, and hydrophobic amino acid residues including M, Nle, I, L, V, F, Y, W and modified forms thereof.
- 5 46. The proteinaceous molecule according to claim 45, wherein X_5 is selected from R, E and V.
47. The proteinaceous molecule according to claim 46, wherein X_5 is V.
48. The proteinaceous molecule according to any one of claims 31-47, wherein X_6 is selected from L, E, K, F and V.
- 10 49. The proteinaceous molecule according to claim 48, wherein X_6 is L or F.
50. The proteinaceous molecule according to claim 49, wherein X_6 is F.
51. The proteinaceous molecule according to any one of claims 31-50, wherein X_7 is selected from R, E and L.
52. The proteinaceous molecule according to claim 51, wherein X_7 is L.
- 15 53. The proteinaceous molecule according to any one of claims 31-52, wherein X_8 is selected from small amino acid residues including A, G, S, T and modified forms thereof, and basic amino acid residues including K, R, Orn and modified forms thereof.
54. The proteinaceous molecule according to claim 53, wherein X_8 is A or K.
55. The proteinaceous molecule according to claim 54, wherein X_8 is A.
- 20 56. The proteinaceous molecule according to any one of claims 31-55, wherein X_9 is selected from G, acidic amino acid residues including D, E and modified forms thereof, and hydrophobic amino acid residues including M, Nle, I, L, V and modified forms thereof.
57. The proteinaceous molecule according to claim 56, wherein X_9 is G, D or V.
58. The proteinaceous molecule according to any one of claims 31-57, wherein X_{10} is selected from basic amino acid residues including K, R and modified forms thereof, and hydrophobic amino acid residues including M, Nle, I, L, V, F, Y, W and modified forms thereof.
- 25 59. The proteinaceous molecule according to claim 58, wherein X_{10} is selected from R and V.
- 30 60. The proteinaceous molecule according to claim 59, wherein X_{10} is R.
61. The proteinaceous molecule according to any one of claims 31-60, wherein X_{11} is selected from small amino acid residues including A, G, S, T and modified forms thereof, hydrophobic amino acid residues including M, Nle, I, L, V, F, Y, W and modified forms thereof, and acidic amino acid residues including D, E and modified forms thereof.
- 35 62. The proteinaceous molecule according to claim 61, wherein X_{11} is selected from M, Nle, A and E.
63. The proteinaceous molecule according to claim 62, wherein X_{11} is A.
64. The proteinaceous molecule according to any one of claims 31-63, wherein X_{12} is selected from hydrophobic amino acid residues including M, Nle, I, L, V, F, Y, W and modified forms thereof, and acidic amino acid residues including D, E and modified forms thereof.
- 40 65. The proteinaceous molecule according to claim 64, wherein X_{12} is M, Nle or E.
66. The proteinaceous molecule according to claim 65, wherein X_{12} is E.
67. The proteinaceous molecule according to any one of claims 31-66, wherein X_{13} is selected from small amino acid residues including A, G, S, T and modified forms thereof,

hydrophobic amino acid residues including M, Nle, I, L, V, F, Y, W and modified forms thereof, and acidic amino acid residues including D, E and modified forms thereof.

68. The proteinaceous molecule according to claim 67, wherein X_{13} is A, D or V.

69. The proteinaceous molecule according to claim 68, wherein X_{13} is A.

5 70. The proteinaceous molecule according to any one of claims 31-69, wherein X_{14} is selected from hydrophobic amino acid residues including M, Nle, I, L, V, F, Y, W and modified forms thereof, and basic amino acid residues including K, R and modified forms thereof.

71. The proteinaceous molecule according to claim 70, wherein X_{14} is V or K.

72. The proteinaceous molecule according to claim 71, wherein X_{14} is K.

10 73. The proteinaceous molecule according to any one of claims 31-72, wherein X_{15} is selected from basic amino acid residues including K, R and modified forms thereof, and aromatic amino acid residues including F, Y, W and modified forms thereof.

74. The proteinaceous molecule according to claim 73, wherein X_{15} is R, K or Y.

75. The proteinaceous molecule according to claim 74, wherein X_{15} is K.

15 76. The proteinaceous molecule according to any one of claims 31-75, wherein X_{16} is K.

77. The proteinaceous molecule according to claim 31, wherein the proteinaceous molecule of Formula I comprises, consists or consists essentially of an amino acid sequence represented by any one of SEQ ID NO: 1-18:

20	LTFIFRLRKGRMMDVKK	[SEQ ID NO: 1];
	LTFIFRLRQGRMMDVKK	[SEQ ID NO: 2];
	LTFIFRLRK(Ac)GRMMDVKK	[SEQ ID NO: 3];
	ATFIFRLRKGRMMDVKK	[SEQ ID NO: 4];
	LKFIFRLRKGRMMDVKK	[SEQ ID NO: 5];
25	AFIFRLRKGRMMDVKK	[SEQ ID NO: 6];
	LTFIFVLRKGRMMDVKK	[SEQ ID NO: 7];
	LTFIFRFRKGRMMDVKK	[SEQ ID NO: 8];
	LTFIFRLLKGRMMDVKK	[SEQ ID NO: 9];
	TFIFRLRAGRMDVKK	[SEQ ID NO: 10];
30	LTFIFRLRKDRMMDVKK	[SEQ ID NO: 11];
	LTFIFRLRKVRMMDVKK	[SEQ ID NO: 12];
	TFIFRLRKGRAMDVKK	[SEQ ID NO: 13];
	LTFIFRLRKGREMDVKK	[SEQ ID NO: 14];
	LTFIFRLRKGRMEDVKK	[SEQ ID NO: 15];
35	TFIFRLRKGRMMAVKK	[SEQ ID NO: 16];
	LTFIFRLRKGRMMVVKK	[SEQ ID NO: 17]; or
	LTFIFRLRKGRMDKKK	[SEQ ID NO: 18].

78. The proteinaceous molecule according to claim 77, wherein the proteinaceous molecule of Formula I comprises, consists or consists essentially of an amino acid sequence represented by SEQ ID NO: 1, 4, 9, 10, 13, 16 or 18.

5 79. The proteinaceous molecule according to any one of claims 31-78, wherein the proteinaceous molecule has any one or more activities selected from the group consisting of: (i) increasing cell death; (ii) increasing MET; (iii) reducing or inhibiting EMT; (iv) inhibiting or reducing maintenance; (v) inhibiting or reducing proliferation; (vi) increasing differentiation; (vii) inhibiting or reducing formation; or (viii) reducing viability of a PD-1-, PD-L1- or PD-L2-overexpressing cell.

10 80. The proteinaceous molecule according to claim 79, wherein the cell is a PD-L1-overexpressing cell.

81. The proteinaceous molecule according to claim 79 or claim 80, wherein the cell is a cancer stem cell or a non-cancer stem cell tumor cell.

15 82. The proteinaceous molecule according to claim 81, wherein the cell is a cancer stem cell tumor cell.

83. The proteinaceous molecule according to any one of claims 31-82, wherein the proteinaceous molecule of Formula I further comprises at least one membrane permeating moiety.

20 84. The proteinaceous molecule according to claim 83, wherein the membrane permeating moiety is a lipid moiety.

85. The proteinaceous molecule according to claim 84, wherein the membrane permeating moiety is a myristoyl group.

86. The proteinaceous molecule according to any one of claims 83-85, wherein the membrane permeating moiety is coupled to the N- or C-terminal amino acid residue.

25 87. The proteinaceous molecule according to claim 86, wherein the membrane permeating moiety is coupled to the N-terminal amino acid residue.

88. The method according to claim 21, wherein the proteinaceous molecule is the isolated or purified proteinaceous molecule according to any one of claims 31-87.

30