CLAIMS

What is claimed is:

1. An immunocytokine comprising: a) an antigen-binding protein specifically recognizing a target antigen; and b) a cytokine or variant thereof, wherein the antigen-binding protein comprises an antigen-binding polypeptide comprising from N' to C': an antigen-binding fragment, a hinge region, and an Fc domain subunit or portion thereof, and wherein the cytokine or variant thereof is positioned at the hinge region;
and wherein the target antigen is PD-1;
the antigen-binding protein specifically binds the target antigen has a KD with a range of 10^{-8}M ＜KD≤~10^{-6}M, and
particularly, wherein the antigen-binding fragment is PD-L2 or PD-L1.

2. The immunocytokine of claim 1,wherein the antigen-binding protein is an antibody specifically recognizing PD1, wherein the antigen-binding polypeptide comprising the hinge region is a heavy chain of the antibody, and wherein the cytokine or variant thereof is positioned at the hinge region of the heavy chain.

~~2~~3. The immunocytokine of claim 1 or 2, wherein in the presence of binding of the antigen-binding protein to the target antigen, the activity of the cytokine or variant thereof increases at least about 20% compared to that in the absence of binding of the antigen-binding protein to the target antigen, alternatively, wherein in the absence of binding of the antigen-binding protein to the target antigen, the activity of the cytokine or variant thereof positioned at the hinge region is no more than about 70% of that of a corresponding cytokine or variant thereof in a free state, alternatively,wherein the cytokine or variant thereof is a cytokine variant, and wherein the activity of the cytokine variant in a free state is no more than about 80% of that of a corresponding wildtype cytokine in a free state.

~~3. The immunocytokine of claim 1 or 2, wherein in the absence of binding of the antigen-binding protein to the target antigen, the activity of the cytokine or variant thereof positioned at the hinge region is no more than about 70% of that of a corresponding cytokine or variant thereof in a free state.~~

~~4. The immunocytokine of any one of claims 1-3, wherein the cytokine or variant thereof is a cytokine variant, and wherein the activity of the cytokine variant in a free state is no more than about 80% of that of a corresponding wildtype cytokine in a free state.~~

~~5~~4. The immunocytokine of any one of claims 1-~~4~~3, wherein the antigen-binding protein comprises two antigen-binding polypeptides each comprising a hinge region, and wherein only one antigen-binding polypeptide comprises the cytokine or variant thereof positioned at the hinge region, alternatively,wherein the antigen-binding protein comprises two antigen-binding polypeptides each comprising a hinge region, and wherein each antigen-binding polypeptide comprises a cytokine or variant thereof positioned at the hinge region, and the two cytokines or variants thereof are the same or different.

~~6. The immunocytokine of any one of claims 1-4, wherein the antigen-binding protein comprises two antigen-binding polypeptides each comprising a hinge region, and wherein each antigen-binding polypeptide comprises a cytokine or variant thereof positioned at the hinge region.~~

~~7. The immunocytokine of any one of claims 1-6, wherein the immunocytokine comprises two or more cytokines or variants thereof, wherein the two or more cytokines or variant thereof are positioned in tandem at the hinge region of the antigen-binding polypeptide.~~

~~8~~5. The immunocytokine of any one of claims 1-~~7~~4, wherein the cytokine or variant thereof is a monomeric or dimericcytokine or variant thereof, and when the cytokine or variant thereof is dimeric, both subunits of the dimeric cytokine or variant thereof are positioned in tandem at the hinge region of the antigen-binding polypeptide, particularly, wherein the cytokine or variant thereof is selected from the group consisting of IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-15, IL-17, IL-18, IL-21, IL-22, IL-23, IL-27, IL-35, IFN-α, IFN-β, IFN-γ, TNF-α, TGF-β, VEGF, erythropoietin, thrombopoietin, G-CSF, M-CSF, SCF, and GM-CSF, and more particularly, wherein the cytokine or variant thereof is IL-2 or variant thereof, alternatively, the cytokine or variant thereof is IL-12 or variant thereof.

~~9. The immunocytokine of any one of claims 1-7, wherein the cytokine or variant thereof is a dimeric cytokine or variant thereof.~~

~~10. The immunocytokine of claim 9, wherein both subunits of the dimeric cytokine or variant thereof are positioned in tandem at the hinge region of the antigen-binding polypeptide.~~

~~11. The immunocytokine of claim 9, wherein the antigen-binding protein comprises two antigen-binding polypeptides each comprising a hinge region, and whereinone subunit of the dimeric cytokine or variant thereof is positioned at the hinge region of one antigen-binding polypeptide, and the other subunit of the dimeric cytokine or variant thereof is positioned at the hinge region of the other antigen-binding polypeptide.~~

~~12. The immunocytokine of any one of claims 6-11, wherein the two or more cytokines or variants thereof are the same.~~

~~13. The immunocytokine of any one of claims 6-11, wherein the two or more cytokines or variants thereof are different.~~

~~14. The immunocytokine of any one of claims 1-13, wherein the antigen-binding protein is a monospecific antigen-binding protein.~~

~~15. The immunocytokine of any one of claims 1-13, wherein the antigen-binding protein is a multispecific antigen-binding protein.~~

~~16. The immunocytokine of any one of claims 1-15, wherein the target antigen is selected from the group consisting of PD-1, PD-L1, PD-L2, CTLA-4, CD3, CD4, CD8, CD25, CD123, and HER2.~~

~~17. The immunocytokine of any one of claims 1-16, wherein the cytokine or variant thereof is selected from the group consisting of IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-15, IL-17, IL-18, IL-21, IL-22, IL-23, IL-27, IL-35, IFN-α, IFN-β, IFN-γ, TNF-α, TGF-β, VEGF, erythropoietin, thrombopoietin, G-CSF, M-CSF, SCF, and GM-CSF.~~

~~18. The immunocytokine of any one of claims 1-17, wherein the cytokine or variant thereof is selected from the group consisting of IL-2, IL-10, IL-12, IL-23, IFN-α, and IFN-γ.~~

~~19. The immunocytokine of claim 17 or 18, wherein the cytokine or variant thereof is IL-2 or variant thereof.~~

~~20~~6. The immunocytokine of any one of claims 1-~~19~~5, wherein the IL-2 variant comprises one or more mutations at a position selected from the group consisting of L18, Q22, F24, K35, R38, F42, K43, E61, P65, Q126, and S130 relative to a wildtype IL-2 comprising the sequence of SEQ ID NO: 1, particularly, wherein the IL-2 variant comprises one or more mutations selected from the group consisting of L18R, Q22E, F24A, R38D, K43E, E61R, P65L, Q126T, and S130R relative to a wildtype IL-2 comprising the sequence of SEQ ID NO: 1, more particularly, wherein the IL-2 variant comprises an R38D/K43E/E61R mutation, an

L18R/Q22E/R38D/K43E/E61R mutation, an R38D/K43E/E61R/Q126T mutation, an L18R/Q22E/R38D/K43E/E61R/Q126T mutation, or an L18R/Q22E/R38D/K43E/E61R/Q126T/S130R mutation relative to a wildtype IL-2 comprising the sequence of SEQ ID NO: 1, and even more particularly, wherein the IL-2 variant comprises the sequence of any one of SEQ ID NOs: 2 and 251-254.

~~21. The immunocytokine of claim 19 or 20, wherein the IL-2 variant comprises one or more mutations selected from the group consisting of L18R, Q22E, F24A, R38D, K43E, E61R, P65L, Q126T, and S130R relative to a wildtype IL-2 comprising the sequence of SEQ ID NO: 1.~~

~~22. The immunocytokine of any one of claims 19-21, wherein the IL-2 variant comprises an R38D/K43E/E61R mutation, an L18R/Q22E/R38D/K43E/E61R mutation, an R38D/K43E/E61R/Q126T mutation, an L18R/Q22E/R38D/K43E/E61R/Q126T mutation, or an L18R/Q22E/R38D/K43E/E61R/Q126T/S130R mutation relative to a wildtype IL-2 comprising the sequence of SEQ ID NO: 1.~~

~~23. The immunocytokine of any one of claims 19-22, wherein the IL-2 variant comprises the sequence of any one of SEQ ID NOs: 2 and 251-254.~~

~~24. The immunocytokine of claim 17 or 18, wherein the cytokine or variant thereof is IFN-α or variant thereof.~~

~~25. The immunocytokine of claim 24, wherein the IFN-α variant comprises one or more mutations at a position selected from the group consisting of R22, L26, F27, L30, K31, D32, R33, H34, D35, F36, S68, T79, K83, Y85, Y89, R120, K121, Y122, Q124, Y129, K131, E132, R144, and E146 relative to an IFN-α comprising the sequence of SEQ ID NO: 3.~~

~~26. The immunocytokine of claim 24 or 25, wherein the IFN-α variant comprises one or more mutations selected from the group consisting of L30A, K31A, D32A, R33A, H34A, and D35A relative to an IFN-α comprising the sequence of SEQ ID NO: 3.~~

~~27. The immunocytokine of any one of claims 24-26, wherein the IFN-α variant comprises the sequence of any of SEQ ID NOs: 4-9.~~

~~28. The immunocytokine of any one of claims 24-27, wherein the IFN-α variant comprises an L30A mutation relative to an IFN-α comprising the sequence of SEQ ID NO: 3.~~

~~29. The immunocytokine of any one of claims 24-28, wherein the IFN-α variant comprises the sequence of SEQ ID NO: 4.~~

30. The immunocytokine of claim 17 or 18, wherein the cytokine or variant thereof is IFN-γ or variant thereof.

31. The immunocytokine of claim 30, wherein the IFN-γ variant comprises one or more mutations within one or both IFN-γ subunits at a position selected from the group consisting of V5, S20, D21, V22, A23, D24, N25, G26, H111, and Q115 relative to a wildtype IFN-γ subunit comprising the sequence of SEQ ID NO: 10.

32. The immunocytokine of claim 30 or 31, wherein the IFN-γ variant comprises one or more mutations within one or both IFN-γ subunits selected from the group consisting of S20A, D21A, D21K, V22A, A23S, A23E, A23Q, A23V, D24A, D24E, N25A, N25K, and H111D relative to a wildtype IFN-γ subunit comprising the sequence of SEQ ID NO: 10.

33. The immunocytokine of any one of claims 30-32, wherein the IFN-γ variant comprises one or more mutations within one or both IFN-γ subunits selected from the group consisting of S20A/D21A, D21K, V22A/A23S, D24A/N25A, A23E/D24E/N25K, A23Q, and A23V relative to a wildtype IFN-γ subunit comprising the sequence of SEQ ID NO: 10.

34. The immunocytokine of any one of claims 30-33, wherein one or both subunits of the IFN-γ variant comprises the sequence of any of SEQ ID NOs: 11-17.

35. The immunocytokine of any one of claims 30-34, wherein the IFN-γ variant comprises an A23V mutation within one or both IFN-γ subunits relative to a wildtype IFN-γ subunit comprising the sequence of SEQ ID NO: 10.

36. The immunocytokine of any one of claims 30-35, wherein the one or both subunits of the IFN-γ variant comprises the sequence of SEQ ID NO: 13.

37. The immunocytokine of any one of claims 30-36, wherein the two subunits of the IFN-γ or variant thereof are connected by a linker.

38. The immunocytokine of claim 37, wherein the IFN-γ variant comprises the sequence of SEQ ID NO: 19.

39. The immunocytokine of claim 17 or 18, wherein the cytokine or variant thereof is IL-10 or variant thereof.

40. The immunocytokine of claim 39, wherein the IL-10 variant comprises one or more mutations within one or both IL-10 subunits at a position selected from the group consisting of N21, M22, R24, D25, L26, R27, D28, A29, F30, S31, R32, H90, and S93 relative to a wildtype IL-10 subunit comprising the sequence of SEQ ID NO: 20.

~~41. The immunocytokine of claim 39 or 40, wherein the IL-10 variant comprises one or more mutations within one or both IL-10 subunits selected from the group consisting of R24A, D25A, L26A, R27A, D28A, A29S, F30A, S31A, and R32A relative to a wildtype IL-10 subunit comprising the sequence of SEQ ID NO: 20.~~

~~42. The immunocytokine of any one of claims 39-41, wherein the IL-10 variant comprises one or more mutations within one or both IL-10 subunits selected from the group consisting of R24A, D25A/L26A, R27A, D28A/A29S, F30A/S31A, and R32A relative to a wildtype IL-10 subunit comprising the sequence of SEQ ID NO: 20.~~

~~43. The immunocytokine of any one of claims 39-42, wherein one or both subunits of the IL-10 variant comprises the sequence of any of SEQ ID NOs: 21-26.~~

~~44. The immunocytokine of any one of claims 39-43, wherein the IL-10 variant comprises an R27A mutation within one or both IL-10 subunits relative to a wildtype IL-10 subunit comprising the sequence of SEQ ID NO: 20.~~

~~45. The immunocytokine of any one of claims 39-44, wherein the one or both subunits of the IL-10 variant comprises the sequence of SEQ ID NO: 23.~~

~~46. The immunocytokine of any one of claims 39-45, wherein the two subunits of the IL-10 or variant thereof are connected by a linker.~~

~~47. The immunocytokine of claim 46, wherein the IL-10 variant comprises the sequence of SEQ ID NO: 28.~~

~~48. The immunocytokine of claim 17 or 18, wherein the cytokine or variant thereof is IL-12 or variant thereof.~~

~~49~~7. The immunocytokine of any one of claim~~s 48~~1-5, wherein the IL-12 variant comprises one or more mutations within the p40 subunit at a position selected from the group consisting of E45, Q56, V57, K58, E59, F60, G61, D62, A63, G64, Q65, and C177 relative to a wildtype p40 subunit comprising the sequence of SEQ ID NO: 30, particularly, wherein the IL-12 variant comprises one or more mutations within the p40 subunit selected from the group consisting of Q56A, V57A, K58A, E59A, F60A, G61A, D62A, A63S, G64A, and Q65A relative to a wildtype p40 subunit comprising the sequence of SEQ ID NO: 30, more particularly,wherein the p40 subunit of the IL-12 variant comprises the sequence of any of SEQ ID NOs: 31-34, even more particularly, wherein the IL-12 variant comprises an E59A/F60A mutation or an F60A mutation

within the p40 subunit relative to a wildtype p40 subunit comprising the sequence of SEQ ID NO: 30.

~~50. The immunocytokine of claim 48 or 49, wherein the IL-12 variant comprises one or more mutations within the p40 subunit selected from the group consisting of Q56A, V57A, K58A, E59A, F60A, G61A, D62A, A63S, G64A, and Q65A relative to a wildtype p40 subunit comprising the sequence of SEQ ID NO: 30.~~

~~51. The immunocytokine of any one of claims 48-50, wherein the p40 subunit of the IL-12 variant comprises the sequence of any of SEQ ID NOs: 31-34.~~

~~52. The immunocytokine of any one of claims 48-51, wherein the IL-12 variant comprises an E59A/F60A mutation or an F60A mutation within the p40 subunit relative to a wildtype p40 subunit comprising the sequence of SEQ ID NO: 30.~~

~~53~~8. The immunocytokine of ~~any one of~~ claim~~s 48-52~~7, wherein the p40 subunit of the IL-12 variant comprises the sequence of SEQ ID NO: 31 or 33, particularly, wherein the p40 subunit and the p35 subunit of the IL-12 or variant thereof are connected by a linker, more particularly,wherein the IL-12 variant comprises the sequence of SEQ ID NO: 36, 275, 331, or 332.

~~54. The immunocytokine of any one of claims 48-53, wherein the p40 subunit and the p35 subunit of the IL-12 or variant thereof are connected by a linker.~~

~~55. The immunocytokine of claim 54, wherein the IL-12 variant comprises the sequence of SEQ ID NO: 36, 275, 331, or 332.~~

~~56. The immunocytokine of claim 17 or 18, wherein the cytokine or variant thereof is IL-23 or variant thereof.~~

~~57. The immunocytokine of claim 56, wherein the IL-23 variant comprises one or more mutations within the p40 subunit at a position selected from the group consisting of E45, Q56, V57, K58, E59, F60, G61, D62, A63, G64, Q65, and C177 relative to a wildtype p40 subunit comprising the sequence of SEQ ID NO: 30.~~

~~58. The immunocytokine of claim 56 or 57, wherein the IL-23 variant comprises one or more mutations within the p40 subunit selected from the group consisting of Q56A, V57A, K58A, E59A, F60A, G61A, D62A, A63S, G64A, and Q65A relative to a wildtype p40 subunit comprising the sequence of SEQ ID NO: 30.~~

59. The immunocytokine of any one of claims 56-58, wherein the p40 subunit of the IL-23 variant comprises the sequence of any of SEQ ID NOs: 31-34.

60. The immunocytokine of any one of claims 56-59, wherein the IL-23 variant comprises an E59A/F60A mutation within the p40 subunit relative to a wildtype p40 subunit comprising the sequence of SEQ ID NO: 30.

61. The immunocytokine of any one of claims 56-60, wherein the p40 subunit of the IL-23 variant comprises the sequence of SEQ ID NO: 31.

62. The immunocytokine of any one of claims 56-61, wherein the p40 subunit and the p19 subunit of the IL-23 or variant thereof are connected by a linker.

63. The immunocytokine of claim 62, wherein the IL-23 variant comprises the sequence of SEQ ID NO: 39.

64. The immunocytokine of any one of claims 37, 46, 54, and 62, wherein the linker comprises the sequence of any of SEQ ID NOs: 227-229.

65. The immunocytokine of any one of claims 1-64, wherein the cytokine or variant thereof is positioned within the hinge region of the antigen-binding polypeptide.

66. The immunocytokine of any one of claims 1-65, wherein the Fc domain subunit or portion thereof comprises a knobs-into-holes (KIH) mutation.

67. The immunocytokine of any one of claims 1-66, wherein the antigen-binding protein is an antibody specifically recognizing the target antigen, wherein the antigen-binding polypeptide comprising the hinge region is a heavy chain of the antibody, and wherein the cytokine or variant thereof is positioned at the hinge region of the heavy chain.

68. The immunocytokine of claim 67, wherein the antibody is an anti-HER2 antibody comprising: i) a VH-CDR1 comprising the sequence of SEQ ID NO: 188; ii) a VH-CDR2 comprising the sequence of SEQ ID NO: 189; iii) a VH-CDR3 comprising the sequence of SEQ ID NO: 190; iv) a VL-CDR1 comprising the sequence of SEQ ID NO: 191; v) a VL-CDR2 comprising the sequence of SEQ ID NO: 192; and vi) a VL-CDR3 comprising the sequence of SEQ ID NO: 193.

69. The immunocytokine of claim 68, wherein the antibody comprises a VH comprising the sequence of SEQ ID NO: 150 and a VL comprising the sequence of SEQ ID NO: 151.

70. The immunocytokine of claim 68 or 69, wherein the antibody comprises a light chain comprising the sequence of SEQ ID NO: 154,

(i) wherein the cytokine or variant thereof is an IL-2 variant comprising the sequence of SEQ ID NO: 2, and wherein the heavy chain comprising the IL-2 variant positioned at the hinge region comprises the sequence of SEQ ID NO: 156;

(ii) wherein the cytokine or variant thereof is an IFN-α variant comprising the sequence of SEQ ID NO: 4, and wherein the heavy chain comprising the IFN-α variant positioned at the hinge region comprises the sequence of SEQ ID NO: 157;

(iii) wherein the cytokine or variant thereof is an IFN-γ variant comprising the sequence of SEQ ID NO: 19, and wherein the heavy chain comprising the IFN-γ variant positioned at the hinge region comprises the sequence of SEQ ID NO: 158;

(iv) wherein the cytokine or variant thereof is an IL-10 variant comprising the sequence of SEQ ID NO: 28, and wherein the heavy chain comprising the IL-10 variant positioned at the hinge region comprises the sequence of SEQ ID NO: 159;

(v) wherein the cytokine or variant thereof is an IL-12 variant comprising the sequence of SEQ ID NO: 36, and wherein the heavy chain comprising the IL-12 variant positioned at the hinge region comprises the sequence of SEQ ID NO: 160; or

(vi) wherein the cytokine or variant thereof is an IL-23 variant comprising the sequence of SEQ ID NO: 39, and wherein the heavy chain comprising the IL-23 variant positioned at the hinge region comprises the sequence of SEQ ID NO: 161.

71. The immunocytokine of any one of claims 68-70, wherein the antibody comprises a second heavy chain not comprising the cytokine or variant thereof positioned at the hinge region, and wherein the second heavy chain comprises the sequence of SEQ ID NO: 155.

72. The immunocytokine of claim 67, wherein the antibody is an anti-CD3 antibody comprising: i) a VH-CDR1 comprising the sequence of SEQ ID NO: 85; ii) a VH-CDR2 comprising the sequence of SEQ ID NO: 86; iii) a VH-CDR3 comprising the sequence of SEQ ID NO: 87; iv) a VL-CDR1 comprising the sequence of SEQ ID NO: 88; v) a VL-CDR2 comprising the sequence of SEQ ID NO: 89; and vi) a VL-CDR3 comprising the sequence of SEQ ID NO: 90.

73. The immunocytokine of claim 72, wherein the antibody comprises a VH comprising the sequence of SEQ ID NO: 91 and a VL comprising the sequence of SEQ ID NO: 92.

74. The immunocytokine of claim 72 or 73, wherein the antibody comprises a light chain comprising the sequence of SEQ ID NO: 94,

(i) wherein the cytokine or variant thereof is an IL-2 variant comprising the sequence of SEQ ID NO: 2, and wherein the heavy chain comprising the IL-2 variant positioned at the hinge region comprises the sequence of SEQ ID NO: 96;

(ii) wherein the cytokine or variant thereof is an IFN-α variant comprising the sequence of SEQ ID NO: 4, and wherein the heavy chain comprising the IFN-α variant positioned at the hinge region comprises the sequence of SEQ ID NO: 97;

(iii) wherein the cytokine or variant thereof is an IFN-γ variant comprising the sequence of SEQ ID NO: 19, and wherein the heavy chain comprising the IFN-γ variant positioned at the hinge region comprises the sequence of SEQ ID NO: 98;

(iv) wherein the cytokine or variant thereof is an IL-10 variant comprising the sequence of SEQ ID NO: 28, and wherein the heavy chain comprising the IL-10 variant positioned at the hinge region comprises the sequence of SEQ ID NO: 99;

(v) wherein the cytokine or variant thereof is an IL-12 variant comprising the sequence of SEQ ID NO: 36, and wherein the heavy chain comprising the IL-12 variant positioned at the hinge region comprises the sequence of SEQ ID NO: 100; or

(vi) wherein the cytokine or variant thereof is an IL-23 variant comprising the sequence of SEQ ID NO: 39, and wherein the heavy chain comprising the IL-23 variant positioned at the hinge region comprises the sequence of SEQ ID NO: 101.

75. The immunocytokine of any one of claims 72-74, wherein the antibody comprises a second heavy chain not comprising the cytokine or variant thereof positioned at the hinge region, and wherein the second heavy chain comprises the sequence of SEQ ID NO: 95.

76. The immunocytokine of claim 67, wherein the antibody is an anti-PD-1 antibody comprising VH-CDR1, VH-CDR2, and VH-CDR3 of a VH comprising the sequence of SEQ ID NO: 102, and VL-CDR1, VL-CDR2, and VL-CDR3 of a VL comprising the sequence of SEQ ID NO: 103.

77. The immunocytokine of claim 76, wherein the antibody comprises a VH comprising the sequence of SEQ ID NO: 102 and a VL comprising the sequence of SEQ ID NO: 103.

78. The immunocytokine of claim 76 or 77, wherein the antibody comprises a light chain comprising the sequence of SEQ ID NO: 106,

(i) wherein the cytokine or variant thereof is an IL-2 variant comprising the sequence of SEQ ID NO: 2, and wherein the heavy chain comprising the IL-2 variant positioned at the hinge region comprises the sequence of SEQ ID NO: 108;

(ii) wherein the cytokine or variant thereof is an IFN-α variant comprising the sequence of SEQ ID NO: 4, and wherein the heavy chain comprising the IFN-α variant positioned at the hinge region comprises the sequence of SEQ ID NO: 109;

(iii) wherein the cytokine or variant thereof is an IFN-γ variant comprising the sequence of SEQ ID NO: 19, and wherein the heavy chain comprising the IFN-γ variant positioned at the hinge region comprises the sequence of SEQ ID NO: 110;

(iv) wherein the cytokine or variant thereof is an IL-10 variant comprising the sequence of SEQ ID NO: 28, and wherein the heavy chain comprising the IL-10 variant positioned at the hinge region comprises the sequence of SEQ ID NO: 111;

(v) wherein the cytokine or variant thereof is an IL-12 variant comprising the sequence of SEQ ID NO: 36, and wherein the heavy chain comprising the IL-12 variant positioned at the hinge region comprises the sequence of SEQ ID NO: 112; or

(vi) wherein the cytokine or variant thereof is an IL-23 variant comprising the sequence of SEQ ID NO: 39, and wherein the heavy chain comprising the IL-23 variant positioned at the hinge region comprises the sequence of SEQ ID NO: 113.

79. The immunocytokine of any one of claims 76-78, wherein the antibody comprises a second heavy chain not comprising the cytokine or variant thereof positioned at the hinge region, and wherein the second heavy chain comprises the sequence of SEQ ID NO: 107.

80. The immunocytokine of claim 67, wherein the antibody is an anti-CD4 antibody comprising: i) a VH-CDR1 comprising the sequence of SEQ ID NO: 67; ii) a VH-CDR2 comprising the sequence of SEQ ID NO: 68; iii) a VH-CDR3 comprising the sequence of SEQ ID NO: 69; iv) a VL-CDR1 comprising the sequence of SEQ ID NO: 70; v) a VL-CDR2 comprising the sequence of SEQ ID NO: 71; and vi) a VL-CDR3 comprising the sequence of SEQ ID NO: 72.

81. The immunocytokine of claim 80, wherein the antibody comprises a VH comprising the sequence of SEQ ID NO: 73 and a VL comprising the sequence of SEQ ID NO: 74.

82. The immunocytokine of claim 80 or 81, wherein the antibody comprises a light chain comprising the sequence of SEQ ID NO: 77,

(i) wherein the cytokine or variant thereof is an IL-2 variant comprising the sequence of SEQ ID NO: 2, and wherein the heavy chain comprising the IL-2 variant positioned at the hinge region comprises the sequence of SEQ ID NO: 79;

(ii) wherein the cytokine or variant thereof is an IFN-α variant comprising the sequence of SEQ ID NO: 4, and wherein the heavy chain comprising the IFN-α variant positioned at the hinge region comprises the sequence of SEQ ID NO: 80;

(iii) wherein the cytokine or variant thereof is an INF-γ variant comprising the sequence of SEQ ID NO: 19, and wherein the heavy chain comprising the IFN-γ variant positioned at the hinge region comprises the sequence of SEQ ID NO: 81;

(iv) wherein the cytokine or variant thereof is an IL-10 variant comprising the sequence of SEQ ID NO: 28, and wherein the heavy chain comprising the IL-10 variant positioned at the hinge region comprises the sequence of SEQ ID NO: 82;

(v) wherein the cytokine or variant thereof is an IL-12 variant comprising the sequence of SEQ ID NO: 36, and wherein the heavy chain comprising the IL-12 variant positioned at the hinge region comprises the sequence of SEQ ID NO: 83; or

(vi) wherein the cytokine or variant thereof is an IL-23 variant comprising the sequence of SEQ ID NO: 39, and wherein the heavy chain comprising the IL-23 variant positioned at the hinge region comprises the sequence of SEQ ID NO: 84.

83. The immunocytokine of any one of claims 80-82, wherein the antibody comprises a second heavy chain not comprising the cytokine or variant thereof positioned at the hinge region, and wherein the second heavy chain comprises the sequence of SEQ ID NO: 78.

84. The immunocytokine of claim 67, wherein the antibody is an anti-CD8 antibody comprising VH-CDR1, VH-CDR2, and VH-CDR3 of a VH comprising the sequence of SEQ ID NO: 114, and VL-CDR1, VL-CDR2, and VL-CDR3 of a VL comprising the sequence of SEQ ID NO: 115.

85. The immunocytokine of claim 84, wherein the antibody comprises a VH comprising the sequence of SEQ ID NO: 114 and a VL comprising the sequence of SEQ ID NO: 115.

86. The immunocytokine of claim 84 or 85, wherein the antibody comprises a light chain comprising the sequence of SEQ ID NO: 117,

(i) wherein the cytokine or variant thereof is an IL-2 variant comprising the sequence of SEQ ID NO: 2, and wherein the heavy chain comprising the IL-2 variant positioned at the hinge region comprises the sequence of SEQ ID NO: 119;

(ii) wherein the cytokine or variant thereof is an IFN-α variant comprising the sequence of SEQ ID NO: 4, and wherein the heavy chain comprising the IFN-α variant positioned at the hinge region comprises the sequence of SEQ ID NO: 120;

(iii) wherein the cytokine or variant thereof is an INF-γ variant comprising the sequence of SEQ ID NO: 19, and wherein the heavy chain comprising the IFN-γ variant positioned at the hinge region comprises the sequence of SEQ ID NO: 121;

(iv) wherein the cytokine or variant thereof is an IL-10 variant comprising the sequence of SEQ ID NO: 28, and wherein the heavy chain comprising the IL-10 variant positioned at the hinge region comprises the sequence of SEQ ID NO: 122;

(v) wherein the cytokine or variant thereof is an IL-12 variant comprising the sequence of SEQ ID NO: 36, and wherein the heavy chain comprising the IL-12 variant positioned at the hinge region comprises the sequence of SEQ ID NO: 123; or

(vi) wherein the cytokine or variant thereof is an IL-23 variant comprising the sequence of SEQ ID NO: 39, and wherein the heavy chain comprising the IL-23 variant positioned at the hinge region comprises the sequence of SEQ ID NO: 124.

87. The immunocytokine of any one of claims 84-86, wherein the antibody comprises a second heavy chain not comprising the cytokine or variant thereof positioned at the hinge region, and wherein the second heavy chain comprises the sequence of SEQ ID NO: 118.

88. The immunocytokine of claim 67, wherein the antibody is an anti-CTLA-4 antibody comprising VH-CDR1, VH-CDR2, and VH-CDR3 of a VH comprising the sequence of SEQ ID NO: 125, and VL-CDR1, VL-CDR2, and VL-CDR3 of a VL comprising the sequence of SEQ ID NO: 126.

89. The immunocytokine of claim 88, wherein the antibody comprises a VH comprising the sequence of SEQ ID NO: 125 and a VL comprising the sequence of SEQ ID NO: 126.

90. The immunocytokine of claim 88 or 89, wherein the antibody comprises a light chain comprising the sequence of SEQ ID NO: 129,

(i) wherein the cytokine or variant thereof is an IL-2 variant comprising the sequence of SEQ ID NO: 2, and wherein the heavy chain comprising the IL-2 variant positioned at the hinge region comprises the sequence of SEQ ID NO: 131;

(ii) wherein the cytokine or variant thereof is an IFN-α variant comprising the sequence of SEQ ID NO: 4, and wherein the heavy chain comprising the IFN-α variant positioned at the hinge region comprises the sequence of SEQ ID NO: 132;

(iii) wherein the cytokine or variant thereof is an INF-γ variant comprising the sequence of SEQ ID NO: 19, and wherein the heavy chain comprising the IFN-γ variant positioned at the hinge region comprises the sequence of SEQ ID NO: 133;

(iv) wherein the cytokine or variant thereof is an IL-10 variant comprising the sequence of SEQ ID NO: 28, and wherein the heavy chain comprising the IL-10 variant positioned at the hinge region comprises the sequence of SEQ ID NO: 134;

(v) wherein the cytokine or variant thereof is an IL-12 variant comprising the sequence of SEQ ID NO: 36, and wherein the heavy chain comprising the IL-12 variant positioned at the hinge region comprises the sequence of SEQ ID NO: 135; or

(vi) wherein the cytokine or variant thereof is an IL-23 variant comprising the sequence of SEQ ID NO: 39, and wherein the heavy chain comprising the IL-23 variant positioned at the hinge region comprises the sequence of SEQ ID NO: 136.

91. The immunocytokine of any one of claims 88-90, wherein the antibody comprises a second heavy chain not comprising the cytokine or variant thereof positioned at the hinge region, and wherein the second heavy chain comprises the sequence of SEQ ID NO: 130.

92. The immunocytokine of claim 67, wherein the antibody is an anti-PD-L1 antibody comprising: i) a VH-CDR1 comprising the sequence of SEQ ID NO: 243; ii) a VH-CDR2 comprising the sequence of SEQ ID NO: 244; iii) a VH-CDR3 comprising the sequence of SEQ ID NO: 245; iv) a VL-CDR1 comprising the sequence of SEQ ID NO: 246; v) a VL-CDR2 comprising the sequence of SEQ ID NO: 247; and vi) a VL-CDR3 comprising the sequence of SEQ ID NO: 248.

93. The immunocytokine of claim 92, wherein the antibody comprises a VH comprising the sequence of SEQ ID NO: 137 and a VL comprising the sequence of SEQ ID NO: 138.

94. The immunocytokine of claim 92 or 93, wherein the antibody comprises a light chain comprising the sequence of SEQ ID NO: 140,

(i) wherein the cytokine or variant thereof is an IL-2 variant comprising the sequence of SEQ ID NO: 2, and wherein the heavy chain comprising the IL-2 variant positioned at the hinge region comprises the sequence of SEQ ID NO: 144;

(ii) wherein the cytokine or variant thereof is an IFN-α variant comprising the sequence of SEQ ID NO: 4, and wherein the heavy chain comprising the IFN-α variant positioned at the hinge region comprises the sequence of SEQ ID NO: 145;

(iii) wherein the cytokine or variant thereof is an INF-γ variant comprising the sequence of SEQ ID NO: 19, and wherein the heavy chain comprising the IFN-γ variant positioned at the hinge region comprises the sequence of SEQ ID NO: 146;

(iv) wherein the cytokine or variant thereof is an IL-10 variant comprising the sequence of SEQ ID NO: 28, and wherein the heavy chain comprising the IL-10 variant positioned at the hinge region comprises the sequence of SEQ ID NO: 147;

(v) wherein the cytokine or variant thereof is an IL-12 variant comprising the sequence of SEQ ID NO: 36, and wherein the heavy chain comprising the IL-12 variant positioned at the hinge region comprises the sequence of SEQ ID NO: 148; or

(vi) wherein the cytokine or variant thereof is an IL-23 variant comprising the sequence of SEQ ID NO: 39, and wherein the heavy chain comprising the IL-23 variant positioned at the hinge region comprises the sequence of SEQ ID NO: 149.

95. The immunocytokine of any one of claims 92-94, wherein the antibody comprises a second heavy chain not comprising the cytokine or variant thereof positioned at the hinge region, and wherein the second heavy chain comprises the sequence of SEQ ID NO: 143.

96. The immunocytokine of claim 67, wherein the antibody is an anti-CD25 antibody comprising VH-CDR1, VH-CDR2, and VH-CDR3 of a VH comprising the sequence of SEQ ID NO: 162, and VL-CDR1, VL-CDR2, and VL-CDR3 of a VL comprising the sequence of SEQ ID NO: 163.

97. The immunocytokine of claim 96, wherein the antibody comprises a VH comprising the sequence of SEQ ID NO: 162 and a VL comprising the sequence of SEQ ID NO: 163.

98. The immunocytokine of claim 96 or 97, wherein the antibody comprises a light chain comprising the sequence of SEQ ID NO: 165,

~~(i) wherein the cytokine or variant thereof is an IL-2 variant comprising the sequence of SEQ ID NO: 2, and wherein the heavy chain comprising the IL-2 variant positioned at the hinge region comprises the sequence of SEQ ID NO: 169;~~

~~(ii) wherein the cytokine or variant thereof is an IFN-α variant comprising the sequence of SEQ ID NO: 4, and wherein the heavy chain comprising the IFN-α variant positioned at the hinge region comprises the sequence of SEQ ID NO: 170;~~

~~(iii) wherein the cytokine or variant thereof is an INF-γ variant comprising the sequence of SEQ ID NO: 19, and wherein the heavy chain comprising the IFN-γ variant positioned at the hinge region comprises the sequence of SEQ ID NO: 171;~~

~~(iv) wherein the cytokine or variant thereof is an IL-10 variant comprising the sequence of SEQ ID NO: 28, and wherein the heavy chain comprising the IL-10 variant positioned at the hinge region comprises the sequence of SEQ ID NO: 172;~~

~~(v) wherein the cytokine or variant thereof is an IL-12 variant comprising the sequence of SEQ ID NO: 36, and wherein the heavy chain comprising the IL-12 variant positioned at the hinge region comprises the sequence of SEQ ID NO: 173; or~~

~~(vi) wherein the cytokine or variant thereof is an IL-23 variant comprising the sequence of SEQ ID NO: 39, and wherein the heavy chain comprising the IL-23 variant positioned at the hinge region comprises the sequence of SEQ ID NO: 174.~~

~~99. The immunocytokine of any one of claims 96-98, wherein the antibody comprises a second heavy chain not comprising the cytokine or variant thereof positioned at the hinge region, and wherein the second heavy chain comprises the sequence of SEQ ID NO: 168.~~

~~100~~9. The immunocytokine of claim ~~67~~1, wherein the antibody is an anti-PD-1 antibody comprising VH-CDR1 and VH-CDR2, and VH-CDR3 of a VH comprising the sequence of SEQ ID NO: 102, and VL-CDR1, VL-CDR2, and VL-CDR3 of a VL comprising the sequence of SEQ ID NO: 103, and wherein the VH-CDR3 comprises any one of following mutations relative to SEQ ID NO: 102: D100N, D100G, D100R, N99G, N99A, or N99M,particularly, wherein the antibody comprises a light chain comprising the sequence of SEQ ID NO: 106, wherein the cytokine or variant thereof is an IL-12 variant comprising the sequence of SEQ ID NO: 36, and wherein the heavy chain comprising the IL-12 variant positioned at the hinge region comprises the sequence of any one of SEQ ID NOs: 268-273.

~~101. The immunocytokine of claim 100, wherein the antibody comprises a light chain comprising the sequence of SEQ ID NO: 106, wherein the cytokine or variant thereof is an IL-12 variant comprising the sequence of SEQ ID NO: 36, and wherein the heavy chain comprising the IL-12 variant positioned at the hinge region comprises the sequence of any one of SEQ ID NOs: 268-273.~~

~~102. The immunocytokine of any one of claims 1-66,~~

~~(i) wherein the antigen-binding fragment is a ligand, and wherein the target antigen is a receptor specifically recognized by the ligand; or~~

~~(ii) wherein the antigen-binding fragment is a receptor, and wherein the target antigen is a ligand specifically recognized by the receptor.~~

~~103. The immunocytokine of claim 102, wherein the ligand or receptor is selected from the group consisting of IL-2, CD25, PD-1, PD-L1, PD-L2, NKG2A, NKG2C, NKG2F, NKG2D, BCMA, APRIL, BAFF, IL-3, IL-13, LLT1, AICL, DNAM-1, and NKp80.~~

~~104~~10. The immunocytokine of any one of claims 1-9 ~~102 or 103~~, wherein the PD-L2 or PD-L1~~ligand~~ comprises the sequence of SEQ ID NO: 176,SEQ ID NO: 250, any of SEQ ID NOs:255-261or any of SEQ ID NOs: 262-265.

~~105~~11. The immunocytokine of claim ~~104~~10,

(i) wherein the cytokine or variant thereof is an IL-2 variant comprising the sequence of SEQ ID NO: 2, and wherein the antigen-binding polypeptide comprising the IL-2 variant positioned at the hinge region comprises the sequence of SEQ ID NO: 180;

(ii) wherein the cytokine or variant thereof is an IFN-α variant comprising the sequence of SEQ ID NO: 4, and wherein the antigen-binding polypeptide comprising the IFN-α variant positioned at the hinge region comprises the sequence of SEQ ID NO: 182;

(iii) wherein the cytokine or variant thereof is an INF-γ variant comprising the sequence of SEQ ID NO: 19, and wherein the antigen-binding polypeptide comprising the IFN-γ variant positioned at the hinge region comprises the sequence of SEQ ID NO: 183;

(iv) wherein the cytokine or variant thereof is an IL-10 variant comprising the sequence of SEQ ID NO: 28, and wherein the antigen-binding polypeptide comprising the IL-10 variant positioned at the hinge region comprises the sequence of SEQ ID NO: 184;

(v) wherein the cytokine or variant thereof is an IL-12 variant comprising the sequence of SEQ ID NO: 36, and wherein the antigen-binding polypeptide comprising the IL-12 variant positioned at the hinge region comprises the sequence of SEQ ID NO: 186;

(vi) wherein the cytokine or variant thereof is an IL-12 variant comprising the sequence of SEQ ID NO: 275, and wherein the antigen-binding polypeptide comprising the IL-12 variant positioned at the hinge region comprises the sequence of SEQ ID NO: 274; or

(vii) wherein the cytokine or variant thereof is an IL-23 variant comprising the sequence of SEQ ID NO: 39, and wherein the antigen-binding polypeptide comprising the IL-23 variant positioned at the hinge region comprises the sequence of SEQ ID NO: 187.

~~106~~12. The immunocytokine of claim ~~104~~ 10 or ~~105~~11, wherein the antigen-binding protein comprises a second antigen-binding polypeptide not comprising the cytokine or variant thereof positioned at the hinge region, and wherein the second antigen-binding polypeptide comprises the sequence of any of SEQ ID NOs: 178, 179, 181, and 185.

~~107. The immunocytokine of claim 102 or 103, wherein the ligand comprises the sequence of any one of SEQ ID NOs: 255-261.~~

~~108. The immunocytokine of claim 107, wherein the cytokine or variant thereof is an IL-12 variant comprising the sequence of SEQ ID NO: 36, and wherein the antigen-binding polypeptide comprising the IL-12 variant positioned at the hinge region comprises the sequence of SEQ ID NO: 277.~~

~~109. The immunocytokine of claim 108, wherein the antigen-binding protein comprises a second antigen-binding polypeptide comprising a second cytokine or variant thereof positioned at the hinge region, and wherein the second antigen-binding polypeptide comprises the sequence of any of SEQ ID NOs: 285-288 and 327.~~

~~110. The immunocytokine of claim 102 or 103, wherein the ligand comprises the sequence of any one of SEQ ID NOs: 262-265.~~

~~111. The immunocytokine of claim 110, wherein the cytokine or variant thereof is an IL-12 variant comprising the sequence of SEQ ID NO: 36, and wherein the antigen-binding polypeptide comprising the IL-12 variant positioned at the hinge region comprises the sequence of SEQ ID NO: 293.~~

112. The immunocytokine of claim 110 or 111, wherein the antigen-binding protein comprises a second antigen-binding polypeptide comprising a second cytokine or variant thereof positioned at the hinge region.

113. A method of selectively activating the activity of a cytokine or variant thereof to a cell expressing a target antigen in an individual, comprising administering to the individual an effective amount of an immunocytokine, wherein the immunocytokine comprises: a) an antigen-binding protein specifically recognizing the target antigen; and b) a cytokine or variant thereof, wherein the antigen-binding protein comprises an antigen-binding polypeptide comprising from N' to C': an antigen-binding fragment, a hinge region, and an Fc domain subunit or portion thereof, and wherein the cytokine or variant thereof is positioned at the hinge region, and wherein the activity of the cytokine or variant thereof is selectively activated upon binding of the antigen-binding protein to the target antigen.

114. The method of claim 113, wherein in the presence of binding of the antigen-binding protein to the target antigen, the activity of the cytokine or variant thereof increases at least about 20% compared to that in the absence of binding of the antigen-binding protein to the target antigen.

115. The method of claim 113 or 114, wherein in the absence of binding of the antigen-binding protein to the target antigen, the activity of the cytokine or variant thereof positioned at the hinge region is no more than about 70% of that of a corresponding cytokine or variant thereof in a free state.

116. The method of any one of claims 113-115, wherein the cytokine or variant thereof is a cytokine variant, and wherein the activity of the cytokine variant in a free state is no more than about 80% of that of a corresponding wildtype cytokine in a free state.

117. The method of any one of claims 113-116, wherein the antigen-binding protein is an antibody specifically recognizing the target antigen, wherein the antigen-binding polypeptide comprising the hinge region is a heavy chain of the antibody, and wherein the cytokine or variant thereof is positioned at the hinge region of the heavy chain.

118. The method of any one of claims 113-116,

(i) wherein the antigen-binding fragment is a ligand, and wherein the target antigen is a receptor specifically recognized by the ligand; or

(ii) wherein the antigen-binding fragment is a receptor, and wherein the target antigen is a ligand specifically recognized by the receptor.

11913. An isolated nucleic acid encoding the immunocytokine of any one of claims 1-11212.

120. A vector comprising the nucleic acid of claim 119.

121. An isolated host cell comprising the nucleic acid of claim 119 or the vector of claim 120.

122. The host cell of claim 121, which is a Chinese hamster ovary (CHO) cell.

123. A method of producing an immunocytokine, comprising: (a) culturing a host cell comprising the nucleic acid of claim 119 or the vector of claim 120, or a host cell of claim 121 or 122, under a condition effective to express the encoded immunocytokine; and (b) obtaining the expressed immunocytokine from said host cell.

12414. A pharmaceutical composition comprising the immunocytokine of any one of claims 1-112, and optionally a pharmaceutical acceptable carrier.

12515. The immunocytokine of any one of claims 1-12A method of for use in treating or preventinga disease or disorder in an individual, particularly, the disease or disorder is a cancer, an infection, an autoimmune disease, an allergy, a graft rejection, or a graft-versus-host disease (GvHD), more particularly,the cancer is selected from the group consisting of lung cancer, liver cancer, renal cancer, colorectal cancer, ovarian cancer, breast cancer, pancreatic cancer, gastric carcinoma, bile duct cancer, squamous cell carcinoma, bladder cancer, esophageal cancer, mesothelioma, melanoma, head and neck cancer, thyroid cancer, sarcoma, prostate cancer, glioblastoma, cervical cancer, thymic carcinoma, leukemia, lymphoma, myeloma, mycoses fungoides, and merkel cell cancer, comprising administering to the individual an effective amount of the immunocytokine of any one of claims 1-112, or the pharmaceutical composition of claim 124.

126. The method of claim 125, wherein the immunocytokine or pharmaceutical composition is administered intravenously or subcutaneously.

127. The method of claim 125 or 126, wherein the immunocytokine or pharmaceutical composition is administered in an amount of about 1μg/kg to about 50mg/kg.

128. The method of any one of claims 125-127, wherein the immunocytokine or pharmaceutical composition is administered once every three weeks.

129. The method of any one of claims 125-128, wherein the disease or disorder is a cancer.

~~130. The method of claim 129, wherein the cancer is selected from the group consisting of lung cancer, liver cancer, renal cancer, colorectal cancer, ovarian cancer, breast cancer, pancreatic cancer, gastric carcinoma, bile duct cancer, squamous cell carcinoma, bladder cancer, esophageal cancer, mesothelioma, melanoma, head and neck cancer, thyroid cancer, sarcoma, prostate cancer, glioblastoma, cervical cancer, thymic carcinoma, leukemia, lymphoma, myeloma, mycoses fungoides, and merkel cell cancer~~.

~~131. The method of claim 129 or 130, wherein the individual has been previously treated with the immunocytokine of any one of claims 1-112, or the pharmaceutical composition of claim 124 against the cancer, and wherein the treatment comprises preventing cancer recurrence in the individual.~~

~~132. The method of claim 129 or 130, wherein the treatment comprises preventing a cancer in the individual.~~

~~132. The method of any one of claims 125-128, wherein the disease or disorder is an infection, an autoimmune disease, an allergy, a graft rejection, or a graft-versus-host disease (GvHD).~~

~~133. A cancer vaccine, comprising the immunocytokine of any one of claims 1-112, or the pharmaceutical composition of claim 124.~~

~~134. A method of preventing a cancer in an individual, comprising administering to the individual an effective amount of the immunocytokine of any one of claims 1-112, the pharmaceutical composition of claim 124, or the cancer vaccine of claim 133.~~