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$\leqslant$~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.

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.

4. The immunocytokine of any one of claims 1-3, 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.

5. The immunocytokine of any one of claims 1-4, 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.

6. The immunocytokine of any one of claims 1-5, 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.

7. The immunocytokine of any one ofclaims1-5, 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.

8. The immunocytokine of claim7, 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.

9. The immunocytokine of claim 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.

10. The immunocytokine of any one of claims 1-9, wherein the PD-L2 or PD-L1 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.

11. The immunocytokine of claim 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.

12. The immunocytokine of claim 10 or 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.

13. An isolated nucleic acid encoding the immunocytokine of any one of claims 1-12.

14. A pharmaceutical composition comprising the immunocytokine of any one of claims 1-12, and optionally a pharmaceutical acceptable carrier.

15. The immunocytokine of any one of claims 1-12 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.