SINGLE-DOMAIN ANTIBODIES AND VARIANTS THEREOF AGAINST PD-1

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority benefits of International Patent Applications No. PCT/CN2018/072589 filed on January 15, 2018, the contents of which are incorporated herein by reference in their entirety.

SUBMISSION OF SEQUENCE LISTING ON ASCII TEXT FILE

[0002] The content of the following submission on ASCII text file is incorporated herein by reference in its entirety: a computer readable form (CRF) of the Sequence Listing (file name: 761422000840SEQLIST.txt, date recorded: January 14, 2018, size: 271 KB).

FIELD OF THE INVENTION

[0003] The present invention relates to constructs comprising a single-domain antibody (sdAb) moiety that specifically recognize PD-1, and methods of making and using thereof.

BACKGROUND OF THE INVENTION

[0004] An immunoinhibitory receptor that is primarily expressed on activated T and B cells, Programmed Cell Death Receptor 1 (PD-1; also referred to as Programmed Death Receptor 1, Programmed cell death protein 1, CD279), is a member of the immunoglobulin superfamily related to CD28 and cytotoxic T-lymphocyte associated protein-4 (CTLA-4, CD152). PD-1 (and the family members alike) is a type I transmembrane glycoprotein containing an extracellular Ig Variable-type (V-type) domain that binds its ligands and a cytoplasmic tail that binds signaling molecules. The cytoplasmic tail of PD-1 contains two tyrosine-based signaling motifs, an ITIM (immunoreceptor tyrosine-based inhibition motif) and an ITSM (immunoreceptor tyrosine-based switch motif).

[0005] PD-1 attenuates T-cell responses when bound by Programmed Cell Death Ligand 1, also referred to as Programmed Death Ligand 1 (PD-L1, CD274, B7-H1), and/or Programmed Cell Death Ligand 2, also referred to as Programmed Death Ligand 2 (PD-L2, CD273, B7-DC). The binding of either of these ligands to PD-1 transduces a signal that inhibits T-cell proliferation, cytokine production, and cytolytic function. Blocking the binding of PD-L1 to PD-1 enhances tumor-specific CD8+ T-cell immunity, facilitating the clearance of tumor cells by the immune system.

[0006] Antibody-mediated blockade of PD-1/PD-L1 interaction has entered clinical trials in the treatment of refractory solid tumors, including melanoma, renal cell carcinoma, colorectal cancer, non-small cell lung cancer, and hematologic malignancies. However, there remains a need of an optimal therapy for treating, stabilizing, preventing, and/or delaying the development of various cancers, especially in view of the resistance or relapse upon PD-1/PD-L1 blockade.

[0007] The disclosures of all publications, patents, patent applications and published patent applications referred to herein are hereby incorporated herein by reference in their entirety.

BRIEF SUMMARY OF THE INVENTION

[0008] The present invention relates to anti-PD-1 constructs comprising an sdAb moiety that specifically recognizes PD-1 (hereinafter referred to as "anti-PD-1 sdAb"), such as anti-PD-1 sdAb, anti-PD-1 HCAb e.g., anti-PD-1 sdAb-Fc fusion protein comprising an anti-PD-1 sdAb fused to a crystalline fragment (Fc) fragment of human immunoglobulin G (IgG), and multispecific (such as bispecific) antigen binding proteins comprising an anti-PD-1 sdAb fused to, for example, other sdAbs, a full-length four-chain antibody or antigen binding fragments thereof (e.g., Fab or scFv), and methods of making and using thereof.

[0009] One aspect of the present application provides an isolated anti-PD-1 construct comprising an sdAb moiety specifically recognizing PD-1, wherein the sdAb moiety comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 37-72, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 109-144, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 181-216, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, the isolated anti-PD-1 construct comprises an sdAb moiety specifically recognizing PD-1, wherein the sdAb moiety comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 37-72, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 109-144, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 181-216. In some embodiments, the sdAb moiety specifically recognizing PD-1 comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 37-72; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 109-144; and a CDR3 comprising the amino acid sequence of

any one of SEQ ID NOs: 181-216; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions. In some embodiments, the amino acid substitutions are in CDR1 and/or CDR2, wherein CDR3 comprises the amino acid sequence of any one of SEQ ID NOs: 181-216. In some embodiments, the sdAb moiety specifically recognizing PD-1 comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 37-72; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 109-144; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 181-216.

[0010] In some embodiments according to any one of the isolated anti-PD-1 constructs described above, the sdAb moiety specifically recognizing PD-1 comprises any one of the following:

(1) a CDR1 comprising the amino acid sequence of SEQ ID NO: 37, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 109, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 181, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions;

(2) a CDR1 comprising the amino acid sequence of SEQ ID NO: 38, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 110, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 182, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions;

(3) a CDR1 comprising the amino acid sequence of SEQ ID NO: 39, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 111, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 183, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions;

(4) a CDR1 comprising the amino acid sequence of SEQ ID NO: 40, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 112, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 184, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions;

(5) a CDR1 comprising the amino acid sequence of SEQ ID NO: 41, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid

sequence of SEQ ID NO: 113, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 185, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions;

(6) a CDR1 comprising the amino acid sequence of SEQ ID NO: 42, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 114, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 186, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions;

(7) a CDR1 comprising the amino acid sequence of SEQ ID NO: 43, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 115, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 187, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions;

(8) a CDR1 comprising the amino acid sequence of SEQ ID NO: 44, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 116, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 188, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions;

(9) a CDR1 comprising the amino acid sequence of SEQ ID NO: 45, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 117, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 189, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions;

(10) a CDR1 comprising the amino acid sequence of SEQ ID NO: 46, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 118, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 190, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions;

(11) a CDR1 comprising the amino acid sequence of SEQ ID NO: 47, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid

sequence of SEQ ID NO: 119, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 191, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions;

(12) a CDR1 comprising the amino acid sequence of SEQ ID NO: 48, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 120, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 192, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions;

(13) a CDR1 comprising the amino acid sequence of SEQ ID NO: 49, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 121, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 193, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions;

(14) a CDR1 comprising the amino acid sequence of SEQ ID NO: 50, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 122, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 194, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions;

(15) a CDR1 comprising the amino acid sequence of SEQ ID NO: 51, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 123, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 195, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions;

(16) a CDR1 comprising the amino acid sequence of SEQ ID NO: 52, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 124, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 196, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions;

(17) a CDR1 comprising the amino acid sequence of SEQ ID NO: 53, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid

sequence of SEQ ID NO: 125, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 197, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions;

(18) a CDR1 comprising the amino acid sequence of SEQ ID NO: 54, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 126, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 198, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions;

(19) a CDR1 comprising the amino acid sequence of SEQ ID NO: 55, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 127, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 199, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions;

(20) a CDR1 comprising the amino acid sequence of SEQ ID NO: 56, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 128, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 200, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions;

(21) a CDR1 comprising the amino acid sequence of SEQ ID NO: 57, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 129, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 201, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions;

(22) a CDR1 comprising the amino acid sequence of SEQ ID NO: 58, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 130, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 202, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions;

(23) a CDR1 comprising the amino acid sequence of SEQ ID NO: 59, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid

sequence of SEQ ID NO: 131, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 203, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions;

(24) a CDR1 comprising the amino acid sequence of SEQ ID NO: 60, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 132, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 204, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions;

(25) a CDR1 comprising the amino acid sequence of SEQ ID NO: 61, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 133, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 205, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions;

(26) a CDR1 comprising the amino acid sequence of SEQ ID NO: 62, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 134, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 206, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions;

(27) a CDR1 comprising the amino acid sequence of SEQ ID NO: 63, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 135, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 207, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions;

(28) a CDR1 comprising the amino acid sequence of SEQ ID NO: 64, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 136, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 208, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions;

(29) a CDR1 comprising the amino acid sequence of SEQ ID NO: 65, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid

sequence of SEQ ID NO: 137, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 209, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions;

(30) a CDR1 comprising the amino acid sequence of SEQ ID NO: 66, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 138, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 210, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions;

(31) a CDR1 comprising the amino acid sequence of SEQ ID NO: 67, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 139, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 211, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions;

(32) a CDR1 comprising the amino acid sequence of SEQ ID NO: 68, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 140, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 212, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions;

(33) a CDR1 comprising the amino acid sequence of SEQ ID NO: 69, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 141, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 213, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions;

(34) a CDR1 comprising the amino acid sequence of SEQ ID NO: 70, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 142, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 214, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions;

(35) a CDR1 comprising the amino acid sequence of SEQ ID NO: 71, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid

sequence of SEQ ID NO: 143, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 215, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; or

(35) a CDR1 comprising the amino acid sequence of SEQ ID NO: 72, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 144, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 216, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions.

[0011] In some embodiments according to any one of the isolated anti-PD-1 constructs described above, the sdAb moiety specifically recognizing PD-1 comprises any one of the following:

(1) a CDR1 comprising the amino acid sequence of SEQ ID NO: 37; a CDR2 comprising the amino acid sequence of SEQ ID NO: 109; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 181; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions;

(2) a CDR1 comprising the amino acid sequence of SEQ ID NO: 38; a CDR2 comprising the amino acid sequence of SEQ ID NO: 110; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 182; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions;

(3) a CDR1 comprising the amino acid sequence of SEQ ID NO: 39; a CDR2 comprising the amino acid sequence of SEQ ID NO: 111; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 183; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions;

(4) a CDR1 comprising the amino acid sequence of SEQ ID NO: 40; a CDR2 comprising the amino acid sequence of SEQ ID NO: 112; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 184; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions;

(5) a CDR1 comprising the amino acid sequence of SEQ ID NO: 41; a CDR2 comprising the amino acid sequence of SEQ ID NO: 113; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 185; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions;

(6) a CDR1 comprising the amino acid sequence of SEQ ID NO: 42; a CDR2 comprising the amino acid sequence of SEQ ID NO: 114; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 186; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions;

(7) a CDR1 comprising the amino acid sequence of SEQ ID NO: 43; a CDR2 comprising the amino acid sequence of SEQ ID NO: 115; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 187; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions;

(8) a CDR1 comprising the amino acid sequence of SEQ ID NO: 44; a CDR2 comprising the amino acid sequence of SEQ ID NO: 116; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 188; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions;

(9) a CDR1 comprising the amino acid sequence of SEQ ID NO: 45; a CDR2 comprising the amino acid sequence of SEQ ID NO: 117; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 189; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions;

(10) a CDR1 comprising the amino acid sequence of SEQ ID NO: 46; a CDR2 comprising the amino acid sequence of SEQ ID NO: 118; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 190; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions;

(11) a CDR1 comprising the amino acid sequence of SEQ ID NO: 47; a CDR2 comprising the amino acid sequence of SEQ ID NO: 119; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 191; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions;

(12) a CDR1 comprising the amino acid sequence of SEQ ID NO: 48; a CDR2 comprising the amino acid sequence of SEQ ID NO: 120; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 192; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions;

(13) a CDR1 comprising the amino acid sequence of SEQ ID NO: 49; a CDR2 comprising the amino acid sequence of SEQ ID NO: 121; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 193; or a

variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions;

(14) a CDR1 comprising the amino acid sequence of SEQ ID NO: 50; a CDR2 comprising the amino acid sequence of SEQ ID NO: 122; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 194; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions;

(15) a CDR1 comprising the amino acid sequence of SEQ ID NO: 51; a CDR2 comprising the amino acid sequence of SEQ ID NO: 123; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 195; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions;

(16) a CDR1 comprising the amino acid sequence of SEQ ID NO: 52; a CDR2 comprising the amino acid sequence of SEQ ID NO: 124; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 196; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions;

(17) a CDR1 comprising the amino acid sequence of SEQ ID NO: 53; a CDR2 comprising the amino acid sequence of SEQ ID NO: 125; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 197; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions;

(18) a CDR1 comprising the amino acid sequence of SEQ ID NO: 54; a CDR2 comprising the amino acid sequence of SEQ ID NO: 126; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 198; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions;

(19) a CDR1 comprising the amino acid sequence of SEQ ID NO: 55; a CDR2 comprising the amino acid sequence of SEQ ID NO: 127; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 199; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions;

(20) a CDR1 comprising the amino acid sequence of SEQ ID NO: 56; a CDR2 comprising the amino acid sequence of SEQ ID NO: 128; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 200; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions;

(21) a CDR1 comprising the amino acid sequence of SEQ ID NO: 57; a CDR2 comprising the amino acid sequence of SEQ ID NO: 129; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 201; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions;

(22) a CDR1 comprising the amino acid sequence of SEQ ID NO: 58; a CDR2 comprising the amino acid sequence of SEQ ID NO: 130; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 202; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions;

(23) a CDR1 comprising the amino acid sequence of SEQ ID NO: 59; a CDR2 comprising the amino acid sequence of SEQ ID NO: 131; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 203; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions;

(24) a CDR1 comprising the amino acid sequence of SEQ ID NO: 60; a CDR2 comprising the amino acid sequence of SEQ ID NO: 132; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 204; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions;

(25) a CDR1 comprising the amino acid sequence of SEQ ID NO: 61; a CDR2 comprising the amino acid sequence of SEQ ID NO: 133; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 205; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions;

(26) a CDR1 comprising the amino acid sequence of SEQ ID NO: 62; a CDR2 comprising the amino acid sequence of SEQ ID NO: 134; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 206; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions;

(27) a CDR1 comprising the amino acid sequence of SEQ ID NO: 63; a CDR2 comprising the amino acid sequence of SEQ ID NO: 135; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 207; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions;

(28) a CDR1 comprising the amino acid sequence of SEQ ID NO: 64; a CDR2 comprising the amino acid sequence of SEQ ID NO: 136; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 208; or a

variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions;

(29) a CDR1 comprising the amino acid sequence of SEQ ID NO: 65; a CDR2 comprising the amino acid sequence of SEQ ID NO: 137; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 209; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions;

(30) a CDR1 comprising the amino acid sequence of SEQ ID NO: 66; a CDR2 comprising the amino acid sequence of SEQ ID NO: 138; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 210; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions;

(31) a CDR1 comprising the amino acid sequence of SEQ ID NO: 67; a CDR2 comprising the amino acid sequence of SEQ ID NO: 139; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 211; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions;

(32) a CDR1 comprising the amino acid sequence of SEQ ID NO: 68; a CDR2 comprising the amino acid sequence of SEQ ID NO: 140; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 212; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions;

(33) a CDR1 comprising the amino acid sequence of SEQ ID NO: 69; a CDR2 comprising the amino acid sequence of SEQ ID NO: 141; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 213; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions;

(34) a CDR1 comprising the amino acid sequence of SEQ ID NO: 70; a CDR2 comprising the amino acid sequence of SEQ ID NO: 142; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 214; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions;

(35) a CDR1 comprising the amino acid sequence of SEQ ID NO: 71; a CDR2 comprising the amino acid sequence of SEQ ID NO: 143; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 215; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions; or

(36) a CDR1 comprising the amino acid sequence of SEQ ID NO: 72; a CDR2 comprising the amino acid sequence of SEQ ID NO: 144; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 216; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions.

[0012] In some embodiments according to any one of the isolated anti-PD-1 constructs described above, the sdAb moiety specifically recognizing PD-1 comprises a V_HH domain comprising the amino acid sequence of any one of the following: a-1) the amino acid residue at position 37 is selected from the group consisting of F, Y, L, I, and V (such as F, Y or V, or such as F); a-2) the amino acid residue at position 44 is selected from the group consisting of A, G, E, D, G, Q, R, S, and L (such as E, Q, or G, or such as E); a-3) the amino acid residue at position 45 is selected from the group consisting of L, R, and C (such as L or R); a-4) the amino acid residue at position 103 is selected from the group consisting of W, R, G, and S (such as W, G, or R, or such as W); and a-5) the amino acid residue at position 108 is Q; or b-1) the amino acid residue at position 37 is selected from the group consisting of F, Y, L, I, and V (such as F, V or Y, or such as F); b-2) the amino acid residue at position 44 is selected from the group consisting of E, Q, and G; b-3) the amino acid residue at position 45 is R; b-4) the amino acid residue at position 103 is selected from the group consisting of W, R, and S (such as W); and b-5) the amino acid residue at position 108 is selected from the group consisting of Q and L (such as Q); wherein the amino acid position is according to Kabat numbering, and wherein position 108 can be optionally humanized to L when position 108 is Q.

[0013] In some embodiments according to any one of the isolated anti-PD-1 constructs described above, the sdAb moiety specifically recognizing PD-1 comprises a V_HH domain comprising the amino acid sequence of any one of SEQ ID NOs: 289-324, or a variant thereof having at least about 80% (such as at least about any of 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) sequence identify to any one of SEQ ID NOs: 289-324. In some embodiments, the sdAb moiety specifically recognizing PD-1 comprises a V_HH domain comprising the amino acid sequence of any one of SEQ ID NOs: 289-324, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the V_HH domain. In some embodiments, the amino acid substitutions are in the CDRs, such as the CDR1, and/or the CDR2, and/or the CDR3 of any one of SEQ ID NOs: 289-324. In some embodiments, the amino acid substitutions are in the FRs, such as the FR1, and/or the FR2, and/or the FR3, and/or the FR4 of any one of SEQ ID NOs: 289-324. In some embodiments, the amino acid substitutions are in both CDRs and FRs. In some embodiments, the sdAb moiety specifically recognizing PD-1 comprises a V_HH domain comprising the amino acid sequence of any one of SEQ ID NOs: 289-324.

[0014] In some embodiments according to any one of the isolated anti-PD-1 constructs described above, the K_d of the binding between the sdAb moiety specifically recognizing PD-1 and PD-1 is about 10^{-5} M to about 10^{-12} M (such as about 10^{-5} M to about 10^{-12} M, about 10^{-7} M to about 10^{-12} M, or about 10^{-8} M to about 10^{-12} M).

[0015] In some embodiments according to any one of the isolated anti-PD-1 constructs described above, the sdAb moiety specifically recognizing PD-1 is camelid, chimeric, human, partially humanized, or fully humanized.

[0016] In some embodiments according to any one of the isolated anti-PD-1 constructs described above, the isolated anti-PD-1 construct is a heavy chain-only antibody (HCAb) comprising the sdAb moiety specifically recognizing PD-1 fused to an Fc fragment via an optional linker. In some embodiments, HCAb is monomeric. In some embodiments, the HCAb is dimeric. In some embodiments, the Fc fragment is a human IgG1 (hIgG1) Fc, effectorless (inert) hIgG1 Fc, hIgG4 Fc, or hIgG4 Fc (S228P). In some embodiments, the Fc fragment comprises the amino acid sequence of any one of SEQ ID NOs: 363-365. In some embodiments, the Fc fragment is hIgG4 Fc (S228P). In some embodiments, the optional linker comprises the amino acid sequence of any one of SEQ ID NOs: 367-376. In some embodiments, the HCAb comprises the amino acid sequence of any one of SEQ ID NOs: 325-360.

[0017] In some embodiments according to any one of the isolated anti-PD-1 construct described above, the isolated anti-PD-1 construct further comprises a second antibody moiety specifically recognizing a second epitope. In some embodiments, the second antibody moiety is a full-length antibody, a Fab, a Fab', a $(Fab')_2$, an Fv, a single chain Fv (scFv), an scFv-scFv, a minibody, a diabody, or an sdAb. In some embodiments, the anti-PD-1 construct is monospecific. In some embodiments, the anti-PD-1 construct is multispecific (such as bispecific). In some embodiments, the second epitope is not from PD-1. In some embodiments, the second epitope is from PD-1 but different from that specifically recognized by the anti-PD-1 sdAb moiety. In some embodiments, the second epitope is the same as that specifically recognized by the anti-PD-1 sdAb moiety. In some embodiments, the sdAb moiety specifically recognizing PD-1 and the second antibody moiety are optionally connected by a peptide linker, such as peptide linker comprising the amino acid sequence of any one of SEQ ID NOs: 367-376. In some embodiments, the second antibody moiety is an sdAb, such as an sdAb specifically recognizing PD-1 or CTLA-4. In some embodiments, the second antibody moiety is a Fab. In some embodiments, the second antibody moiety is an scFv. In some embodiments, the second antibody moiety is a full-length antibody consisting of two heavy chains and two light chains. In some embodiments, the Fc fragment of the heavy chain is IgG1 Fc, effectorless IgG1 Fc, IgG2 Fc, IgG4 Fc, or IgG4 Fc (S228P), such as any of SEQ ID NOs: 363-365. In some embodiments, the N-terminus of the sdAb moiety specifically recognizing PD-1 is fused to the C-

terminus of at least one of the heavy chains of the full-length antibody. In some embodiments, the C-terminus of the sdAb moiety specifically recognizing PD-1 is fused to the N-terminus of at least one of the heavy chains of the full-length antibody. In some embodiments, the N-terminus of the sdAb moiety specifically recognizing PD-1 is fused to the C-terminus of at least one of the light chains of the full-length antibody. In some embodiments, the C-terminus of the sdAb moiety specifically recognizing PD-1 is fused to the N-terminus of at least one of the light chains of the full-length antibody. In some embodiments, the isolated anti-PD-1 construct comprises four identical sdAb moieties specifically recognizing PD-1 as described above, the C-terminus of each anti-PD-1 sdAb moiety is fused to the N-terminus of each chain of the full-length antibody via an optional peptide linker. In some embodiments, the isolated anti-PD-1 construct comprises four identical sdAb moieties specifically recognizing PD-1 as described above, two anti-PD-1 sdAb moieties are fused to each other via an optional peptide linker, the other two anti-PD-1 sdAb moieties are fused to each other via an optional peptide linker, and the C-terminus of each of the anti-PD-1 sdAb moiety fusion polypeptide is fused to the N-terminus of each heavy chain of the full-length antibody via an optional peptide linker. In some embodiments the isolated anti-PD-1 construct consists of four polypeptide chains with structures from the N-terminus to the C-terminus as follows: (1) V_L-C_L; (2) anti-PD-1 sdAb-V_H-C_H1-C_H2-C_H3; (3) anti-PD-1 sdAb-V_H-C_H1-C_H2-C_H3; and (4) V_L-C_L, wherein V_H and V_L of polypeptide chains (1) and (2) forms an antigen binding site that specifically binds a first copy of the second epitope (*e.g.*, TIGIT, LAG-3, TIM-3, CTLA-4, PD-1 (such as a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein)), V_H and V_L of polypeptide chains (3) and (4) forms an antigen binding site that specifically binds a second copy of the second epitope (*e.g.*, TIGIT, LAG-3, TIM-3, CTLA-4, PD-1 (such as a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein)), and each anti-PD-1 sdAb specifically binds a copy of PD-1. In some embodiments the isolated anti-PD-1 construct consists of four polypeptide chains with structures from the N-terminus to the C-terminus as follows: (1) V_L-C_L; (2) V_H-C_H1-C_H2-C_H3-anti-PD-1 sdAb; (3) V_H-C_H1-C_H2-C_H3-anti-PD-1 sdAb; and (4) V_L-C_L, wherein V_H and V_L of polypeptide chains (1) and (2) forms an antigen binding site that specifically binds a first copy of the second epitope (*e.g.*, TIGIT, LAG-3, TIM-3, CTLA-4, PD-1 (such as a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein)), V_H and V_L of polypeptide chains (3) and (4) forms an antigen binding site that specifically binds a second copy of the second epitope (*e.g.*, TIGIT, LAG-3, TIM-3, CTLA-4, PD-1 (such as a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein)), and each anti-PD-1 sdAb specifically binds a copy of PD-1. In some embodiments the isolated anti-PD-1 construct consists of four polypeptide chains with structures from the N-terminus to the C-terminus as follows: (1) anti-PD-1 sdAb-V_L-C_L; (2) V_H-C_H1-C_H2-C_H3; (3) V_H-C_H1-C_H2-C_H3; and (4) anti-PD-1 sdAb-V_L-C_L, wherein V_H and V_L

of polypeptide chains (1) and (2) forms an antigen binding site that specifically binds a first copy of the second epitope (*e.g.*, TIGIT, LAG-3, TIM-3, CTLA-4, PD-1 (such as a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein)), V_H and V_L of polypeptide chains (3) and (4) forms an antigen binding site that specifically binds a second copy of the second epitope (*e.g.*, TIGIT, LAG-3, TIM-3, CTLA-4, PD-1 (such as a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein)), and each anti-PD-1 sdAb specifically binds a copy of PD-1. In some embodiments the isolated anti-PD-1 construct consists of four polypeptide chains with structures from the N-terminus to the C-terminus as follows: (1) V_L-C_L-anti-PD-1 sdAb; (2) V_H-C_H1-C_H2-C_H3; (3) V_H-C_H1-C_H2-C_H3; and (4) V_L-C_L-anti-PD-1 sdAb, wherein V_H and V_L of polypeptide chains (1) and (2) forms an antigen binding site that specifically binds a first copy of the second epitope (*e.g.*, TIGIT, LAG-3, TIM-3, CTLA-4, PD-1 (such as a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein)), V_H and V_L of polypeptide chains (3) and (4) forms an antigen binding site that specifically binds a second copy of the second epitope (*e.g.*, TIGIT, LAG-3, TIM-3, CTLA-4, PD-1 (such as a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein)), and each anti-PD-1 sdAb specifically binds a copy of PD-1. In some embodiments the isolated anti-PD-1 construct consists of four polypeptide chains with structures from the N-terminus to the C-terminus as follows: (1) anti-PD-1 sdAb-V_L-C_L; (2) anti-PD-1 sdAb-V_H-C_H1-C_H2-C_H3; (3) anti-PD-1 sdAb-V_H-C_H1-C_H2-C_H3; and (4) anti-PD-1 sdAb-V_L-C_L, wherein V_H and V_L of polypeptide chains (1) and (2) forms an antigen binding site that specifically binds a first copy of the second epitope (*e.g.*, TIGIT, LAG-3, TIM-3, CTLA-4, PD-1 (such as a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein)), V_H and V_L of polypeptide chains (3) and (4) forms an antigen binding site that specifically binds a second copy of the second epitope (*e.g.*, TIGIT, LAG-3, TIM-3, CTLA-4, PD-1 (such as a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein)), and each anti-PD-1 sdAb specifically binds a copy of PD-1. In some embodiments the isolated anti-PD-1 construct consists of four polypeptide chains with structures from the N-terminus to the C-terminus as follows: (1) V_L-C_L; (2) anti-PD-1 sdAb-anti-PD-1 sdAb-V_H-C_H1-C_H2-C_H3; (3) anti-PD-1 sdAb-anti-PD-1 sdAb-V_H-C_H1-C_H2-C_H3; and (4) V_L-C_L, wherein V_H and V_L of polypeptide chains (1) and (2) forms an antigen binding site that specifically binds a first copy of the second epitope (*e.g.*, TIGIT, LAG-3, TIM-3, CTLA-4, PD-1 (such as a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein)), V_H and V_L of polypeptide chains (3) and (4) forms an antigen binding site that specifically binds a second copy of the second epitope (*e.g.*, TIGIT, LAG-3, TIM-3, CTLA-4, PD-1 (such as a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein)), and each anti-PD-1 sdAb specifically binds a copy of PD-1. In some embodiments the isolated anti-PD-1 construct consists of four polypeptide chains with structures

from the N-terminus to the C-terminus as follows: (1) V_L-C_L; (2) V_H-C_H1-anti-PD-1 sdAb-C_H2-C_H3; (3) V_H-C_H1-anti-PD-1 sdAb-C_H2-C_H3; and (4) V_L-C_L, wherein V_H and V_L of polypeptide chains (1) and (2) forms an antigen binding site that specifically binds a first copy of the second epitope (*e.g.*, TIGIT, LAG-3, TIM-3, CTLA-4, PD-1 (such as a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein)), V_H and V_L of polypeptide chains (3) and (4) forms an antigen binding site that specifically binds a second copy of the second epitope (*e.g.*, TIGIT, LAG-3, TIM-3, CTLA-4, PD-1 (such as a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein)), and each anti-PD-1 sdAb specifically binds a copy of PD-1. In some embodiments the isolated anti-PD-1 construct consists of two polypeptide chains each with a structure from the N-terminus to the C-terminus as follows: V_L-V_H-anti-PD-1 sdAb-C_H2-C_H3, wherein V_H and V_L of each polypeptide chain forms a scFv domain that specifically binds a copy of the second epitope (*e.g.*, TIGIT, LAG-3, TIM-3, CTLA-4, PD-1 (such as a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein)), and each anti-PD-1 sdAb specifically binds a copy of PD-1. In some embodiments the isolated anti-PD-1 construct consists of four polypeptide chains with structures from the N-terminus to the C-terminus as follows: (1) V_L-C_L-anti-PD-1 sdAb-C_L; (2) V_H-C_H1-anti-PD-1 sdAb-C_H1-C_H2-C_H3; (3) V_H-C_H1-anti-PD-1 sdAb-C_H1-C_H2-C_H3; and (4) V_L-C_L-anti-PD-1 sdAb-C_L, wherein V_H and V_L of polypeptide chains (1) and (2) forms an antigen binding site that specifically binds a first copy of the second epitope (*e.g.*, TIGIT, LAG-3, TIM-3, CTLA-4, PD-1 (such as a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein)), V_H and V_L of polypeptide chains (3) and (4) forms an antigen binding site that specifically binds a second copy of the second epitope (*e.g.*, TIGIT, LAG-3, TIM-3, CTLA-4, PD-1 (such as a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein)), and each anti-PD-1 sdAb specifically binds a copy of PD-1. In some embodiments the isolated anti-PD-1 construct consists of four polypeptide chains with structures from the N-terminus to the C-terminus as follows: (1) anti-PD-1 sdAb-C_L; (2) V_L-V_H-anti-PD-1 sdAb-C_H1-C_H2-C_H3; (3) V_L-V_H-anti-PD-1 sdAb-C_H1-C_H2-C_H3; and (4) anti-PD-1 sdAb-C_L, wherein V_H and V_L of polypeptide chains (2) and (3) each forms an scFv that specifically binds a copy of the second epitope (*e.g.*, TIGIT, LAG-3, TIM-3, CTLA-4, PD-1 (such as a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein)), and each anti-PD-1 sdAb specifically binds a copy of PD-1. In some embodiments, the full-length antibody (or antigen binding portion comprising a V_H and a V_L) specifically recognizes TIGIT. In some embodiments, the anti-TIGIT full-length antibody (or antigen binding portion comprising a V_H and a V_L) comprises a V_H comprising HC-CDR1, HC-CDR2, and HC-CDR3 of a heavy chain comprising the amino acid sequence of SEQ ID NO: 377, and a V_L comprising LC-CDR1, LC-CDR2, and LC-CDR3 of a light chain comprising the amino acid sequence of SEQ ID NO: 378. In some embodiments, the anti-TIGIT full-

length antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 377, and a light chain comprising the amino acid sequence of SEQ ID NO: 378. In some embodiments, the full-length antibody (or antigen binding portion comprising a V_H and a V_L) specifically recognizing TIGIT is derived from tiragolumab. In some embodiments, the full-length antibody (or antigen binding portion comprising a V_H and a V_L) specifically recognizes LAG-3. In some embodiments, the anti-LAG-3 full-length antibody (or antigen binding portion comprising a V_H and a V_L) comprises a V_H comprising HC-CDR1, HC-CDR2, and HC-CDR3 of a heavy chain comprising the amino acid sequence of SEQ ID NO: 379, and a V_L comprising LC-CDR1, LC-CDR2, and LC-CDR3 of a light chain comprising the amino acid sequence of SEQ ID NO: 380. In some embodiments, the anti-LAG-3 full-length antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 379, and a light chain comprising the amino acid sequence of SEQ ID NO: 380. In some embodiments, the full-length antibody (or antigen binding portion comprising a V_H and a V_L) specifically recognizing LAG-3 is derived from relatlimab. In some embodiments, the full-length antibody (or antigen binding portion comprising a V_H and a V_L) specifically recognizes TIM-3. In some embodiments, the anti-TIM-3 full-length antibody (or antigen binding portion comprising a V_H and a V_L) comprises a V_H comprising HC-CDR1, HC-CDR2, and HC-CDR3 of a heavy chain comprising the amino acid sequence of SEQ ID NO: 381, and a V_L comprising LC-CDR1, LC-CDR2, and LC-CDR3 of a light chain comprising the amino acid sequence of SEQ ID NO: 382. In some embodiments, the anti-TIM-3 full-length antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 381, and a light chain comprising the amino acid sequence of SEQ ID NO: 382. In some embodiments, the full-length antibody (or antigen binding portion comprising a V_H and a V_L) specifically recognizing TIM-3 is derived from MBG453. In some embodiments, the full-length antibody (or antigen binding portion comprising a V_H and a V_L) specifically recognizes CTLA-4. In some embodiments, the anti-CTLA-4 full-length antibody (or antigen binding portion comprising a V_H and a V_L) comprises a V_H comprising HC-CDR1, HC-CDR2, and HC-CDR3 of a heavy chain comprising the amino acid sequence of SEQ ID NO: 383, and a V_L comprising LC-CDR1, LC-CDR2, and LC-CDR3 of a light chain comprising the amino acid sequence of SEQ ID NO: 384. In some embodiments, the anti-CTLA-4 full-length antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 383, and a light chain comprising the amino acid sequence of SEQ ID NO: 384. In some embodiments, the full-length antibody (or antigen binding portion comprising a V_H and a V_L) specifically recognizing CTLA-4 is derived from ipilimumab (*e.g.*, Yervoy®). In some embodiments, the full-length antibody (or antigen binding portion comprising a V_H and a V_L) specifically recognizes PD-1 (such as a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein). In some embodiments, the anti-PD-1 full-length antibody (or antigen binding portion comprising a V_H and a V_L) comprises a V_H comprising HC-CDR1, HC-CDR2, and HC-CDR3 of a heavy chain comprising the amino

acid sequence of SEQ ID NO: 385, and a V_L comprising LC-CDR1, LC-CDR2, and LC-CDR3 of a light chain comprising the amino acid sequence of SEQ ID NO: 386. In some embodiments, the anti-PD-1 full-length antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 385, and a light chain comprising the amino acid sequence of SEQ ID NO: 386. In some embodiments, the full-length antibody (or antigen binding portion comprising a V_H and a V_L) specifically recognizing PD-1 is derived from pembrolizumab (*e.g.*, Keytruda®) or nivolumab (*e.g.*, Opdivo®).

[0018] In some embodiments according to any one of the isolated anti-PD-1 constructs described above, the isolated anti-PD-1 construct further comprises a biologically active protein or fragments thereof.

[0019] Further provided is an isolated anti-PD-1 construct comprising an sdAb moiety specifically recognizing PD-1, wherein the sdAb moiety comprises CDR1, CDR2, and CDR3 of any one of SEQ ID NOs: 289-324.

[0020] Further provided is an isolated anti-PD-1 construct (e.g., anti-PD-1 sdAb, anti-PD-1 HCAb (e.g., anti-PD-1 sdAb-Fc fusion), PD-1×TIGIT BABP, PD-1×LAG-3 BABP, PD-1×TIM-3 BABP, PD-1×CTLA-4 BABP, or PD-1×PD-1 BABP) that specifically binds to PD-1 competitively with the any of the isolated anti-PD-1 constructs described above.

[0021] Further provided is a pharmaceutical composition comprising any one of the isolated anti-PD-1 constructs described above, and optionally a pharmaceutical acceptable carrier.

[0022] Another aspect of the present application provides a method of treating an individual having a PD-1-related disease (such as cancer, or immune-related disease), comprising administering to the individual an effective amount of any one of the pharmaceutical compositions described above. In some embodiments, the PD-1-related disease is cancer. In some embodiments, the cancer is a solid tumor, such as a colon cancer. In some embodiments, the PD-1-related disease is an immune-related disease. In some embodiments, immune-related disease is associated with a T cell dysfunctional disorder. In some embodiments, the T cell dysfunctional disorder is characterized by T cell anergy or decreased ability to secrete cytokines, proliferate or execute cytolytic activity. In some embodiments, the T cell dysfunctional disorder is characterized by T cell exhaustion. In some embodiments, the immune-related disease is selected from the group consisting of unresolved acute infection, chronic infection, and tumor immunity. In some embodiments, the PD-1 related disease is a pathogenic infection. In some embodiments, the method further comprises administering to the individual an additional therapy (e.g., cancer therapy), such as surgery, radiation, chemotherapy, immunotherapy, hormone therapy, or a combination thereof. In some embodiments, the additional therapy is immunotherapy. In some embodiments, the immunotherapy

comprises administering to the individual an effective amount of a second pharmaceutical composition comprising an immunomodulator, such as an immune checkpoint inhibitor (e.g., antibody specifically recognizing TIGIT, LAG-3, TIM-3, CTLA-4, PD-1, or PD-L1). In some embodiments, the pharmaceutical composition is administered systemically, such as intravenously (i.v.) or intraperitoneally (i.p.). In some embodiments, the pharmaceutical composition is administered locally, such as intratumorally. In some embodiments, the individual is a human.

[0023] Further provided is an isolated nucleic acid encoding any one of the isolated anti-PD-1 constructs described above. In some embodiments, the isolated nucleic acid comprises the nucleic acid sequence of any one of SEQ ID NOs: 253-288.

[0024] Further provided is a vector comprising any one of the isolated nucleic acids described above.

[0025] Further provided is an isolated host cell comprising any one of the isolated nucleic acid or vector described above.

[0026] Further provided is a kit comprising any one of the isolated anti-PD-1 construct, isolated nucleic acid, vector, or isolated host cell described above.

[0027] Another aspect of the present application provides a method of producing any one of isolated anti-PD-1 constructs described above, comprising culturing a host cell comprising any one of the isolated nucleic acid or vector described above, or culturing any one of the isolated host cell described above, under conditions effective to express the encoded anti-PD-1 construct; and obtaining the expressed anti-PD-1 construct from said host cell. In some embodiments, the method further comprises producing a host cell comprising any one of the isolated nucleic acid or vector described above.

BRIEF DESCRIPTION OF THE DRAWINGS

[0028] FIGs. 1A-1B depict immune response evaluation of the first camel after PD-1 immunization. FIG. 1A depicts immune response evaluation of pre-immune serum and immune serum of the first camel after the 6^{th} immunization. FIG. 1B depicts immune response evaluation of heavy chain antibodies (IgG2 and IgG3) after the 6^{th} immunization (terminal bleed). Heavy chain antibodies fractionated from pre-immune serum were used as negative controls.

[0029] FIGs. 2A-2B depict immune response evaluation of the second camel after PD-1 immunization. FIG. 2A depicts immune response evaluation of pre-immune serum and immune serum of the 2^{nd} camel after the 6^{th} immunization. FIG. 2B depicts immune response evaluation of heavy chain antibodies (IgG2 and IgG3) after the 6^{th} immunization (terminal bleed). Heavy chain antibodies fractionated from pre-immune serum were used as negative controls.

[0030] FIGs. 3A-3F depict the affinities of selected six camelid sdAbs measured by surface plasma resonance. The k_{on}, k_{off} and K_D values are summarized in FIG. 3G.

[0031] FIGs. 4A-4B depict binding abilities of generated sdAbs to human PD-1 expressing cells. FIG. 4A depicts the binding of AS06962 sdAb, AS07424 sdAb and A31543 sdAb to human PD-1 expressing cells by flow cytometry. EC_{50} data are summarized in FIG. 4B.

[0032] FIGs. 5A-5B depict ligand competition activity evaluation of generated sdAbs measured by flow cytometry. FIG. 5A depicts ligand competition activity evaluation of AS06962 sdAb and A31543 sdAb measured by flow cytometry, using PD-L1 Fc ligand and PD-1 expressing cell line. Keytruda® was used as a positive anti-PD-1 control antibody. IC_{50} is summarized in FIG. 5B.

[0033] FIGs. 6B-6L depict the affinities of generated camelid HCAbs measured by surface plasma resonance. Keytruda® was used as a positive anti-PD-1 control antibody (FIG. 6K). The k_{on}, k_{off} and K_D parameters are summarized in FIG. 6A.

[0034] FIGs. 7A-7X depict binding abilities of generated camelid HCAbs to human PD-1 expressing cells by flow cytometry. FIGs. 7A-7V depict FACS based binding of generated camelid HCAbs to human PD-1 expressing cells. Keytruda® was used as a positive anti-PD-1 control antibody (FIG. 7W). EC_{50} is summarized in FIG. 7X.

[0035] FIGs. 8A-8X depict ligand competition activity evaluation of generated camelid HCAbs measured by flow cytometry. FIGs. 8A-8V depict FACS based ligand competition assay of generated camelid HCAbs using PD-L1 Fc ligand and PD-1 expressing cell line. Keytruda® was used as a positive anti-PD-1 control antibody (FIG. 8W). IC_{50} is summarized in FIG. 8X.

[0036] FIGs. 9A-9H depict biological activity evaluation of generated camelid HCAbs measured by NFAT-induced luciferase reporter activity. FIGs. 9A-9F depict RLU induction through NFAT response elements from the IL-2 promoter in the presence of generated camelid HCAbs during PD-1 effector cells and PD-L1 cells incubation. Keytruda® was used as a positive anti-PD-1 control antibody (FIG. 9G). EC_{50} is summarized in FIG. 9H.

[0037] FIGs. 10A-10C depict functional activity evaluation of camelid HCAbs AS15140_HCAb, AS15156_HCAb, and AS15193_HCAb by mixed lymphocyte reaction (MLR) assay. Keytruda® was used as a positive anti-PD-1 control antibody (FIG. 10D). EC_{50} is summarized in FIG. 10E.

[0038] FIG. 11 depicts sequence alignment of parent AS15193 sdAb, its corresponding four humanized versions, and the human acceptor.

[0039] FIGs. 12A-12E depict the affinities of four humanized HCAbs (FIGs. 12B-12E) and the parent HCAb (AS15193_HCAb, FIG. 12A) measured by surface plasma resonance. The k_{on}, k_{off} and K_D values are summarized in FIG. 12F.

[0040] FIGs. 13A-13E depict binding abilities of four humanized HCAbs (FIGs. 13B-13E) and the parent HCAb (AS15193_HCAb, FIG. 13A) to human PD-1 expressing cells. EC_{50} is summarized in FIG. 13F.

[0041] FIGs. 14A-14F depict ligand competition activity evaluation of four humanized HCAbs measured by flow cytometry. FIGs. 14B-14E depict FACS based ligand competition assay of humanized HCAbs using PD-L1-Fc ligand and PD-1 expressing cell line. Parent HCAb AS15193_HCAb was used as a positive anti-PD-1 control antibody (FIG. 14A). IC_{50} is summarized in FIG. 14F.

[0042] FIGs. 15A-15F depict biological activity evaluation of four humanized HCAbs and their parent HCAb measured by NFAT-induced luciferase reporter activity. FIGs. 15B-15E depict RLU induction through NFAT response elements from the IL-2 promoter in the presence of humanized HCAbs during PD-1 effector cells and PD-L1 cells incubation. Parent HCAb AS15193_HCAb was used as a positive anti-PD-1 control antibody (FIG. 15A). EC_{50} is summarized in FIG. 15F.

[0043] FIG. 16 depicts a schematic structure of an exemplary BABP comprising a monospecific full-length antibody having two identical heavy chains and two identical light chains, and two identical anti-PD-1 sdAbs, wherein the C-terminus of each anti-PD-1 sdAb is fused to the N-terminus of one heavy chain via an optional peptide linker. The two anti-PD-1 sdAbs specifically bind a first epitope (PD-1). The full-length antibody has two antigen binding sites that specifically bind a second epitope. For example, the BABP can consist of four polypeptide chains with structures from the N-terminus to the C-terminus as follows: (1) V_L-C_L; (2) V_HH-V_H-C_H1-C_H2-C_H3; (3) V_HH-V_H-C_H1-C_H2-C_H3; and (4) V_L-C_L, wherein V_H and V_L of polypeptide chains (1) and (2) forms an antigen binding site that specifically binds a first copy of the second epitope, V_H and V_L of polypeptide chains (3) and (4) forms an antigen binding site that specifically binds a second copy of the second epitope, and each V_HH specifically binds a copy of the first epitope (PD-1). In alternative formats, each anti-PD-1 sdAb may be omitted, or replaced with two identical or different anti-PD-1 sdAbs fused to each other. The monospecific full-length antibody may be replaced with a bispecific full-length antibody to further expand binding specificity.

[0044] FIG. 17 depicts a schematic structure of an exemplary BABP comprising a monospecific full-length antibody having two identical heavy chains and two identical light chains, and two identical anti-PD-1 sdAbs, wherein the N-terminus of each anti-PD-1 sdAb is fused to the C-terminus of one heavy chain via an optional peptide linker. The two anti-PD-1 sdAbs specifically bind a first epitope (PD-1).

The full-length antibody has two antigen binding sites that specifically bind a second epitope. For example, the BABP can consist of four polypeptide chains with structures from the N-terminus to the C-terminus as follows: (1) V_L-C_L; (2) V_H-C_H1-C_H2-C_H3-V_HH; (3) V_H-C_H1-C_H2-C_H3-V_HH; and (4) V_L-C_L, wherein V_H and V_L of polypeptide chains (1) and (2) forms an antigen binding site that specifically binds a first copy of the second epitope, V_H and V_L of polypeptide chains (3) and (4) forms an antigen binding site that specifically binds a second copy of the second epitope, and each V_HH specifically binds a copy of the first epitope (PD-1). In alternative formats, each anti-PD-1 sdAb may be omitted, or replaced with two identical or different anti-PD-1 sdAbs fused to each other. The monospecific full-length antibody may be replaced with a bispecific full-length antibody to further expand binding specificity.

[0045] FIG. 18 depicts a schematic structure of an exemplary BABP comprising a monospecific full-length antibody having two identical heavy chains and two identical light chains, and two identical anti-PD-1 sdAbs, wherein the C-terminus of each anti-PD-1 sdAb is fused to the N-terminus of one light chain via an optional peptide linker. The two anti-PD-1 sdAbs specifically bind a first epitope (PD-1). The full-length antibody has two antigen binding sites that specifically bind a second epitope. For example, the BABP can consist of four polypeptide chains with structures from the N-terminus to the C-terminus as follows: (1) V_HH-V_L-C_L; (2) V_H-C_H1-C_H2-C_H3; (3) V_H-C_H1-C_H2-C_H3; and (4) V_HH-V_L-C_L, wherein V_H and V_L of polypeptide chains (1) and (2) forms an antigen binding site that specifically binds a first copy of the second epitope, V_H and V_L of polypeptide chains (3) and (4) forms an antigen binding site that specifically binds a second copy of the second epitope, and each V_HH specifically binds a copy of the first epitope (PD-1). In alternative formats, each anti-PD-1 sdAb may be omitted, or replaced with two identical or different anti-PD-1 sdAbs fused to each other. The monospecific full-length antibody may be replaced with a bispecific full-length antibody to further expand binding specificity.

[0046] FIG. 19 depicts a schematic structure of an exemplary BABP comprising a monospecific full-length antibody having two identical heavy chains and two identical light chains, and two identical anti-PD-1 sdAbs, wherein the N-terminus of each anti-PD-1 sdAb is fused to the C-terminus of one light chain via an optional peptide linker. The two anti-PD-1 sdAbs specifically bind a first epitope. The full-length antibody has two antigen binding sites that specifically bind a second epitope. For example, the BABP can consist of four polypeptide chains with structures from the N-terminus to the C-terminus as follows: (1) V_L-C_L-V_HH; (2) V_H-C_H1-C_H2-C_H3; (3) V_H-C_H1-C_H2-C_H3; and (4) V_L-C_L-V_HH, wherein V_H and V_L of polypeptide chains (1) and (2) forms an antigen binding site that specifically binds a first copy of the second epitope, V_H and V_L of polypeptide chains (3) and (4) forms an antigen binding site that specifically binds a second copy of the second epitope, and each V_HH specifically binds a copy of the first epitope (PD-1). In alternative formats, each anti-PD-1 sdAb may be omitted, or replaced with two

identical or different anti-PD-1 sdAbs fused to each other. The monospecific full-length antibody may be replaced with a bispecific full-length antibody to further expand binding specificity.

[0047] FIG. 20 depicts a schematic structure of an exemplary BABP comprising a monospecific full-length antibody having two identical heavy chains and two identical light chains, and four identical anti-PD-1 sdAbs, wherein the C-terminus of each anti-PD-1 sdAb is fused to the N-terminus of heavy chain or light chain of the monospecific full-length antibody via an optional peptide linker. Each anti-PD-1 sdAb specifically binds to a first epitope (PD-1). The full-length antibody has two antigen binding sites that each specifically binds a second epitope. For example, the BABP can consist of four polypeptide chains with structures from the N-terminus to the C-terminus as follows: (1) V_HH-V_L-C_L; (2) V_HH-V_H-C_H1-C_H2-C_H3; (3) V_HH-V_H-C_H1-C_H2-C_H3; and (4) V_HH-V_L-C_L, wherein V_H and V_L of polypeptide chains (1) and (2) forms an antigen binding site that specifically binds a first copy of the second epitope, V_H and V_L of polypeptide chains (3) and (4) forms an antigen binding site that specifically binds a second copy of the second epitope, and each V_HH specifically binds a copy of the first epitope (PD-1). In alternative formats, each anti-PD-1 sdAb may be omitted, or replaced with two identical or different anti-PD-1 sdAbs fused to each other. The monospecific full-length antibody may be replaced with a bispecific full-length antibody to further expand binding specificity.

[0048] FIG. 21 depicts a schematic structure of an exemplary BABP comprising a monospecific full-length antibody having two identical heavy chains and two identical light chains, and four identical anti-PD-1 sdAbs, wherein fused to the N-terminus of each heavy chain are two identical anti-PD-1 sdAbs, the two anti-PD-1 sdAbs are fused to each other via an optional peptide linker, and the two anti-PD-1 sdAbs are fused to the N-terminus of each heavy chain via an optional peptide linker. Each anti-PD-1 sdAb specifically binds a first epitope (PD-1). The full-length antibody has two antigen binding sites that each specifically binds a second epitope. For example, the BABP can consist of four polypeptide chains with structures from the N-terminus to the C-terminus as follows: (1) V_L-C_L; (2) V_HH-V_HH-V_H-C_H1-C_H2-C_H3; (3) V_HH-V_HH-V_H-C_H1-C_H2-C_H3; and (4) V_L-C_L, wherein V_H and V_L of polypeptide chains (1) and (2) forms an antigen binding site that specifically binds a first copy of the second epitope, V_H and V_L of polypeptide chains (3) and (4) forms an antigen binding site that specifically binds a second copy of the second epitope, and each V_HH specifically binds a copy of the first epitope (PD-1). In alternative formats, each anti-PD-1 sdAb may be omitted, or replaced with two identical or different anti-PD-1 sdAbs fused to each other. The monospecific full-length antibody may be replaced with a bispecific full-length antibody to further expand binding specificity.

[0049] FIG. 22 depicts a schematic structure of an exemplary BABP comprising two identical antigen-binding (Fab) fragments, two identical anti-PD-1 sdAbs, and an Fc region, wherein the N-terminus of

each anti-PD-1 sdAb is fused to the C-terminus of the C_H1 region of the Fab fragment via an optional peptide linker and the C-terminus of each anti-PD-1 sdAb is fused to the N-terminus of the C_H2 region of the Fc region. Each anti-PD-1 sdAb specifically binds a first epitope (PD-1). Each Fab fragment specifically binds a second epitope. For example, the BABP can consist of four polypeptide chains with structures from the N-terminus to the C-terminus as follows: (1) V_L-C_L; (2) V_H-C_H1-V_HH-C_H2-C_H3; (3) V_H-C_H1-V_HH-C_H2-C_H3; and (4) V_L-C_L, wherein V_H and V_L of polypeptide chains (1) and (2) forms an antigen binding site that specifically binds a first copy of the second epitope, V_H and V_L of polypeptide chains (3) and (4) forms an antigen binding site that specifically binds a second copy of the second epitope, and each V_HH specifically binds a copy of the first epitope (PD-1). In alternative formats, each anti-PD-1 sdAb may be omitted, or replaced with two identical or different anti-PD-1 sdAbs fused to each other. In alternative formats, to expand specificity, the two Fab fragments can specifically bind different epitopes, and/or the V_HH fragments can specifically bind different epitopes.

[0050] FIG. 23 depicts a schematic structure of an exemplary BABP comprising two identical single chain variable fragments (scFvs), two identical anti-PD-1 sdAbs, and an Fc region, wherein the N-terminus of each anti-PD-1 sdAb is fused to the C-terminus of an scFv via an optional peptide linker and the C-terminus of each anti-PD-1 sdAb is fused to the N-terminus of the Fc region. Each anti-PD-1 sdAb specifically binds a first epitope (PD-1). Each scFv specifically binds a second epitope. For example, the BABP can consist of two polypeptide chains each with a structure from the N-terminus to the C-terminus as follows: V_L-V_H-V_HH-C_H2-C_H3, wherein V_H and V_L of each polypeptide chain forms a scFv domain that specifically binds a copy of the second epitope, and each V_HH specifically binds a copy of the first epitope (PD-1). In alternative formats, the scFv domain can comprise from the N-terminus to the C-terminus: V_H-V_L. In alternative formats, each anti-PD-1 sdAb may be omitted, or replaced with two identical or different anti-PD-1 sdAbs fused to each other. Additionally, to expand specificity, the two scFvs can specifically bind different epitopes, and/or the V_HH fragments can specifically bind different epitopes.

[0051] FIG. 24 depicts a schematic structure of an exemplary BABP comprising two identical Fab fragments, two identical Fab-like fragments each comprising two V_HH fragments, and an Fc region. In each Fab-like fragment, the V_H and V_L regions are each replaced by an anti-PD-1 sdAb. Each Fab-like fragment specifically binds a first epitope (PD-1). Each Fab fragment specifically binds a second epitope. For example, the BABP can consist of four polypeptide chains with structures from the N-terminus to the C-terminus as follows: (1) V_L-C_L-V_HH-C_L; (2) V_H-C_H1-V_HH-C_H1-C_H2-C_H3; (3) V_H-C_H1-V_HH-C_H1-C_H2-C_H3; and (4) V_L-C_L-V_HH-C_L, wherein V_H and V_L of polypeptide chains (1) and (2) forms an antigen binding site that specifically binds a first copy of the second epitope, V_H and V_L of polypeptide chains (3)

and (4) forms an antigen binding site that specifically binds a second copy of the second epitope, and each V_HH specifically binds a copy of the first epitope (PD-1). In alternative formats, to expand specificity, the two Fab fragments can specifically bind different epitopes, and/or the Fab-like fragments can specifically bind different epitopes (e.g., different epitopes from PD-1).

[0052] FIG. 25 depicts a schematic structure of an exemplary BABP comprising two identical scFvs, two identical Fab-like fragments each comprising two V_HH fragments, and an Fc region. In each Fab-like fragment, the V_H and V_L regions are each replaced by an anti-PD-1 sdAb. Each Fab-like fragment specifically binds a first epitope (PD-1). Each scFv specifically binds a second epitope. For example, the BABP can consist of four polypeptide chains with structures from the N-terminus to the C-terminus as follows: (1) V_HH-C_L; (2) V_L-V_H-V_HH-C_H1-C_H2-C_H3; (3) V_L-V_H-V_HH-C_H1-C_H2-C_H3; and (4) V_HH-C_L, wherein V_H and V_L of polypeptide chains (2) and (3) each forms an scFv that specifically binds a copy of the second epitope, and each V_HH specifically binds a copy of the first epitope (PD-1). In alternative formats, the C-terminus of the scFv may be fused to the N-terminus of the chain in the Fab-like fragment comprising V_HH-C_L; and/or the scFv domain can comprise from the N-terminus to the C-terminus: V_H-V_L. Additionally, to expand specificity, the two scFvs can specifically bind different epitopes, and/or the V_HH fragments can specifically bind different epitopes (e.g., different epitopes from PD-1).

[0053] FIGs. 26A-26B depict schematic structure of exemplary anti-PD-1 HCAbs. FIG. 26A depicts a schematic structure of an exemplary monospecific bivalent anti-PD-1 HCAb. FIG. 26B depicts a schematic structure of an exemplary bispecific bivalent anti-PD-1 HCAb.

[0054] FIG. 27 depicts the in vivo efficacy study of two humanized HCAbs AS15193VH8M1_HCAb and AS15193VH18M1_HCAb..

[0055] FIG. 28 depicts the purification summary of selected sdAbs.

[0056] FIG. 29 depicts the purification summary of selected HCAbs.

DETAILED DESCRIPTION OF THE INVENTION

[0057] The present invention provides novel sdAbs specifically recognizing PD-1 (hereinafter also referred to as "anti-PD-1 sdAb") and its antibody variants (for example, a larger protein or polypeptide comprising the anti-PD-1 sdAb, such as anti-PD-1 sdAb-Fc fusion protein (e.g., anti-PD-1 HCAb), anti-PD-1 sdAb fused to a full-length antibody, Fab, or scFv, or multispecific antigen binding proteins (MABPs, such as bispecific antigen binding proteins (BABPs)) comprising the anti-PD-1 sdAb), uses thereof for treating PD-1-related diseases (such as cancer) and methods of making thereof.

[0058] sdAbs are different from conventional 4-chain antibodies by having a single monomeric antibody variable domain, such as heavy chain variable domain (V_HH), which can exhibit high affinity to an antigen without the aid of a light chain. Camelid V_HH is known as the smallest functional antigen-binding fragment with a molecular weight of approximately 15 kDa.

[0059] Accordingly, one aspect of the present application provides an isolated anti-PD-1 construct comprising an sdAb moiety specifically recognizing PD-1. The isolated anti-PD-1 construct can be, for example, an anti-PD-1 sdAb (e.g. natural, humanized, or human), a polypeptide comprising multiple anti-PD-1 sdAbs described herein fused together, an anti-PD-1 sdAb-Fc fusion protein (e.g., anti-PD-1 HCAb) comprising an anti-PD-1 sdAb described herein fused to an Fc fragment (*e.g.*, a human IgG1 Fc, effectorless (inert) hIgG1 Fc, hIgG4 Fc, or hIgG4 Fc (S228P)), or a MABP comprising the anti-PD-1 sdAb described herein fused to a full-length antibody (such as an antibody specifically recognizing TIGIT, LAG-3, TIM-3, CTLA-4, or PD-1 (*e.g.*, a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein)) or antigen binding fragment thereof that comprises a heavy chain variable domain (V_H) and a light chain variable domain (V_L). The anti-PD-1 construct can be monospecific or multispecific (such as bispecific), monovalent or multivalent (such as bivalent).

[0060] Also provided are compositions (such as pharmaceutical compositions), kits and articles of manufacture comprising the anti-PD-1 construct described herein, methods of making thereof, and methods of treating PD-1-related disease (such as cancer) using the anti-PD-1 construct described herein.

I. Definitions

[0061] The term "epitope" means a protein determinant capable of specific binding to an antibody. Epitopes usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and usually have specific three dimensional structural characteristics, as well as specific charge characteristics. Conformational and non-conformational epitopes are distinguished in that the binding to the former but not the latter is lost in the presence of denaturing solvents.

[0062] As used herein, "treatment" or "treating" is an approach for obtaining beneficial or desired results including clinical results. For purposes of this invention, beneficial or desired clinical results include, but are not limited to, one or more of the following: alleviating one or more symptoms resulting from the disease, diminishing the extent of the disease, stabilizing the disease (*e.g.*, preventing or delaying the worsening of the disease), preventing or delaying the spread (*e.g.*, metastasis) of the disease, preventing or delaying the recurrence of the disease, delay or slowing the progression of the disease, ameliorating the disease state, providing a remission (partial or total) of the disease, decreasing the dose

of one or more other medications required to treat the disease, delaying the progression of the disease, increasing the quality of life, and/or prolonging survival. Also encompassed by "treatment" is a reduction of pathological consequence of cancer. The methods of the invention contemplate any one or more of these aspects of treatment.

[0063] The term "prevent," and similar words such as "prevented," "preventing" *etc.*, indicate an approach for preventing, inhibiting, or reducing the likelihood of the recurrence of, a disease or condition, *e.g.*, cancer. It also refers to delaying the recurrence of a disease or condition or delaying the recurrence of the symptoms of a disease or condition. As used herein, "prevention" and similar words also includes reducing the intensity, effect, symptoms and/or burden of a disease or condition prior to recurrence of the disease or condition.

[0064] As used herein, "delaying" the development of cancer means to defer, hinder, slow, retard, stabilize, and/or postpone development of the disease. This delay can be of varying lengths of time, depending on the history of the disease and/or individual being treated. A method that "delays" development of cancer is a method that reduces probability of disease development in a given time frame and/or reduces the extent of the disease in a given time frame, when compared to not using the method. Such comparisons are typically based on clinical studies, using a statistically significant number of individuals. Cancer development can be detectable using standard methods, including, but not limited to, computerized axial tomography (CAT Scan), Magnetic Resonance Imaging (MRI), abdominal ultrasound, clotting tests, arteriography, or biopsy. Development may also refer to cancer progression that may be initially undetectable and includes occurrence, recurrence, and onset.

[0065] The term "effective amount" used herein refers to an amount of an agent or a combination of agents, sufficient to treat a specified disorder, condition or disease such as ameliorate, palliate, lessen, and/or delay one or more of its symptoms. In reference to cancer, an effective amount comprises an amount sufficient to cause a tumor to shrink and/or to decrease the growth rate of the tumor (such as to suppress tumor growth) or to prevent or delay other unwanted cell proliferation. In some embodiments, an effective amount is an amount sufficient to delay development. In some embodiments, an effective amount is an amount sufficient to prevent or delay recurrence. An effective amount can be administered in one or more administrations. The effective amount of the drug or composition may: (i) reduce the number of cancer cells; (ii) reduce tumor size; (iii) inhibit, retard, slow to some extent and preferably stop cancer cell infiltration into peripheral organs; (iv) inhibit (*i.e.*, slow to some extent and preferably stop) tumor metastasis; (v) inhibit tumor growth; (vi) prevent or delay occurrence and/or recurrence of tumor; and/or (vii) relieve to some extent one or more of the symptoms associated with the cancer.

[0066] As used herein, an "individual" or a "subject" refers to a mammal, including, but not limited to, human, bovine, horse, feline, canine, rodent, or primate. In some embodiments, the individual is a human.

[0067] The terms "antibody," "antigen binding portion," or "antibody moiety" are used in their broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, polyclonal antibodies, multispecific antibodies (*e.g.*, bispecific antibodies), full-length antibodies and antigen-binding fragments thereof, so long as they exhibit the desired antigen-binding activity.

[0068] The basic 4-chain antibody unit is a heterotetrameric glycoprotein composed of two identical light (L) chains and two identical heavy (H) chains. An IgM antibody consists of 5 of the basic heterotetramer units along with an additional polypeptide called a J chain, and contains 10 antigen-binding sites, while IgA antibodies comprise from 2-5 of the basic 4-chain units which can polymerize to form polyvalent assemblages in combination with the J chain. In the case of IgGs, the 4-chain unit is generally about 150,000 Daltons. Each L chain is linked to an H chain by one covalent disulfide bond, while the two H chains are linked to each other by one or more disulfide bonds depending on the H chain isotype. Each H and L chain also has regularly spaced intrachain disulfide bridges. Each H chain has at the N-terminus, a variable domain (V_H) followed by three constant domains (C_H) for each of the α and γ chains and four C_H domains for μ and ε isotypes. Each L chain has at the N-terminus, a variable domain (V_L) followed by a constant domain at its other end. The V_L is aligned with the V_H and the C_L is aligned with the first constant domain of the heavy chain (C_H1). Particular amino acid residues are believed to form an interface between the light chain and heavy chain variable domains. The pairing of a V_H and V_L together forms a single antigen-binding site. For the structure and properties of the different classes of antibodies, see *e.g.*, *Basic and Clinical Immunology,* 8th Edition, Daniel P. Sties, Abba I. Terr and Tristram G. Parsolw (eds), Appleton & Lange, Norwalk, Conn., 1994, page 71 and Chapter 6. The L chain from any vertebrate species can be assigned to one of two clearly distinct types, called kappa and lambda, based on the amino acid sequences of their constant domains. Depending on the amino acid sequence of the constant domain of their heavy chains (C_H), immunoglobulins can be assigned to different classes or isotypes. There are five classes of immunoglobulins: IgA, IgD, IgE, IgG and IgM, having heavy chains designated α, δ, ε, γ and μ, respectively. The γ and α classes are further divided into subclasses on the basis of relatively minor differences in the C_H sequence and function, *e.g.*, humans express the following subclasses: IgG1, IgG2A, IgG2B, IgG3, IgG4, IgA1 and IgA2.

[0069] The term "heavy chain-only antibody" or "HCAb" refers to a functional antibody, which comprises heavy chains, but lacks the light chains usually found in 4-chain antibodies. Camelid animals (such as camels, llamas, or alpacas) are known to produce HCAbs.

[0070] The term "single-domain antibody" or "sdAb" refers to a single antigen-binding polypeptide having three complementary determining regions (CDRs). The sdAb alone is capable of binding to the antigen without pairing with a corresponding CDR-containing polypeptide. In some cases, single-domain antibodies are engineered from camelid HCAbs, and their heavy chain variable domains are referred herein as "V_HHs" (Variable domain of the heavy chain of the Heavy chain antibody). Camelid sdAb is one of the smallest known antigen-binding antibody fragments (see, *e.g.,* Hamers-Casterman *et al.*, Nature 363:446-8 (1993); Greenberg *et al.*, Nature 374:168-73 (1995); Hassanzadeh-Ghassabeh *et al.*, Nanomedicine (Lond), 8:1013-26 (2013)). A basic V_HH has the following structure from the N-terminus to the C-terminus: FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4, in which FR1 to FR4 refer to framework regions 1 to 4, respectively, and in which CDR1 to CDR3 refer to the complementarity determining regions 1 to 3.

[0071] An "isolated" antibody (or construct) is one that has been identified, separated and/or recovered from a component of its production environment (*e.g.*, natural or recombinant). Preferably, the isolated polypeptide is free of association with all other components from its production environment. Contaminant components of its production environment, such as that resulting from recombinant transfected cells, are materials that would typically interfere with research, diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. In preferred embodiments, the polypeptide will be purified: (1) to greater than 95% by weight of antibody as determined by, for example, the Lowry method, and in some embodiments, to greater than 99% by weight; (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator; or (3) to homogeneity by SDS-PAGE under non-reducing or reducing conditions using Coomassie Blue or, preferably, silver stain. Isolated antibody (or construct) includes the antibody *in situ* within recombinant cells since at least one component of the antibody's natural environment will not be present. Ordinarily, however, an isolated polypeptide, antibody, or construct will be prepared by at least one purification step.

[0072] The "variable region" or "variable domain" of an antibody refers to the amino-terminal domains of the heavy or light chain of the antibody. The variable domains of the heavy chain and light chain may be referred to as "V_H" and "V_L", respectively. These domains are generally the most variable parts of the antibody (relative to other antibodies of the same class) and contain the antigen binding sites. Heavy-chain only antibodies from the *Camelid* species have a single heavy chain variable region, which is referred to as "V_HH". V_HH is thus a special type of V_H.

[0073] The term "variable" refers to the fact that certain segments of the variable domains differ extensively in sequence among antibodies. The V domain mediates antigen binding and defines the

specificity of a particular antibody for its particular antigen. However, the variability is not evenly distributed across the entire span of the variable domains. Instead, it is concentrated in three segments called complementary determining regions (CDRs) or hypervariable regions (HVRs) both in the heavy chain and light chain variable domains. The more highly conserved portions of variable domains are called the framework regions (FR). The variable domains of native heavy and light chains each comprise four FR regions, largely adopting a beta-sheet configuration, connected by three CDRs, which form loops connecting, and in some cases forming part of, the beta-sheet structure. The CDRs in each chain are held together in close proximity by the FR regions and, with the CDRs from the other chain, contribute to the formation of the antigen binding site of antibodies (see Kabat *et al.*, *Sequences of Immunological Interest*, Fifth Edition, National Institute of Health, Bethesda, Md. (1991)). The constant domains are not involved directly in the binding of antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody-dependent cellular toxicity.

[0074] The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations and/or post-translation modifications (*e.g.*, isomerizations, amidations) that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. In contrast to polyclonal antibody preparations which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they are synthesized by the hybridoma culture, uncontaminated by other immunoglobulins. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by a variety of techniques, including, for example, the hybridoma method (*e.g.*, Kohler and Milstein., *Nature,* 256:495-97 (1975); Hongo *et al.*, *Hybridoma,* 14 (3): 253-260 (1995), Harlow *et al.*, *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling *et al.*, in: *Monoclonal Antibodies and T-Cell Hybridomas* 563-681 (Elsevier, N.Y., 1981)), recombinant DNA methods (see, *e.g.*, U.S. Pat. No. 4,816,567), phage-display technologies (see, *e.g.*, Clackson *et al.*, *Nature,* 352: 624-628 (1991); Marks *et al.*, *J. Mol. Biol.* 222: 581-597 (1992); Sidhu *et al.*, *J. Mol. Biol.* 338(2): 299-310 (2004); Lee *et al.*, *J. Mol. Biol.* 340(5): 1073-1093 (2004); Fellouse, *Proc. Natl. Acad. Sci. USA* 101(34): 12467-12472 (2004); and Lee *et al.*, *J. Immunol. Methods* 284(1-2): 119-132 (2004), and technologies for producing human or human-like antibodies in animals that have parts or all of the human immunoglobulin loci or genes encoding human immunoglobulin sequences (see, *e.g.*, WO 1998/24893;

WO 1996/34096; WO 1996/33735; WO 1991/10741; Jakobovits *et al.*, *Proc. Natl. Acad. Sci. USA* 90: 2551 (1993); Jakobovits *et al.*, *Nature* 362: 255-258 (1993); Bruggemann *et al.*, *Year in Immunol.* 7:33 (1993); U.S. Pat. Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; and 5,661,016; Marks *et al.*, *Bio/Technology* 10: 779-783 (1992); Lonberg *et al.*, *Nature* 368: 856-859 (1994); Morrison, *Nature* 368: 812-813 (1994); Fishwild *et al.*, *Nature Biotechnol.* 14: 845-851 (1996); Neuberger, *Nature Biotechnol.* 14: 826 (1996); and Lonberg and Huszar, *Intern. Rev. Immunol.* 13: 65-93 (1995).

[0075] The terms "full-length antibody", "intact antibody", or "whole antibody" are used interchangeably to refer to an antibody in its substantially intact form, as opposed to an antibody fragment. Specifically, full-length 4-chain antibodies include those with heavy and light chains including an Fc region. Full-length heavy-chain only antibodies include the heavy chain variable domain (such as V_HH) and an Fc region. The constant domains may be native sequence constant domains (*e.g.*, human native sequence constant domains) or amino acid sequence variants thereof. In some cases, the intact antibody may have one or more effector functions.

[0076] An "antibody fragment" or "antigen-binding fragment" comprises a portion of an intact antibody, preferably the antigen binding and/or the variable region of the intact antibody. Examples of antibody fragments include, but are not limited to Fab, Fab′, F(ab′)$_2$ and Fv fragments; diabodies; linear antibodies (see U.S. Pat. No. 5,641,870, Example 2; Zapata *et al.*, *Protein Eng.* 8(10): 1057-1062 (1995)); single-chain antibody (scFv) molecules; single-domain antibodies (such as V_HH), and multispecific antibodies formed from antibody fragments. Papain digestion of antibodies produced two identical antigen-binding fragments, called "Fab" fragments, and a residual "Fc" fragment, a designation reflecting the ability to crystallize readily. The Fab fragment consists of an entire L chain along with the variable domain of the H chain (V_H), and the first constant domain of one heavy chain (C_H1). Each Fab fragment is monovalent with respect to antigen binding, *i.e.*, it has a single antigen-binding site. Pepsin treatment of an antibody yields a single large F(ab′)$_2$ fragment which roughly corresponds to two disulfide linked Fab fragments having different antigen-binding activity and is still capable of cross-linking antigen. Fab′ fragments differ from Fab fragments by having a few additional residues at the carboxy-terminus of the C_H1 domain including one or more cysteines from the antibody hinge region. Fab′-SH is the designation herein for Fab′ in which the cysteine residue(s) of the constant domains bear a free thiol group. F(ab′)$_2$ antibody fragments originally were produced as pairs of Fab′ fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

[0077] The term "constant domain" refers to the portion of an immunoglobulin molecule having a more conserved amino acid sequence relative to the other portion of the immunoglobulin, the variable

domain, which contains the antigen-binding site. The constant domain contains the C_H1, C_H2 and C_H3 domains (collectively, C_H) of the heavy chain and the CHL (or C_L) domain of the light chain.

[0078] The "light chains" of antibodies (immunoglobulins) from any mammalian species can be assigned to one of two clearly distinct types, called kappa ("κ") and lambda ("λ"), based on the amino acid sequences of their constant domains.

[0079] "Fv" is the minimum antibody fragment which contains a complete antigen-recognition and -binding site. This fragment consists of a dimer of one heavy- and one light-chain variable region domain in tight, non-covalent association. From the folding of these two domains emanate six hypervariable loops (3 loops each from the H and L chain) that contribute the amino acid residues for antigen binding and confer antigen binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

[0080] "Single-chain Fv" also abbreviated as "sFv" or "scFv" are antibody fragments that comprise the V_H and V_L antibody domains connected into a single polypeptide chain. Preferably, the scFv polypeptide further comprises a polypeptide linker between the V_H and V_L domains which enables the scFv to form the desired structure for antigen binding. For a review of the scFv, see Pluckthun in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenburg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994).

[0081] The term "diabodies" refers to small antibody fragments prepared by constructing sFv fragments (see preceding paragraph) with short linkers (about 5-10 residues) between the V_H and V_L domains such that inter-chain but not intra-chain pairing of the V domains is achieved, thereby resulting in a bivalent fragment, *i.e.*, a fragment having two antigen-binding sites. Bispecific diabodies are heterodimers of two "crossover" sFv fragments in which the V_H and V_L domains of the two antibodies are present on different polypeptide chains. Diabodies are described in greater detail in, for example, EP 404,097; WO 93/11161; Hollinger *et al.*, *Proc. Natl. Acad. Sci. USA* 90: 6444-6448 (1993).

[0082] The monoclonal antibodies herein specifically include "chimeric" antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is(are) identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Pat.

No. 4,816,567; Morrison *et al.*, *Proc. Natl. Acad. Sci. USA,* 81:6851-6855 (1984)). "Humanized antibody" is used as a subset of "chimeric antibodies".

[0083] "Humanized" forms of non-human (*e.g.*, llama or camelid) antibodies are chimeric antibodies that contain minimal sequence derived from non-human immunoglobulin. In some embodiments, a humanized antibody is a human immunoglobulin (recipient antibody) in which residues from an CDR (hereinafter defined) of the recipient are replaced by residues from an CDR of a non-human species (donor antibody) such as mouse, rat, rabbit, camel, llama, alpaca, or non-human primate having the desired specificity, affinity, and/or capacity. In some instances, framework ("FR") residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications may be made to further refine antibody performance, such as binding affinity. In general, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin sequence, and all or substantially all of the FR regions are those of a human immunoglobulin sequence, although the FR regions may include one or more individual FR residue substitutions that improve antibody performance, such as binding affinity, isomerization, immunogenicity, *etc*. The number of these amino acid substitutions in the FR is typically no more than 6 in the H chain, and in the L chain, no more than 3. The humanized antibody optionally will also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see, *e.g.*, Jones *et al.*, *Nature* 321:522-525 (1986); Riechmann *et al.*, *Nature* 332:323-329 (1988); and Presta, *Curr. Op. Struct. Biol.* 2:593-596 (1992). See also, for example, Vaswani and Hamilton, *Ann. Allergy, Asthma & Immunol.* 1:105-115 (1998); Harris, *Biochem. Soc. Transactions* 23:1035-1038 (1995); Hurle and Gross, *Curr. Op. Biotech.* 5:428-433 (1994); and U.S. Pat. Nos. 6,982,321 and 7,087,409.

[0084] A "human antibody" is an antibody that possesses an amino-acid sequence corresponding to that of an antibody produced by a human and/or has been made using any of the techniques for making human antibodies as disclosed herein. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues. Human antibodies can be produced using various techniques known in the art, including phage-display libraries. Hoogenboom and Winter, *J. Mol. Biol.,* 227:381 (1991); Marks *et al.*, *J. Mol. Biol.,* 222:581 (1991). Also available for the preparation of human monoclonal antibodies are methods described in Cole *et al.*, *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, p. 77 (1985); Boerner *et al.*, *J. Immunol.,* 147(1):86-95 (1991). See also van Dijk and van de Winkel, *Curr. Opin. Pharmacol.,* 5: 368-74 (2001). Human antibodies can be

prepared by administering the antigen to a transgenic animal that has been modified to produce such antibodies in response to antigenic challenge, but whose endogenous loci have been disabled, *e.g.*, immunized xenomice (see, *e.g.*, U.S. Pat. Nos. 6,075,181 and 6,150,584 regarding XENOMOUSE™ technology). See also, for example, Li *et al.*, *Proc. Natl. Acad. Sci. USA,* 103:3557-3562 (2006) regarding human antibodies generated via a human B-cell hybridoma technology.

[0085] The term "hypervariable region," "HVR," or "HV," when used herein refers to the regions of an antibody variable domain which are hypervariable in sequence and/or form structurally defined loops. Generally, single-domain antibodies comprise three HVRs (or CDRs): HVR1 (or CDR1), HVR2 (or CDR2), and HVR3 (or CDR3). HVR3 (or CDR3) displays the most diversity of the three HVRs, and is believed to play a unique role in conferring fine specificity to antibodies. See, *e.g.*, Hamers-Casterman *et al.*, *Nature* 363:446-448 (1993); Sheriff *et al.*, *Nature Struct. Biol.* 3:733-736 (1996).

[0086] The term "Complementarity Determining Region" or "CDR" are used to refer to hypervariable regions as defined by the Kabat system. See Kabat *et al.*, *Sequences of Proteins of Immunological Interest,* 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991).

[0087] A number of HVR delineations are in use and are encompassed herein. The Kabat Complementarity Determining Regions (CDRs) are based on sequence variability and are the most commonly used (Kabat *et al.*, *Sequences of Proteins of Immunological Interest,* 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)). Chothia refers instead to the location of the structural loops (Chothia and Lesk, *J. Mol. Biol.* 196:901-917 (1987)). The AbM HVRs represent a compromise between the Kabat HVRs and Chothia structural loops, and are used by Oxford Molecular's AbM antibody modeling software. The "contact" HVRs are based on an analysis of the available complex crystal structures. The residues from each of these HVRs are noted below in Table 1.

Table 1. HVR delineations.

Loop	Kabat	AbM	Chothia	Contact
L1	L24-L34	L24-L34	L26-L32	L30-L36
L2	L50-L56	L50-L56	L50-L52	L46-L55
L3	L89-L97	L89-L97	L91-L96	L89-L96
H1	H31-H35B	H26-H35B	H26-H32	H30-H35B
		(Kabat Numbering)		
H1	H31-H35	H26-H35	H26-H32	H30-H35
		(Chothia Numbering)		
H2	H50-H65	H50-H58	H53-H55	H47-H58
H3	H95-H102	H95-H102	H96-H101	H93-H101

[0088] HVRs may comprise "extended HVRs" as follows: 24-36 or 24-34 (L1), 46-56 or 50-56 (L2) and 89-97 or 89-96 (L3) in the V_L and 26-35 (H1), 50-65 or 49-65 (H2) and 93-102, 94-102, or 95-102 (H3) in the V_H. The variable domain residues are numbered according to Kabat *et al.*, supra, for each of these definitions.

[0089] The amino acid residues of a single-domain antibody (such as V_HH) are numbered according to the general numbering for V_H domains given by Kabat *et al.* ("Sequence of proteins of immunological interest", US Public Health Services, NIH Bethesda, Md., Publication No. 91), as applied to V_HH domains from Camelids in the article of Riechmann and Muyldermans, J. Immunol. Methods 2000 Jun. 23; 240 (1-2): 185-195. According to this numbering, FR1 of a V_HH comprises the amino acid residues at positions 1-30, CDR1 of a V_HH comprises the amino acid residues at positions 31-35, FR2 of a V_HH comprises the amino acids at positions 36-49, CDR2 of a V_HH comprises the amino acid residues at positions 50-65, FR3 of a V_HH comprises the amino acid residues at positions 66-94, CDR3 of a V_HH comprises the amino acid residues at positions 95-102, and FR4 of a V_HH comprises the amino acid residues at positions 103-113. In this respect, it should be noted that—as is well known in the art for V_H domains and for V_HH domains—the total number of amino acid residues in each of the CDRs may vary and may not correspond to the total number of amino acid residues indicated by the Kabat numbering (that is, one or more positions according to the Kabat numbering may not be occupied in the actual sequence, or the actual sequence may contain more amino acid residues than the number allowed for by the Kabat numbering).

[0090] The expression "variable-domain residue-numbering as in Kabat" or "amino-acid-position numbering as in Kabat," and variations thereof, refers to the numbering system used for heavy-chain variable domains or light-chain variable domains of the compilation of antibodies in Kabat *et al.*, supra. Using this numbering system, the actual linear amino acid sequence may contain fewer or additional amino acids corresponding to a shortening of, or insertion into, a FR or HVR of the variable domain. For example, a heavy-chain variable domain may include a single amino acid insert (residue 52a according to Kabat) after residue 52 of H2 and inserted residues (*e.g.* residues 82a, 82b, and 82c, etc. according to Kabat) after heavy-chain FR residue 82. The Kabat numbering of residues may be determined for a given antibody by alignment at regions of homology of the sequence of the antibody with a "standard" Kabat numbered sequence.

[0091] Unless indicated otherwise herein, the numbering of the residues in an immunoglobulin heavy chain is that of the EU index as in Kabat *et al.*, supra. The "EU index as in Kabat" refers to the residue numbering of the human IgG1 EU antibody.

[0092] "Framework" or "FR" residues are those variable-domain residues other than the HVR residues as herein defined.

[0093] A "human consensus framework" or "acceptor human framework" is a framework that represents the most commonly occurring amino acid residues in a selection of human immunoglobulin V_L or V_H framework sequences. Generally, the selection of human immunoglobulin V_L or V_H sequences is from a subgroup of variable domain sequences. Generally, the subgroup of sequences is a subgroup as in Kabat *et al.*, *Sequences of Proteins of Immunological Interest,* 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991). Examples include for the V_L, the subgroup may be subgroup kappa I, kappa II, kappa III or kappa IV as in Kabat *et al.*, supra. Additionally, for the VH, the subgroup may be subgroup I, subgroup II, or subgroup III as in Kabat *et al.* Alternatively, a human consensus framework can be derived from the above in which particular residues, such as when a human framework residue is selected based on its homology to the donor framework by aligning the donor framework sequence with a collection of various human framework sequences. An acceptor human framework "derived from" a human immunoglobulin framework or a human consensus framework may comprise the same amino acid sequence thereof, or it may contain pre-existing amino acid sequence changes. In some embodiments, the number of pre-existing amino acid changes are 10 or less, 9 or less, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less, 3 or less, or 2 or less.

[0094] An "affinity-matured" antibody is one with one or more alterations in one or more CDRs thereof that result in an improvement in the affinity of the antibody for antigen, compared to a parent antibody that does not possess those alteration(s). In some embodiments, an affinity-matured antibody has nanomolar or even picomolar affinities for the target antigen. Affinity-matured antibodies are produced by procedures known in the art. For example, Marks *et al.*, *Bio/Technology* 10:779-783 (1992) describes affinity maturation by V_H- and V_L-domain shuffling. Random mutagenesis of CDR and/or framework residues is described by, for example: Barbas *et al. Proc Nat. Acad. Sci. USA* 91:3809-3813 (1994); Schier *et al. Gene* 169:147-155 (1995); Yelton *et al. J. Immunol.* 155:1994-2004 (1995); Jackson *et al.*, *J. Immunol.* 154(7):3310-9 (1995); and Hawkins *et al*, *J. Mol. Biol.* 226:889-896 (1992).

[0095] As used herein, the term "specifically binds," "specifically recognizes," or is "specific for" refers to measurable and reproducible interactions such as binding between a target and an antigen binding protein (such as an sdAb), which is determinative of the presence of the target in the presence of a heterogeneous population of molecules including biological molecules. For example, an antigen binding protein (such as an sdAb) that specifically binds a target (which can be an epitope) is an antigen binding protein (such as an sdAb) that binds this target with greater affinity, avidity, more readily, and/or with greater duration than it binds other targets. In some embodiments, the extent of binding of an antigen

binding protein (such as an sdAb) to an unrelated target is less than about 10% of the binding of the antigen binding protein (such as an sdAb) to the target as measured, *e.g.*, by a radioimmunoassay (RIA). In some embodiments, an antigen binding protein (such as an sdAb) that specifically binds a target has a dissociation constant (K_d) of $\leqslant 10^{-5}$ M, $\leqslant 10^{-6}$ M, $\leqslant 10^{-7}$ M, $\leqslant 10^{-8}$ M, $\leqslant 10^{-9}$ M, $\leqslant 10^{-10}$ M, $\leqslant 10^{-11}$ M, or $\leqslant 10^{-12}$ M. In some embodiments, an antigen binding protein specifically binds an epitope on a protein that is conserved among the protein from different species. In some embodiments, specific binding can include, but does not require exclusive binding. Binding specificity of the antibody or antigen-binding domain can be determined experimentally by methods known in the art. Such methods comprise, but are not limited to Western blots, ELISA-, RIA-, ECL-, IRMA-, EIA-, BIAcore-tests and peptide scans.

[0096] The term "specificity" refers to selective recognition of an antigen binding protein (such as an sdAb) for a particular epitope of an antigen. Natural antibodies, for example, are monospecific. The term "multispecific" as used herein denotes that an antigen binding protein has polyepitopic specificity (*i.e.*, is capable of specifically binding to two, three, or more, different epitopes on one biological molecule or is capable of specifically binding to epitopes on two, three, or more, different biological molecules). "Bispecific" as used herein denotes that an antigen binding protein has two different antigen-binding specificities. Unless otherwise indicated, the order in which the antigens bound by a bispecific antibody listed is arbitrary. That is, for example, the terms "anti-TIGIT/PD-1," "anti-PD-1/TIGIT," "TIGIT×PD-1," "PD-1×TIGIT," "PD-1/TIGIT," "TIGIT/PD-1," "PD-1-TIGIT," and "TIGIT-PD-1" may be used interchangeably to refer to bispecific antibodies that specifically bind to both TIGIT and PD-1. The term "monospecific" as used herein denotes an antigen binding protein (such as an sdAb) that has one or more binding sites each of which bind the same epitope of the same antigen.

[0097] The term "valent" as used herein denotes the presence of a specified number of binding sites in an antigen binding protein. A natural antibody for example or a full length antibody has two binding sites and is bivalent. As such, the terms "trivalent", "tetravalent", "pentavalent" and "hexavalent" denote the presence of two binding site, three binding sites, four binding sites, five binding sites, and six binding sites, respectively, in an antigen binding protein.

[0098] "Antibody effector functions" refer to those biological activities attributable to the Fc region (a native sequence Fc region or amino acid sequence variant Fc region) of an antibody, and vary with the antibody isotype. Examples of antibody effector functions include: C1q binding and complement dependent cytotoxicity; Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (*e.g.*, B cell receptors); and B cell activation. "Reduced or minimized" antibody effector function means that which is reduced by at least 50% (alternatively 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%) from the wild type or

unmodified antibody. The determination of antibody effector function is readily determinable and measurable by one of ordinary skill in the art. In a preferred embodiment, the antibody effector functions of complement binding, complement dependent cytotoxicity and antibody dependent cytotoxicity are affected. In some embodiments, effector function is eliminated through a mutation in the constant region that eliminated glycosylation, *e.g.*, "effectorless mutation." In one aspect, the effectorless mutation is an N297A or DANA mutation (D265A+N297A) in the C_H2 region. Shields *et al.*, *J. Biol. Chem.* 276 (9): 6591-6604 (2001). Alternatively, additional mutations resulting in reduced or eliminated effector function include: K322A and L234A/L235A (LALA). Alternatively, effector function can be reduced or eliminated through production techniques, such as expression in host cells that do not glycosylate (*e.g.*, *E. coli.*) or in which result in an altered glycosylation pattern that is ineffective or less effective at promoting effector function (*e.g.*, Shinkawa *et al.*, *J. Biol. Chem.* 278(5): 3466-3473 (2003).

[0099] "Antibody-dependent cell-mediated cytotoxicity" or ADCC refers to a form of cytotoxicity in which secreted Ig bound onto Fc receptors (FcRs) present on certain cytotoxic cells (*e.g.*, natural killer (NK) cells, neutrophils and macrophages) enable these cytotoxic effector cells to bind specifically to an antigen-bearing target cell and subsequently kill the target cell with cytotoxins. The antibodies "arm" the cytotoxic cells and are required for killing of the target cell by this mechanism. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. Fc expression on hematopoietic cells is summarized in Table 2 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol.* 9: 457-92 (1991). To assess ADCC activity of a molecule of interest, an *in vitro* ADCC assay, such as that described in U.S. Pat. No. 5,500,362 or 5,821,337 may be performed. Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and natural killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo*, *e.g.*, in an animal model such as that disclosed in Clynes *et al.*, *PNAS USA* 95:652-656 (1998).

[0100] The term "Fc region" or "fragment crystallizable region" herein is used to define a C-terminal region of an immunoglobulin heavy chain, including native-sequence Fc regions and variant Fc regions. Although the boundaries of the Fc region of an immunoglobulin heavy chain might vary, the human IgG heavy-chain Fc region is usually defined to stretch from an amino acid residue at position Cys226, or from Pro230, to the carboxyl-terminus thereof. The C-terminal lysine (residue 447 according to the EU numbering system) of the Fc region may be removed, for example, during production or purification of the antibody, or by recombinantly engineering the nucleic acid encoding a heavy chain of the antibody. Accordingly, a composition of intact antibodies may comprise antibody populations with all K447 residues removed, antibody populations with no K447 residues removed, and antibody populations

having a mixture of antibodies with and without the K447 residue. Suitable native-sequence Fc regions for use in the antibodies described herein include human IgG1, IgG2 (IgG2A, IgG2B), IgG3 and IgG4.

[0101] "Fc receptor" or "FcR" describes a receptor that binds the Fc region of an antibody. The preferred FcR is a native sequence human FcR. Moreover, a preferred FcR is one which binds an IgG antibody (a gamma receptor) and includes receptors of the FcγRI, FcγRII, and FcγRIII subclasses, including allelic variants and alternatively spliced forms of these receptors, FcγRII receptors include FcγRIIA (an "activating receptor") and FcγRIIB (an "inhibiting receptor"), which have similar amino acid sequences that differ primarily in the cytoplasmic domains thereof. Activating receptor FcγRIIA contains an immunoreceptor tyrosine-based activation motif (ITAM) in its cytoplasmic domain. Inhibiting receptor FcγRIIB contains an immunoreceptor tyrosine-based inhibition motif (ITIM) in its cytoplasmic domain. (See M. Daëron, *Annu. Rev. Immunol.* 15:203-234 (1997). FcRs are reviewed in Ravetch and Kinet, *Annu. Rev. Immunol.* 9: 457-92 (1991); Capel *et al.*, *Immunomethods* 4: 25-34 (1994); and de Haas *et al.*, *J. Lab. Clin. Med.* 126: 330-41 (1995). Other FcRs, including those to be identified in the future, are encompassed by the term "FcR" herein.

[0102] The term "Fc receptor" or "FcR" also includes the neonatal receptor, FcRn, which is responsible for the transfer of maternal IgGs to the fetus. Guyer *et al.*, *J. Immunol.* 117: 587 (1976) and Kim *et al.*, *J. Immunol.* 24: 249 (1994). Methods of measuring binding to FcRn are known (see, *e.g.*, Ghetie and Ward, *Immunol. Today* 18: (12): 592-8 (1997); Ghetie *et al.*, *Nature Biotechnology* 15 (7): 637-40 (1997); Hinton *et al.*, *J. Biol. Chem.* 279 (8): 6213-6 (2004); WO 2004/92219 (Hinton *et al.*). Binding to FcRn *in vivo* and serum half-life of human FcRn high-affinity binding polypeptides can be assayed, *e.g.*, in transgenic mice or transfected human cell lines expressing human FcRn, or in primates to which the polypeptides having a variant Fc region are administered. WO 2004/42072 (Presta) describes antibody variants which improved or diminished binding to FcRs. See also, *e.g.*, Shields *et al.*, *J. Biol. Chem.* 9(2): 6591-6604 (2001).

[0103] "Complement dependent cytotoxicity" or "CDC" refers to the lysis of a target cell in the presence of complement. Activation of the classical complement pathway is initiated by the binding of the first component of the complement system (C1q) to antibodies (of the appropriate subclass) which are bound to their cognate antigen. To assess complement activation, a CDC assay, *e.g.*, as described in Gazzano-Santoro *et al.*, *J. Immunol. Methods* 202: 163 (1996), may be performed. Antibody variants with altered Fc region amino acid sequences and increased or decreased C1q binding capability are described in U.S. Pat. No. 6,194,551B1 and WO99/51642. The contents of those patent publications are specifically incorporated herein by reference. See, also, Idusogie *et al. J. Immunol.* 164: 4178-4184 (2000).

[0104] "Binding affinity" generally refers to the strength of the sum total of non-covalent interactions between a single binding site of a molecule (*e.g.*, an antibody) and its binding partner (*e.g.*, an antigen). Unless indicated otherwise, as used herein, "binding affinity" refers to intrinsic binding affinity that reflects a 1:1 interaction between members of a binding pair. Binding affinity can be indicated by K_d, K_{off}, K_{on}, or K_a. The term "K_{off}", as used herein, is intended to refer to the off rate constant for dissociation of an antibody (or antigen-binding domain) from the antibody/antigen complex, as determined from a kinetic selection set up, expressed in units of s^{-1}. The term "K_{on}", as used herein, is intended to refer to the on rate constant for association of an antibody (or antigen-binding domain) to the antigen to form the antibody/antigen complex, expressed in units of $M^{-1}s^{-1}$. The term equilibrium dissociation constant "K_D" or "K_d", as used herein, refers to the dissociation constant of a particular antibody-antigen interaction, and describes the concentration of antigen required to occupy one half of all of the antibody-binding domains present in a solution of antibody molecules at equilibrium, and is equal to K_{off}/K_{on}, expressed in units of M. The measurement of K_d presupposes that all binding agents are in solution. In the case where the antibody is tethered to a cell wall, *e.g.*, in a yeast expression system, the corresponding equilibrium rate constant is expressed as EC50, which gives a good approximation of K_d. The affinity constant, K_a, is the inverse of the dissociation constant, K_d, expressed in units of M^{-1}. The dissociation constant (K_D or K_d) is used as an indicator showing affinity of antibodies to antigens. For example, easy analysis is possible by the Scatchard method using antibodies marked with a variety of marker agents, as well as by using BiacoreX (made by Amersham Biosciences), which is an over-the-counter, measuring kit, or similar kit, according to the user's manual and experiment operation method attached with the kit. The K_D value that can be derived using these methods is expressed in units of M (Mols). An antibody or antigen-binding fragment thereof that specifically binds to a target may have a dissociation constant (K_d) of, for example, $\leqslant 10^{-5}$ M, $\leqslant 10^{-6}$ M, $\leqslant 10^{-7}$ M, $\leqslant 10^{-8}$ M, $\leqslant 10^{-9}$ M, $\leqslant 10^{-10}$ M, $\leqslant 10^{-11}$ M, or $\leqslant 10^{-12}$ M.

[0105] Half maximal inhibitory concentration (IC_{50}) is a measure of the effectiveness of a substance (such as an antibody) in inhibiting a specific biological or biochemical function. It indicates how much of a particular drug or other substance (inhibitor, such as an antibody) is needed to inhibit a given biological process (e.g., the binding between PD-1 and PD-L1/PD-L2, or component of a process, *i.e.* an enzyme, cell, cell receptor or microorganism) by half. The values are typically expressed as molar concentration. IC_{50} is comparable to an "EC_{50}" for agonist drug or other substance (such as an antibody). EC_{50} also represents the plasma concentration required for obtaining 50% of a maximum effect *in vivo*. As used herein, an "IC_{50}" is used to indicate the effective concentration of an antibody (such as an anti-PD-1 sdAb) needed to neutralize 50% of the antigen bioactivity (such as PD-1 bioactivity) *in vitro*. IC_{50} or EC_{50} can be

measured by bioassays such as inhibition of ligand binding by FACS analysis (competition binding assay), cell based cytokine release assay, or amplified luminescent proximity homogeneous assay (AlphaLISA).

[0106] "Percent (%) amino acid sequence identity" and "homology" with respect to a peptide, polypeptide or antibody sequence are defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the specific peptide or polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or MEGALIGN™ (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared.

[0107] An "isolated" nucleic acid molecule encoding a construct, antibody, or antigen-binding fragment thereof described herein is a nucleic acid molecule that is identified and separated from at least one contaminant nucleic acid molecule with which it is ordinarily associated in the environment in which it was produced. Preferably, the isolated nucleic acid is free of association with all components associated with the production environment. The isolated nucleic acid molecules encoding the polypeptides and antibodies described herein is in a form other than in the form or setting in which it is found in nature. Isolated nucleic acid molecules therefore are distinguished from nucleic acid encoding the polypeptides and antibodies described herein existing naturally in cells. An isolated nucleic acid includes a nucleic acid molecule contained in cells that ordinarily contain the nucleic acid molecule, but the nucleic acid molecule is present extrachromosomally or at a chromosomal location that is different from its natural chromosomal location.

[0108] The term "control sequences" refers to DNA sequences necessary for the expression of an operably linked coding sequence in a particular host organism. The control sequences that are suitable for prokaryotes, for example, include a promoter, optionally an operator sequence, and a ribosome binding site. Eukaryotic cells are known to utilize promoters, polyadenylation signals, and enhancers.

[0109] Nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are

contiguous, and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, the synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

[0110] The term "vector," as used herein, refers to a nucleic acid molecule capable of propagating another nucleic acid to which it is linked. The term includes the vector as a self-replicating nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been introduced. Certain vectors are capable of directing the expression of nucleic acids to which they are operatively linked. Such vectors are referred to herein as "expression vectors."

[0111] The term "transfected" or "transformed" or "transduced" as used herein refers to a process by which exogenous nucleic acid is transferred or introduced into the host cell. A "transfected" or "transformed" or "transduced" cell is one which has been transfected, transformed or transduced with exogenous nucleic acid. The cell includes the primary subject cell and its progeny.

[0112] The terms "host cell," "host cell line," and "host cell culture" are used interchangeably and refer to cells into which exogenous nucleic acid has been introduced, including the progeny of such cells. Host cells include "transformants" and "transformed cells," which include the primary transformed cell and progeny derived therefrom without regard to the number of passages. Progeny may not be completely identical in nucleic acid content to a parent cell, but may contain mutations. Mutant progeny that have the same function or biological activity as screened or selected for in the originally transformed cell are included herein.

[0113] "Adjuvant setting" refers to a clinical setting in which an individual has had a history of cancer, and generally (but not necessarily) been responsive to therapy, which includes, but is not limited to, surgery (*e.g.*, surgery resection), radiotherapy, and chemotherapy. However, because of their history of cancer, these individuals are considered at risk of development of the disease. Treatment or administration in the "adjuvant setting" refers to a subsequent mode of treatment. The degree of risk (*e.g.*, when an individual in the adjuvant setting is considered as "high risk" or "low risk") depends upon several factors, most usually the extent of disease when first treated.

[0114] "Neoadjuvant setting" refers to a clinical setting in which the method is carried out before the primary/definitive therapy.

[0115] The term "pharmaceutical formulation" of "pharmaceutical composition" refers to a preparation that is in such form as to permit the biological activity of the active ingredient to be effective, and that contains no additional components that are unacceptably toxic to a subject to which the formulation

would be administered. Such formulations are sterile. A "sterile" formulation is aseptic or free from all living microorganisms and their spores.

[0116] It is understood that embodiments of the invention described herein include "consisting" and/or "consisting essentially of" embodiments.

[0117] Reference to "about" a value or parameter herein includes (and describes) variations that are directed to that value or parameter *per se*. For example, description referring to "about X" includes description of "X".

[0118] As used herein, reference to "not" a value or parameter generally means and describes "other than" a value or parameter. For example, the method is not used to treat cancer of type X means the method is used to treat cancer of types other than X.

[0119] As used herein and in the appended claims, the singular forms "a," "or," and "the" include plural referents unless the context clearly dictates otherwise.

II. Anti-PD-1 construct

(I) Anti-PD-1 single-domain antibody moiety

[0120] The isolated anti-PD-1 construct described herein comprises a single-domain antibody (sdAb) moiety that specifically recognizes PD-1 (or "anti-PD-1 sdAb"). In some embodiments, the isolated anti-PD-1 construct is an anti-PD-1 sdAb.

[0121] In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 37-72, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 109-144, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 181-216, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, the K_d of the binding between the anti-PD-1 sdAb moiety and PD-1 is about 10^{-5} M to about 10^{-12} M (such as about 10^{-7} M to about 10^{-12} M, or about 10^{-8} M to about 10^{-12} M). In some embodiments, the anti-PD-1 sdAb moiety is camelid, chimeric, human, partially humanized, or fully humanized.

[0122] In some embodiments, the anti-PD-1 sdAb moiety comprises a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 181-216, and the amino acid substitutions are in CDR1 and/or CDR2. Thus, in some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1

comprising the amino acid sequence of any one of SEQ ID NOs: 37-72, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 109-144, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 181-216. In some embodiments, the K_d of the binding between the anti-PD-1 sdAb moiety and PD-1 is about 10^{-5} M to about 10^{-12} M (such as about 10^{-7} M to about 10^{-12} M, or about 10^{-8} M to about 10^{-12} M). In some embodiments, the anti-PD-1 sdAb moiety is camelid, chimeric, human, partially humanized, or fully humanized.

[0123] In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 37-72; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 109-144; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 181-216; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions. In some embodiments, the amino acid substitutions are in CDR1 and/or CDR2. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 37-72; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 109-144; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 181-216; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions, wherein the amino acid substitutions are in CDR1 and/or CDR2. In some embodiments, the K_d of the binding between the anti-PD-1 sdAb moiety and PD-1 is about 10^{-5} M to about 10^{-12} M (such as about 10^{-7} M to about 10^{-12} M, or about 10^{-8} M to about 10^{-12} M). In some embodiments, the anti-PD-1 sdAb moiety is camelid, chimeric, human, partially humanized, or fully humanized.

[0124] In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 37-72; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 109-144; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 181-216. In some embodiments, the K_d of the binding between the anti-PD-1 sdAb moiety and PD-1 is about 10^{-5} M to about 10^{-12} M (such as about 10^{-7} M to about 10^{-12} M, or about 10^{-8} M to about 10^{-12} M). In some embodiments, the anti-PD-1 sdAb moiety is camelid, chimeric, human, partially humanized, or fully humanized.

[0125] The sequences of the CDRs noted herein are provided in Table 3. The CDRs can be combined in various pair-wise combinations to generate a number of anti-PD-1 sdAb moieties.

[0126] For example, in some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 37, or a variant thereof comprising up to

about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 109, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 181, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 37; a CDR2 comprising the amino acid sequence of SEQ ID NO: 109; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 181; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions. In some embodiments, the amino acid substitutions are in CDR1 and/or CDR2. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 37; a CDR2 comprising the amino acid sequence of SEQ ID NO: 109; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 181. In some embodiments, the K_d of the binding between the anti-PD-1 sdAb moiety and PD-1 is about 10^{-5} M to about 10^{-12} M (such as about 10^{-7} M to about 10^{-12} M, or about 10^{-8} M to about 10^{-12} M). In some embodiments, the anti-PD-1 sdAb moiety is camelid, chimeric, human, partially humanized, or fully humanized.

[0127] In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 38, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 110, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 182, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 38; a CDR2 comprising the amino acid sequence of SEQ ID NO: 110; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 182; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions. In some embodiments, the amino acid substitutions are in CDR1 and/or CDR2. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 38; a CDR2 comprising the amino acid sequence of SEQ ID NO: 110; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 182. In some embodiments, the K_d of the binding between the anti-PD-1 sdAb moiety and PD-1 is about 10^{-5} M to about 10^{-12} M (such as about 10^{-7} M to about 10^{-12} M, or about 10^{-8} M to about 10^{-12} M). In some embodiments, the anti-PD-1 sdAb moiety is camelid, chimeric, human, partially humanized, or fully humanized.

[0128] In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 39, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 111, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 183, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 39; a CDR2 comprising the amino acid sequence of SEQ ID NO: 111; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 183; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions. In some embodiments, the amino acid substitutions are in CDR1 and/or CDR2. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 39; a CDR2 comprising the amino acid sequence of SEQ ID NO: 111; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 183. In some embodiments, the K_d of the binding between the anti-PD-1 sdAb moiety and PD-1 is about 10^{-5} M to about 10^{-12} M (such as about 10^{-7} M to about 10^{-12} M, or about 10^{-8} M to about 10^{-12} M). In some embodiments, the anti-PD-1 sdAb moiety is camelid, chimeric, human, partially humanized, or fully humanized.

[0129] In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 40, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 112, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 184, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 40; a CDR2 comprising the amino acid sequence of SEQ ID NO: 112; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 184; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions. In some embodiments, the amino acid substitutions are in CDR1 and/or CDR2. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 40; a CDR2 comprising the amino acid sequence of SEQ ID NO: 112; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 184. In some embodiments, the K_d of the binding between the anti-PD-1 sdAb moiety and PD-1 is about 10^{-5} M to about 10^{-12} M (such as about 10^{-7} M to about 10^{-12} M, or about 10^{-8} M to about 10^{-12} M). In some embodiments, the anti-PD-1 sdAb moiety is camelid, chimeric, human, partially humanized, or fully humanized.

[0130] In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 41, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 113, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 185, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 41; a CDR2 comprising the amino acid sequence of SEQ ID NO: 113; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 185; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions. In some embodiments, the amino acid substitutions are in CDR1 and/or CDR2. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 41; a CDR2 comprising the amino acid sequence of SEQ ID NO: 113; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 185. In some embodiments, the K_d of the binding between the anti-PD-1 sdAb moiety and PD-1 is about 10^{-5} M to about 10^{-12} M (such as about 10^{-7} M to about 10^{-12} M, or about 10^{-8} M to about 10^{-12} M). In some embodiments, the anti-PD-1 sdAb moiety is camelid, chimeric, human, partially humanized, or fully humanized.

[0131] In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 42, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 114, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 186, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 42; a CDR2 comprising the amino acid sequence of SEQ ID NO: 114; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 186; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions. In some embodiments, the amino acid substitutions are in CDR1 and/or CDR2. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 42; a CDR2 comprising the amino acid sequence of SEQ ID NO: 114; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 186. In some embodiments, the K_d of the binding between the anti-PD-1 sdAb moiety and PD-1 is about 10^{-5} M to about 10^{-12} M (such as about 10^{-7} M to about 10^{-12} M, or about 10^{-8} M to about 10^{-12} M). In some embodiments, the anti-PD-1 sdAb moiety is camelid, chimeric, human, partially humanized, or fully humanized.

[0132] In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 43, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 115, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 187, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 43; a CDR2 comprising the amino acid sequence of SEQ ID NO: 115; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 187; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions. In some embodiments, the amino acid substitutions are in CDR1 and/or CDR2. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 43; a CDR2 comprising the amino acid sequence of SEQ ID NO: 115; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 187. In some embodiments, the K_d of the binding between the anti-PD-1 sdAb moiety and PD-1 is about 10^{-5} M to about 10^{-12} M (such as about 10^{-7} M to about 10^{-12} M, or about 10^{-8} M to about 10^{-12} M). In some embodiments, the anti-PD-1 sdAb moiety is camelid, chimeric, human, partially humanized, or fully humanized.

[0133] In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 44, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 116, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 188, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 44; a CDR2 comprising the amino acid sequence of SEQ ID NO: 116; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 188; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions. In some embodiments, the amino acid substitutions are in CDR1 and/or CDR2. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 44; a CDR2 comprising the amino acid sequence of SEQ ID NO: 116; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 188. In some embodiments, the K_d of the binding between the anti-PD-1 sdAb moiety and PD-1 is about 10^{-5} M to about 10^{-12} M (such as about 10^{-7} M to about 10^{-12} M, or about 10^{-8} M to about 10^{-12} M). In some embodiments, the anti-PD-1 sdAb moiety is camelid, chimeric, human, partially humanized, or fully humanized.

[0134] In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 45, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 117, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 189, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 45; a CDR2 comprising the amino acid sequence of SEQ ID NO: 117; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 189; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions. In some embodiments, the amino acid substitutions are in CDR1 and/or CDR2. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 45; a CDR2 comprising the amino acid sequence of SEQ ID NO: 117; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 189. In some embodiments, the K_d of the binding between the anti-PD-1 sdAb moiety and PD-1 is about 10^{-5} M to about 10^{-12} M (such as about 10^{-7} M to about 10^{-12} M, or about 10^{-8} M to about 10^{-12} M). In some embodiments, the anti-PD-1 sdAb moiety is camelid, chimeric, human, partially humanized, or fully humanized.

[0135] In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 46, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 118, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 190, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 46; a CDR2 comprising the amino acid sequence of SEQ ID NO: 118; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 190; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions. In some embodiments, the amino acid substitutions are in CDR1 and/or CDR2. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 46; a CDR2 comprising the amino acid sequence of SEQ ID NO: 118; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 190. In some embodiments, the K_d of the binding between the anti-PD-1 sdAb moiety and PD-1 is about 10^{-5} M to about 10^{-12} M (such as about 10^{-7} M to about 10^{-12} M, or about 10^{-8} M to about 10^{-12} M). In some embodiments, the anti-PD-1 sdAb moiety is camelid, chimeric, human, partially humanized, or fully humanized.

[0136] In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 47, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 119, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 191, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 47; a CDR2 comprising the amino acid sequence of SEQ ID NO: 119; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 191; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions. In some embodiments, the amino acid substitutions are in CDR1 and/or CDR2. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 47; a CDR2 comprising the amino acid sequence of SEQ ID NO: 119; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 191. In some embodiments, the K_d of the binding between the anti-PD-1 sdAb moiety and PD-1 is about 10^{-5} M to about 10^{-12} M (such as about 10^{-7} M to about 10^{-12} M, or about 10^{-8} M to about 10^{-12} M). In some embodiments, the anti-PD-1 sdAb moiety is camelid, chimeric, human, partially humanized, or fully humanized.

[0137] In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 48, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 120, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 192, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 48; a CDR2 comprising the amino acid sequence of SEQ ID NO: 120; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 192; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions. In some embodiments, the amino acid substitutions are in CDR1 and/or CDR2. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 48; a CDR2 comprising the amino acid sequence of SEQ ID NO: 120; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 192. In some embodiments, the K_d of the binding between the anti-PD-1 sdAb moiety and PD-1 is about 10^{-5} M to about 10^{-12} M (such as about 10^{-7} M to about 10^{-12} M, or about 10^{-8} M to about 10^{-12} M). In some embodiments, the anti-PD-1 sdAb moiety is camelid, chimeric, human, partially humanized, or fully humanized.

[0138] In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 49, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 121, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 193, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 49; a CDR2 comprising the amino acid sequence of SEQ ID NO: 121; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 193; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions. In some embodiments, the amino acid substitutions are in CDR1 and/or CDR2. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 49; a CDR2 comprising the amino acid sequence of SEQ ID NO: 121; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 193. In some embodiments, the K_d of the binding between the anti-PD-1 sdAb moiety and PD-1 is about 10^{-5} M to about 10^{-12} M (such as about 10^{-7} M to about 10^{-12} M, or about 10^{-8} M to about 10^{-12} M). In some embodiments, the anti-PD-1 sdAb moiety is camelid, chimeric, human, partially humanized, or fully humanized.

[0139] In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 50, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 122, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 194, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 50; a CDR2 comprising the amino acid sequence of SEQ ID NO: 122; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 194; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions. In some embodiments, the amino acid substitutions are in CDR1 and/or CDR2. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 50; a CDR2 comprising the amino acid sequence of SEQ ID NO: 122; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 194. In some embodiments, the K_d of the binding between the anti-PD-1 sdAb moiety and PD-1 is about 10^{-5} M to about 10^{-12} M (such as about 10^{-7} M to about 10^{-12} M, or about 10^{-8} M to about 10^{-12} M). In some embodiments, the anti-PD-1 sdAb moiety is camelid, chimeric, human, partially humanized, or fully humanized.

[0140] In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 51, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 123, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 195, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 51; a CDR2 comprising the amino acid sequence of SEQ ID NO: 123; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 195; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions. In some embodiments, the amino acid substitutions are in CDR1 and/or CDR2. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 51; a CDR2 comprising the amino acid sequence of SEQ ID NO: 123; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 195. In some embodiments, the K_d of the binding between the anti-PD-1 sdAb moiety and PD-1 is about 10^{-5} M to about 10^{-12} M (such as about 10^{-7} M to about 10^{-12} M, or about 10^{-8} M to about 10^{-12} M). In some embodiments, the anti-PD-1 sdAb moiety is camelid, chimeric, human, partially humanized, or fully humanized.

[0141] In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 52, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 124, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 196, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 52; a CDR2 comprising the amino acid sequence of SEQ ID NO: 124; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 196; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions. In some embodiments, the amino acid substitutions are in CDR1 and/or CDR2. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 52; a CDR2 comprising the amino acid sequence of SEQ ID NO: 124; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 196. In some embodiments, the K_d of the binding between the anti-PD-1 sdAb moiety and PD-1 is about 10^{-5} M to about 10^{-12} M (such as about 10^{-7} M to about 10^{-12} M, or about 10^{-8} M to about 10^{-12} M). In some embodiments, the anti-PD-1 sdAb moiety is camelid, chimeric, human, partially humanized, or fully humanized.

[0142] In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 53, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 125, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 197, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 53; a CDR2 comprising the amino acid sequence of SEQ ID NO: 125; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 197; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions. In some embodiments, the amino acid substitutions are in CDR1 and/or CDR2. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 53; a CDR2 comprising the amino acid sequence of SEQ ID NO: 125; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 197. In some embodiments, the K_d of the binding between the anti-PD-1 sdAb moiety and PD-1 is about 10^{-5} M to about 10^{-12} M (such as about 10^{-7} M to about 10^{-12} M, or about 10^{-8} M to about 10^{-12} M). In some embodiments, the anti-PD-1 sdAb moiety is camelid, chimeric, human, partially humanized, or fully humanized.

[0143] In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 54, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 126, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 198, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 54; a CDR2 comprising the amino acid sequence of SEQ ID NO: 126; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 198; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions. In some embodiments, the amino acid substitutions are in CDR1 and/or CDR2. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 54; a CDR2 comprising the amino acid sequence of SEQ ID NO: 126; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 198. In some embodiments, the K_d of the binding between the anti-PD-1 sdAb moiety and PD-1 is about 10^{-5} M to about 10^{-12} M (such as about 10^{-7} M to about 10^{-12} M, or about 10^{-8} M to about 10^{-12} M). In some embodiments, the anti-PD-1 sdAb moiety is camelid, chimeric, human, partially humanized, or fully humanized.

[0144] In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 55, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 127, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 199, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 55; a CDR2 comprising the amino acid sequence of SEQ ID NO: 127; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 199; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions. In some embodiments, the amino acid substitutions are in CDR1 and/or CDR2. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 55; a CDR2 comprising the amino acid sequence of SEQ ID NO: 127; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 199. In some embodiments, the K_d of the binding between the anti-PD-1 sdAb moiety and PD-1 is about 10^{-5} M to about 10^{-12} M (such as about 10^{-7} M to about 10^{-12} M, or about 10^{-8} M to about 10^{-12} M). In some embodiments, the anti-PD-1 sdAb moiety is camelid, chimeric, human, partially humanized, or fully humanized.

[0145] In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 56, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 128, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 200, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 56; a CDR2 comprising the amino acid sequence of SEQ ID NO: 128; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 200; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions. In some embodiments, the amino acid substitutions are in CDR1 and/or CDR2. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 56; a CDR2 comprising the amino acid sequence of SEQ ID NO: 128; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 200. In some embodiments, the K_d of the binding between the anti-PD-1 sdAb moiety and PD-1 is about 10^{-5} M to about 10^{-12} M (such as about 10^{-7} M to about 10^{-12} M, or about 10^{-8} M to about 10^{-12} M). In some embodiments, the anti-PD-1 sdAb moiety is camelid, chimeric, human, partially humanized, or fully humanized.

[0146] In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 57, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 129, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 201, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 57; a CDR2 comprising the amino acid sequence of SEQ ID NO: 129; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 201; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions. In some embodiments, the amino acid substitutions are in CDR1 and/or CDR2. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 57; a CDR2 comprising the amino acid sequence of SEQ ID NO: 129; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 201. In some embodiments, the K_d of the binding between the anti-PD-1 sdAb moiety and PD-1 is about 10^{-5} M to about 10^{-12} M (such as about 10^{-7} M to about 10^{-12} M, or about 10^{-8} M to about 10^{-12} M). In some embodiments, the anti-PD-1 sdAb moiety is camelid, chimeric, human, partially humanized, or fully humanized.

[0147] In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 58, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 130, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 202, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 58; a CDR2 comprising the amino acid sequence of SEQ ID NO: 130; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 202; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions. In some embodiments, the amino acid substitutions are in CDR1 and/or CDR2. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 58; a CDR2 comprising the amino acid sequence of SEQ ID NO: 130; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 202. In some embodiments, the K_d of the binding between the anti-PD-1 sdAb moiety and PD-1 is about 10^{-5} M to about 10^{-12} M (such as about 10^{-7} M to about 10^{-12} M, or about 10^{-8} M to about 10^{-12} M). In some embodiments, the anti-PD-1 sdAb moiety is camelid, chimeric, human, partially humanized, or fully humanized.

[0148] In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 59, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 131, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 203, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 59; a CDR2 comprising the amino acid sequence of SEQ ID NO: 131; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 203; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions. In some embodiments, the amino acid substitutions are in CDR1 and/or CDR2. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 59; a CDR2 comprising the amino acid sequence of SEQ ID NO: 131; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 203. In some embodiments, the K_d of the binding between the anti-PD-1 sdAb moiety and PD-1 is about 10^{-5} M to about 10^{-12} M (such as about 10^{-7} M to about 10^{-12} M, or about 10^{-8} M to about 10^{-12} M). In some embodiments, the anti-PD-1 sdAb moiety is camelid, chimeric, human, partially humanized, or fully humanized.

[0149] In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 60, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 132, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 204, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 60; a CDR2 comprising the amino acid sequence of SEQ ID NO: 132; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 204; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions. In some embodiments, the amino acid substitutions are in CDR1 and/or CDR2. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 60; a CDR2 comprising the amino acid sequence of SEQ ID NO: 132; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 204. In some embodiments, the K_d of the binding between the anti-PD-1 sdAb moiety and PD-1 is about 10^{-5} M to about 10^{-12} M (such as about 10^{-7} M to about 10^{-12} M, or about 10^{-8} M to about 10^{-12} M). In some embodiments, the anti-PD-1 sdAb moiety is camelid, chimeric, human, partially humanized, or fully humanized.

[0150] In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 61, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 133, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 205, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 61; a CDR2 comprising the amino acid sequence of SEQ ID NO: 133; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 205; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions. In some embodiments, the amino acid substitutions are in CDR1 and/or CDR2. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 61; a CDR2 comprising the amino acid sequence of SEQ ID NO: 133; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 205. In some embodiments, the K_d of the binding between the anti-PD-1 sdAb moiety and PD-1 is about 10^{-5} M to about 10^{-12} M (such as about 10^{-7} M to about 10^{-12} M, or about 10^{-8} M to about 10^{-12} M). In some embodiments, the anti-PD-1 sdAb moiety is camelid, chimeric, human, partially humanized, or fully humanized.

[0151] In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 62, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 134, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 206, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 62; a CDR2 comprising the amino acid sequence of SEQ ID NO: 134; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 206; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions. In some embodiments, the amino acid substitutions are in CDR1 and/or CDR2. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 62; a CDR2 comprising the amino acid sequence of SEQ ID NO: 134; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 206. In some embodiments, the K_d of the binding between the anti-PD-1 sdAb moiety and PD-1 is about 10^{-5} M to about 10^{-12} M (such as about 10^{-7} M to about 10^{-12} M, or about 10^{-8} M to about 10^{-12} M). In some embodiments, the anti-PD-1 sdAb moiety is camelid, chimeric, human, partially humanized, or fully humanized.

[0152] In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 63, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 135, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 207, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 63; a CDR2 comprising the amino acid sequence of SEQ ID NO: 135; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 207; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions. In some embodiments, the amino acid substitutions are in CDR1 and/or CDR2. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 63; a CDR2 comprising the amino acid sequence of SEQ ID NO: 135; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 207. In some embodiments, the K_d of the binding between the anti-PD-1 sdAb moiety and PD-1 is about 10^{-5} M to about 10^{-12} M (such as about 10^{-7} M to about 10^{-12} M, or about 10^{-8} M to about 10^{-12} M). In some embodiments, the anti-PD-1 sdAb moiety is camelid, chimeric, human, partially humanized, or fully humanized.

[0153] In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 64, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 136, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 208, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 64; a CDR2 comprising the amino acid sequence of SEQ ID NO: 136; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 208; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions. In some embodiments, the amino acid substitutions are in CDR1 and/or CDR2. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 64; a CDR2 comprising the amino acid sequence of SEQ ID NO: 136; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 208. In some embodiments, the K_d of the binding between the anti-PD-1 sdAb moiety and PD-1 is about 10^{-5} M to about 10^{-12} M (such as about 10^{-7} M to about 10^{-12} M, or about 10^{-8} M to about 10^{-12} M). In some embodiments, the anti-PD-1 sdAb moiety is camelid, chimeric, human, partially humanized, or fully humanized.

[0154] In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 65, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 137, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 209, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 65; a CDR2 comprising the amino acid sequence of SEQ ID NO: 137; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 209; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions. In some embodiments, the amino acid substitutions are in CDR1 and/or CDR2. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 65; a CDR2 comprising the amino acid sequence of SEQ ID NO: 137; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 209. In some embodiments, the K_d of the binding between the anti-PD-1 sdAb moiety and PD-1 is about 10^{-5} M to about 10^{-12} M (such as about 10^{-7} M to about 10^{-12} M, or about 10^{-8} M to about 10^{-12} M). In some embodiments, the anti-PD-1 sdAb moiety is camelid, chimeric, human, partially humanized, or fully humanized.

[0155] In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 66, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 138, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 210, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 66; a CDR2 comprising the amino acid sequence of SEQ ID NO: 138; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 210; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions. In some embodiments, the amino acid substitutions are in CDR1 and/or CDR2. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 66; a CDR2 comprising the amino acid sequence of SEQ ID NO: 138; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 210. In some embodiments, the K_d of the binding between the anti-PD-1 sdAb moiety and PD-1 is about 10^{-5} M to about 10^{-12} M (such as about 10^{-7} M to about 10^{-12} M, or about 10^{-8} M to about 10^{-12} M). In some embodiments, the anti-PD-1 sdAb moiety is camelid, chimeric, human, partially humanized, or fully humanized.

[0156] In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 67, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 139, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 211, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 67; a CDR2 comprising the amino acid sequence of SEQ ID NO: 139; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 211; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions. In some embodiments, the amino acid substitutions are in CDR1 and/or CDR2. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 67; a CDR2 comprising the amino acid sequence of SEQ ID NO: 139; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 211. In some embodiments, the K_d of the binding between the anti-PD-1 sdAb moiety and PD-1 is about 10^{-5} M to about 10^{-12} M (such as about 10^{-7} M to about 10^{-12} M, or about 10^{-8} M to about 10^{-12} M). In some embodiments, the anti-PD-1 sdAb moiety is camelid, chimeric, human, partially humanized, or fully humanized.

[0157] In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 68, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 140, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 212, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 68; a CDR2 comprising the amino acid sequence of SEQ ID NO: 140; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 212; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions. In some embodiments, the amino acid substitutions are in CDR1 and/or CDR2. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 68; a CDR2 comprising the amino acid sequence of SEQ ID NO: 140; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 212. In some embodiments, the K_d of the binding between the anti-PD-1 sdAb moiety and PD-1 is about 10^{-5} M to about 10^{-12} M (such as about 10^{-7} M to about 10^{-12} M, or about 10^{-8} M to about 10^{-12} M). In some embodiments, the anti-PD-1 sdAb moiety is camelid, chimeric, human, partially humanized, or fully humanized.

[0158] In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 69, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 141, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 213, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 69; a CDR2 comprising the amino acid sequence of SEQ ID NO: 141; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 213; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions. In some embodiments, the amino acid substitutions are in CDR1 and/or CDR2. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 69; a CDR2 comprising the amino acid sequence of SEQ ID NO: 141; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 213. In some embodiments, the K_d of the binding between the anti-PD-1 sdAb moiety and PD-1 is about 10^{-5} M to about 10^{-12} M (such as about 10^{-7} M to about 10^{-12} M, or about 10^{-8} M to about 10^{-12} M). In some embodiments, the anti-PD-1 sdAb moiety is camelid, chimeric, human, partially humanized, or fully humanized.

[0159] In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 70, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 142, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 214, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 70; a CDR2 comprising the amino acid sequence of SEQ ID NO: 142; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 214; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions. In some embodiments, the amino acid substitutions are in CDR1 and/or CDR2. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 70; a CDR2 comprising the amino acid sequence of SEQ ID NO: 142; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 214. In some embodiments, the K_d of the binding between the anti-PD-1 sdAb moiety and PD-1 is about 10^{-5} M to about 10^{-12} M (such as about 10^{-7} M to about 10^{-12} M, or about 10^{-8} M to about 10^{-12} M). In some embodiments, the anti-PD-1 sdAb moiety is camelid, chimeric, human, partially humanized, or fully humanized.

[0160] In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 71, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 143, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 215, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 71; a CDR2 comprising the amino acid sequence of SEQ ID NO: 143; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 215; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions. In some embodiments, the amino acid substitutions are in CDR1 and/or CDR2. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 71; a CDR2 comprising the amino acid sequence of SEQ ID NO: 143; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 215. In some embodiments, the K_d of the binding between the anti-PD-1 sdAb moiety and PD-1 is about 10^{-5} M to about 10^{-12} M (such as about 10^{-7} M to about 10^{-12} M, or about 10^{-8} M to about 10^{-12} M). In some embodiments, the anti-PD-1 sdAb moiety is camelid, chimeric, human, partially humanized, or fully humanized.

[0161] In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 72, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 144, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 216, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 72; a CDR2 comprising the amino acid sequence of SEQ ID NO: 144; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 216; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions. In some embodiments, the amino acid substitutions are in CDR1 and/or CDR2. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 72; a CDR2 comprising the amino acid sequence of SEQ ID NO: 144; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 216. In some embodiments, the K_d of the binding between the anti-PD-1 sdAb moiety and PD-1 is about 10^{-5} M to about 10^{-12} M (such as about 10^{-7} M to about 10^{-12} M, or about 10^{-8} M to about 10^{-12} M). In some embodiments, the anti-PD-1 sdAb moiety is camelid, chimeric, human, partially humanized, or fully humanized.

[0162] The anti-PD-1 sdAb moiety may comprise one or more "hallmark residues" in one or more of the FR sequences. In some embodiments, the anti-PD-1 sdAb moiety comprises a V_HH domain comprising the amino acid sequence of any one of the following: a-1) the amino acid residue at position 37 is selected from the group consisting of F, Y, L, I, and V (such as F, Y or V, or such as F); a-2) the amino acid residue at position 44 is selected from the group consisting of A, G, E, D, G, Q, R, S, and L (such as E, Q, or G, or such as E); a-3) the amino acid residue at position 45 is selected from the group consisting of L, R, and C (such as L or R); a-4) the amino acid residue at position 103 is selected from the group consisting of W, R, G, and S (such as W, G, or R, or such as W); and a-5) the amino acid residue at position 108 is Q; or b-1) the amino acid residue at position 37 is selected from the group consisting of F, Y, L, I, and V (such as F, V or Y, or such as F); b-2) the amino acid residue at position 44 is selected from the group consisting of E, Q, and G; b-3) the amino acid residue at position 45 is R; b-4) the amino acid residue at position 103 is selected from the group consisting of W, R, and S (such as W); and b-5) the amino acid residue at position 108 is selected from the group consisting of Q and L (such as Q); wherein the amino acid position is according to Kabat numbering. It should be noted that these "hallmark residues" at amino acid positions 37, 44, 45, 103 and 108 according to Kabat numbering apply to anti-PD-1 sdAb moieties of natural V_HH sequences, and can be substituted during humanization. For example, Q at amino acid position 108 according to Kabat numbering can be optionally humanized to L. Other humanized substitutions will be clear to those skilled in the art. For example, potentially useful humanizing substitutions can be determined by comparing the FR sequences of a naturally occurring V_HH with the corresponding FR sequences of one or more closely related human V_H, then introducing one or more of such potentially useful humanizing substitutions into said V_HH using methods known in the art (also as described herein). The resulting humanized V_HH sequences can be tested for their PD-1 binding affinity, for stability, for ease and level of expression, and/or for other desired properties. Possible residue substitutions may also come from an antibody V_H domain wherein the V_H/V_L interface comprises one or more highly charged amino acid residues. The anti-PD-1 sdAb moiety described herein can be partially or fully humanized. Preferably, the resulting humanized anti-PD-1 sdAb binds to PD-1 with K_d, K_{on}, and K_{off} as described herein.

[0163] In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a V_HH domain comprising the amino acid sequence of any one of SEQ ID NOs: 289-324, or a variant thereof having at least about 80% (such as at least about any of 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) sequence identify to any one of SEQ ID NOs: 289-324. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a V_HH domain comprising the amino acid sequence of any one of SEQ ID NOs: 289-324, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the V_HH domain. In some embodiments, the anti-PD-1 sdAb moiety

comprising the V_HH domain comprising the amino acid sequence of any one of SEQ ID NOs: 289-324, or a variant thereof comprises amino acid substitutions in CDRs, such as the CDR1, and/or the CDR2, and/or the CDR3 of any one of SEQ ID NOs: 289-324. In some embodiments, the anti-PD-1 sdAb moiety comprising the V_HH domain comprising the amino acid sequence of any one of SEQ ID NOs: 289-324, or a variant thereof comprises CDR1, CDR2, and CDR3 of any one of SEQ ID NOs: 289-324, and the amino acid substitutions are in FRs, such as the FR1, and/or the FR2, and/or the FR3, and/or the FR4 of any one of SEQ ID NOs: 289-324. In some embodiments, the anti-PD-1 sdAb moiety comprising the V_HH domain comprising the amino acid sequence of any one of SEQ ID NOs: 289-324, or a variant thereof comprises amino acid substitutions in both CDRs and FRs. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising a V_HH domain comprising the amino acid sequence of any one of SEQ ID NOs: 289-324. In some embodiments, there is provided an anti-PD-1 sdAb moiety comprising CDR1, CDR2, and CDR3 of any one of SEQ ID NO: 289-324. In some embodiments, the K_d of the binding between the anti-PD-1 sdAb moiety and PD-1 is about 10^{-5} M to about 10^{-12} M (such as about 10^{-7} M to about 10^{-12} M, or about 10^{-8} M to about 10^{-12} M). In some embodiments, the anti-PD-1 sdAb moiety is camelid, chimeric, human, partially humanized, or fully humanized.

[0164] In some embodiments, there is provided an anti-PD-1 sdAb moiety (hereinafter referred to as "competing anti-PD-1 sdAb moiety" or "competing anti-PD-1 sdAb") or anti-PD-1 construct comprising an anti-PD-1 sdAb moiety (hereinafter referred to as "competing anti-PD-1 construct") that specifically binds to PD-1 competitively with any one of the anti-PD-1 sdAb moiety described herein. In some embodiments, competitive binding may be determined using an ELISA assay. In some embodiments, there is provided an anti-PD-1 sdAb moiety (or an anti-PD-1 construct comprising an anti-PD-1 sdAb moiety) that specifically binds to PD-1 competitively with an anti-PD-1 sdAb moiety comprising the amino acid sequence of any one of SEQ ID NOs: 289-324. In some embodiments, there is provided an anti-PD-1 sdAb moiety (or an anti-PD-1 construct comprising an anti-PD-1 sdAb moiety) that specifically binds to PD-1 competitively with an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 37-72; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 109-144; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 181-216. In some embodiments, the K_d of the binding between the competing anti-PD-1 sdAb moiety and PD-1 is about 10^{-5} M to about 10^{-12} M (such as about 10^{-7} M to about 10^{-12} M, or about 10^{-8} M to about 10^{-12} M). In some embodiments, the competing anti-PD-1 sdAb moiety is camelid, chimeric, human, partially humanized, or fully humanized.

Single-domain antibodies

[0165] Exemplary sdAbs include, but are not limited to, heavy chain variable domains from heavy-chain only antibodies (*e.g.*, V_HH (Variable domain of the heavy chain of the Heavy chain antibody) in *Camelidae* or V_{NAR} (Variable domain of the shark New Antigen Receptor) in cartilaginous fish), binding molecules naturally devoid of light chains, single domains (such as V_H or V_L) derived from conventional 4-chain antibodies, humanized heavy-chain only antibodies, human single-domain antibodies produced by transgenic mice or rats expressing human heavy chain segments, and engineered domains and single domain scaffolds other than those derived from antibodies. The sdAbs may be derived from any species including, but not limited to mouse, rat, human, camel, llama, lamprey, fish, shark, goat, rabbit, and bovine. sdAbs contemplated herein also include naturally occurring sdAb molecules from species other than *Camelidae* and sharks.

[0166] In some embodiments, the sdAb is derived from a naturally occurring single-domain antigen binding molecule known as heavy chain antibody devoid of light chains (also referred herein as "heavy chain-only antibodies", or "HCAb"). Such single domain molecules are disclosed in WO 94/04678 and Hamers-Casterman, C. *et al.* (1993) *Nature* 363:446-448, for example. For clarity reasons, the variable domain derived from a heavy chain molecule naturally devoid of light chain is known herein as a V_HH to distinguish it from the conventional V_H of four chain immunoglobulins. Such a V_HH molecule can be derived from antibodies raised in *Camelidae* species, for example, camel, llama, vicuna, dromedary, alpaca and guanaco. Other species besides Camelidae may produce heavy chain molecules naturally devoid of light chain, and such V_HHs are within the scope of the present application.

[0167] In some embodiments, the sdAb is derived from a variable region of the immunoglobulin found in cartilaginous fish. For example, the sdAb can be derived from the immunoglobulin isotype known as Novel Antigen Receptor (NAR) found in the serum of shark. Methods of producing single domain molecules derived from a variable region of NAR ("IgNARs") are described in WO 03/014161 and Streltsov (2005) *Protein Sci.* 14:2901-2909.

[0168] In some embodiments, the sdAb is recombinant, CDR-grafted, humanized, camelized, de-immunized and/or *in vitro* generated (*e.g.*, selected by phage display). In some embodiments, the amino acid sequence of the framework regions may be altered by "camelization" of specific amino acid residues in the framework regions. Camelization refers to the replacing or substitution of one or more amino acid residues in the amino acid sequence of a (naturally occurring) V_H domain from a conventional 4-chain antibody by one or more of the amino acid residues that occur at the corresponding position(s) in a V_HH domain of a heavy chain antibody. This can be performed in a manner known *per se*, which will be clear to the skilled person, for example on the basis of the further description herein. Such "camelizing"

substitutions are preferably inserted at amino acid positions that form and/or are present at the V_H-V_L interface, and/or at the so-called Camelidae hallmark residues, as defined herein (*see* for example WO 94/04678, Davies and Riechmann FEBS Letters 339: 285-290, 1994; Davies and Riechmann Protein Engineering 9 (6): 531-537, 1996; Riechmann J. Mol. Biol. 259: 957-969, 1996; and Riechmann and Muyldermans J. Immunol. Meth. 231: 25-38, 1999).

[0169] In some embodiments, the sdAb is a human sdAb produced by transgenic mice or rats expressing human heavy chain segments. See, *e.g.*, US20090307787A1, U.S. Pat. No. 8,754,287, US20150289489A1, US20100122358A1, and WO2004049794. In some embodiments, the sdAb is affinity-matured.

[0170] In some embodiments, naturally occurring V_HH domains against a particular antigen or target, can be obtained from (naïve or immune) libraries of Camelid V_HH sequences. Such methods may or may not involve screening such a library using said antigen or target, or at least one part, fragment, antigenic determinant or epitope thereof using one or more screening techniques known *per se*. Such libraries and techniques are for example described in WO 99/37681, WO 01/90190, WO 03/025020 and WO 03/035694. Alternatively, improved synthetic or semi-synthetic libraries derived from (naïve or immune) V_HH libraries may be used, such as V_HH libraries obtained from (naïve or immune) V_HH libraries by techniques such as random mutagenesis and/or CDR shuffling, as for example described in WO 00/43507.

[0171] In some embodiments, the sdAbs are generated from conventional 4-chain antibodies. *See*, for example, EP 0 368 684, Ward *et al.* (Nature 1989 Oct. 12; 341 (6242): 544-6), Holt *et al.*, Trends Biotechnol., 2003, 21(11):484-490; WO 06/030220; and WO 06/003388.

[0172] Because of the unique properties of sdAbs, using V_HH domains as single antigen-binding proteins or as antigen-binding domains (*i.e.* as part of a larger protein or polypeptide) offers a number of significant advantages over the conventional V_H and V_L, scFv and conventional antibody fragments (such as Fab or $(Fab')_2$): 1) only a single domain is required to bind an antigen with high affinity, so there is no need to have a second domain, nor to assure that these two domains are present in the correct spatial conformation and configuration (*e.g.* no need to pair the heavy chain and light chain during folding, no need to use a specially designed linker such as for scFv); 2) V_HH domains and other sdAbs can be expressed from a single gene and require no post-translational folding or modifications; 3) V_HH domains and other sdAbs can be easily engineered into multivalent and/or multispecific formats (such as those described in the present application); 4) V_HH domains and other sdAbs are highly soluble and do not have a tendency to aggregate (as with the mouse-derived "dAbs" described by Ward *et al.*, Nature. 1989 Oct 12;341(6242):544-6); 5) V_HH domains and other sdAbs are highly stable against heat, pH, proteases and other denaturing agents or conditions; 6) V_HH domains and other sdAbs are easy and relatively cheap to

prepare (even on a large production scale), such as using microbial fermentation, there is no need to use mammalian expression system (required by production of, for example, conventional antibody fragments); 7) V_HH domains and other sdAbs are relatively small (approximately 15 kDa, or 10 times smaller than a conventional IgG) compared to conventional 4-chain antibodies and antigen-binding fragments thereof, thus have high(er) tissue penetration ability, such as for solid tumors and other dense tissues; and 8) V_HH domains and other sdAbs can exhibit so-called "cavity-binding properties" (due to their extended CDR3 loop compared to that of conventional V_H domains) and can therefore access targets and epitopes not accessible to conventional 4-chain antibodies and antigen-binding fragments thereof, for example, it has been shown that V_HH domains and other sdAbs can inhibit enzymes (*see* for example WO1997049805; Transue *et al.*, Proteins. 1998 Sep 1;32(4):515-22; Lauwereys *et al.*, EMBO J. 1998 Jul 1;17(13):3512-20).

PD-1

[0173] The terms "programmed cell death protein 1", "PD-1", "PD-1 antigen", "PD-1 epitope", and "Programmed Death 1" are used interchangeably, and include variants, isoforms, species homologs of human PD-1, and analogs having at least one common epitope with PD-1.

[0174] The amino acid sequence of human PD-1 is disclosed at Genbank Accession Number NP_005009. The region of amino acids 1-20 is the leader peptide; 21-170 is the extracellular domain; 171-191 is the transmembrane domain; and 192-288 is the cytoplasmic domain.

[0175] A particular human PD-1 sequence will generally be at least 90% identical in amino acids sequence to human PD-1 of Genbank Accession Number NP_005009 and contains amino acid residues that identify the amino acid sequence as being human when compared to PD-1 amino acid sequences of other species (*e.g.*, murine). In some embodiments, a human PD-1 may be at least about 95%, 96%, 97%, 98%, or 99% identical in amino acid sequence to the human PD-1 of Genbank Accession Number NP_005009. In some embodiments, a human PD-1 sequence will display no more than 10 amino acid differences from the human PD-1 of Genbank Accession Number NP_005009. In some embodiments, the human PD-1 may display no more than 5, 4, 3, 2, or 1 amino acid difference from the human PD-1 of Genbank Accession Number NP_005009. Percent identity can be determined as described herein. In some embodiments, a human PD-1 sequence may differ from the human PD-1 of Genbank Accession Number NP_005009 by having, for example, conserved mutations or mutations in non-conserved regions and the PD-1 has substantially the same biological function as the human PD-1 of Genbank Accession Number NP_005009. For example, a biological function of human PD-1 is having an epitope in the extracellular domain of PD-1 that is specifically bound by an anti-PD-1 construct of the instant disclosure or a biological function of human PD-1 is modulation of T cell activity. In some embodiments, the anti-PD-1 sdAb moiety described herein specifically recognizes a PD-1

polypeptide with 100% amino acid sequence identity to the human PD-1 of Genbank Accession Number NP_005009. In some embodiments, the anti-PD-1 sdAb moiety described herein specifically recognizes a PD-1 polypeptide comprising an amino acid sequence of SEQ ID NO: 361 or 362.

[0176] In some embodiments, the anti-PD-1 sdAb moiety may cross-react with PD-1 from species other than human, or other proteins which are structurally related to human PD-1 (e.g., human PD-1 homologs). In some embodiments, the anti-PD-1 sdAb moiety is completely specific for human PD-1 and not exhibit species or other types of cross-reactivity. In some embodiments, the anti-PD-1 sdAb moiety specifically recognizes a soluble isoform of human PD-1. In some embodiments, the anti-PD-1 sdAb moiety specifically recognizes a membrane-bound isoform of human PD-1 (e.g., SEQ ID NO: 361).

[0177] In some embodiments, the anti-PD-1 sdAb moiety described herein specifically recognizes the extracellular domain (ECD) of PD-1. In some embodiments, the anti-PD-1 sdAb moiety specifically recognizes the N-terminal portion of the PD-1 ECD. In some embodiments, the anti-PD-1 sdAb moiety specifically recognizes the C-terminal portion of the PD-1 ECD. In some embodiments, the anti-PD-1 sdAb moiety specifically recognizes the middle portion of the PD-1 ECD. In some embodiments, the ECD of PD-1 specifically recognized by the anti-PD-1 sdAb moiety is at least about 95%, 96%, 97%, 98%, or 99% identical in amino acid sequence to the ECD of the human PD-1 of Genbank Accession Number NP_005009. In some embodiments, the ECD of PD-1 specifically recognized by the anti-PD-1 sdAb moiety is 100% identical in amino acid sequence to the ECD of the human PD-1 of Genbank Accession Number NP_005009. In some embodiments, the anti-PD-1 sdAb moiety specifically recognizes a PD-1 polypeptide comprising an amino acid sequence of SEQ ID NO: 362.

Antibody affinity

[0178] Binding specificity of the antibody or antigen-binding domain can be determined experimentally by methods known in the art. Such methods comprise, but are not limited to Western blots, ELISA-, RIA-, ECL-, IRMA-, EIA-, BIAcore-tests and peptide scans.

[0179] In some embodiments, the K_d of the binding between the anti-PD-1 sdAb moiety and PD-1 is about 10^{-5} M to about 10^{-6} M, about 10^{-6} M to about 10^{-7} M, about 10^{-7} M to about 10^{-8} M, about 10^{-8} M to about 10^{-9} M, about 10^{-9} M to about 10^{-10} M, about 10^{-10} M to about 10^{-11} M, about 10^{-11} M to about 10^{-12} M, about 10^{-5} M to about 10^{-12} M, about 10^{-6} M to about 10^{-12} M, about 10^{-7} M to about 10^{-12} M, about 10^{-8} M to about 10^{-12} M, about 10^{-9} M to about 10^{-12} M, about 10^{-10} M to about 10^{-12} M, about 10^{-5} M to about 10^{-11} M, about 10^{-7} M to about 10^{-11} M, about 10^{-8} M to about 10^{-11} M, about 10^{-9} M to about 10^{-11} M, about 10^{-5} M to about 10^{-10} M, about 10^{-7} M to about 10^{-10} M, about 10^{-8} M to about 10^{-10} M, about 10^{-5} M

to about 10^{-9} M, about 10^{-7} M to about 10^{-9} M, about 10^{-5} M to about 10^{-8} M, or about 10^{-6} M to about 10^{-8} M.

[0180] In some embodiments, the K_{on} of the binding between the anti-PD-1 sdAb moiety and PD-1 is about 10^{2} $M^{-1}s^{-1}$ to about 10^{4} $M^{-1}s^{-1}$, about 10^{4} $M^{-1}s^{-1}$ to about 10^{6} $M^{-1}s^{-1}$, about 10^{6} $M^{-1}s^{-1}$ to about 10^{7} $M^{-1}s^{-1}$, about 10^{2} $M^{-1}s^{-1}$ to about 10^{7} $M^{-1}s^{-1}$, about 10^{3} $M^{-1}s^{-1}$ to about 10^{7} $M^{-1}s^{-1}$, about 10^{4} $M^{-1}s^{-1}$ to about 10^{7} $M^{-1}s^{-1}$, about 10^{5} $M^{-1}s^{-1}$ to about 10^{7} $M^{-1}s^{-1}$, about 10^{3} $M^{-1}s^{-1}$ to about 10^{6} $M^{-1}s^{-1}$, or about 10^{4} $M^{-1}s^{-1}$ to about 10^{6} $M^{-1}s^{-1}$.

[0181] In some embodiments, the K_{off} of the binding between the anti-PD-1 sdAb moiety and PD-1 is about 1 s^{-1} to about 10^{-2} s^{-1}, about 10^{-2} s^{-1} to about 10^{-4} s^{-1}, about 10^{-4} s^{-1} to about 10^{-5} s^{-1}, about 10^{-5} s^{-1} to about 10^{-6} s^{-1}, about 1 s^{-1} to about 10^{-6} s^{-1}, about 10^{-2} s^{-1} to about 10^{-6} s^{-1}, about 10^{-3} s^{-1} to about 10^{-6} s^{-1}, about 10^{-4} s^{-1} to about 10^{-6} s^{-1}, about 10^{-2} s^{-1} to about 10^{-5} s^{-1}, or about 10^{-3} s^{-1} to about 10^{-5} s^{-1}.

[0182] In some embodiments, the EC_{50} of the anti-PD-1 sdAb moiety is less than 10 nM in an amplified luminescent proximity homogeneous assay (AlphaLISA). In some embodiments, the EC_{50} of the anti-PD-1 sdAb moiety is less than 500 nM in an inhibition of ligand binding by FACS analysis (competition binding assay), or cell based cytokine release assay. In some embodiments, the EC_{50} of the anti-PD-1 sdAb moiety is less than 1 nM (such as about 0.001 nM to about 0.01 nM, about 0.01 nM to about 0.1 nM, about 0.1 nM to about 1 nM, *etc.*), about 1 nM to about 10 nM, about 10 nM to about 50 nM, about 50 nM to about 100 nM, about 100 nM to about 200 nM, about 200 nM to about 300 nM, about 300 nM to about 400 nM, or about 400 nM to about 500 nM.

[0183] In some embodiments, the IC_{50} of the anti-PD-1 sdAb moiety is less than 10 nM in an AlphaLISA. In some embodiments, the IC_{50} of the anti-PD-1 sdAb moiety is less than 500 nM in an inhibition of ligand binding by FACS analysis (competition binding assay), or cell based cytokine release assay. In some embodiments, the IC_{50} of the anti-PD-1 sdAb moiety is less than 1 nM (such as about 0.001 nM to about 0.01 nM, about 0.01 nM to about 0.1 nM, about 0.1 nM to about 1 nM, *etc.*), about 1 nM to about 10 nM, about 10 nM to about 50 nM, about 50 nM to about 100 nM, about 100 nM to about 200 nM, about 200 nM to about 300 nM, about 300 nM to about 400 nM, or about 400 nM to about 500 nM.

Chimeric or humanized antibodies

[0184] In some embodiments, the anti-PD-1 sdAb moiety provided herein is a chimeric antibody. Certain chimeric antibodies are described, *e.g.*, in U.S. Patent No. 4,816,567; and Morrison et al., *Proc. Natl. Acad. Sci. USA*, 81:6851-6855 (1984)). In one example, a chimeric antibody comprises a non-human variable region (*e.g.*, a variable region derived from a camelid species, such as llama) and a human

constant region. In a further example, a chimeric antibody is a "class switched" antibody in which the class or subclass has been changed from that of the parent antibody. Chimeric antibodies include antigen-binding fragments thereof.

[0185] In some embodiments, a chimeric antibody is a humanized antibody. Typically, a non-human antibody is humanized to reduce immunogenicity to humans, while retaining the specificity and affinity of the parental non-human antibody. Generally, a humanized antibody comprises one or more variable domains in which HVRs, e.g., CDRs, (or portions thereof) are derived from a non-human antibody, and FRs (or portions thereof) are derived from human antibody sequences. A humanized antibody optionally will also comprise at least a portion of a human constant region. In some embodiments, some FR residues in a humanized antibody are substituted with corresponding residues from a non-human antibody (*e.g.*, the antibody from which the HVR residues are derived), *e.g.*, to restore or improve antibody specificity or affinity.

[0186] Humanized antibodies and methods of making them are reviewed, *e.g.*, in Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008), and are further described, e.g., in Riechmann et al., *Nature* 332:323-329 (1988); Queen et al., *Proc. Nat'l Acad. Sci. USA* 86:10029-10033 (1989); US Patent Nos. 5, 821,337, 7,527,791, 6,982,321, and 7,087,409; Kashmiri *et al.*, *Methods* 36:25-34 (2005) (describing SDR (a-CDR) grafting); Padlan, *Mol. Immunol.* 28:489-498 (1991) (describing "resurfacing"); Dall'Acqua et al., *Methods* 36:43-60 (2005) (describing "FR shuffling"); and Osbourn et al., *Methods* 36:61-68 (2005) and Klimka et al., *Br. J. Cancer*, 83:252-260 (2000) (describing the "guided selection" approach to FR shuffling).

[0187] Human framework regions that may be used for humanization include but are not limited to: framework regions selected using the "best-fit" method (*see, e.g.*, Sims et al. *J. Immunol.* 151:2296 (1993)); framework regions derived from the consensus sequence of human antibodies of a particular subgroup of light or heavy chain variable regions (see, e.g., Carter et al. *Proc. Natl. Acad. Sci. USA*, 89:4285 (1992); and Presta et al. *J. Immunol.*, 151:2623 (1993)); human mature (somatically mutated) framework regions or human germline framework regions (see, e.g., Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008)); and framework regions derived from screening FR libraries (see, e.g., Baca et al., *J. Biol. Chem.* 272:10678-10684 (1997) and Rosok et al., *J. Biol. Chem.* 271:22611-22618 (1996)).

[0188] In some embodiments, the anti-PD-1 sdAbs are modified, such as humanized, without diminishing the native affinity of the domain for antigen and while reducing its immunogenicity with respect to a heterologous species. For example, the amino acid residues of the antibody variable domain (V_HH) of an llama antibody can be determined, and one or more of the Camelid amino acids, for example, in the framework regions, are replaced by their human counterpart as found in the human consensus

sequence, without that polypeptide losing its typical character, *i.e.* the humanization does not significantly affect the antigen binding capacity of the resulting polypeptide. Humanization of Camelid single-domain antibodies requires the introduction and mutagenesis of a limited amount of amino acids in a single polypeptide chain. This is in contrast to humanization of scFv, Fab', $(Fab')_2$ and IgG, which requires the introduction of amino acid changes in two chains, the light and the heavy chain and the preservation of the assembly of both chains.

[0189] sdAbs comprising a V_HH domain can be humanized to have human-like sequences. In some embodiments, the FR regions of the V_HH domain used herein comprise at least about any one of 50%, 60%, 70%, 80%, 90%, 95% or more of amino acid sequence homology to human VH framework regions. One exemplary class of humanized V_HH domains is characterized in that the V_HHs carry an amino acid from the group consisting of glycine, alanine, valine, leucine, isoleucine, proline, phenylalanine, tyrosine, tryptophan, methionine, serine, threonine, asparagine, or glutamine at position 45, such as, for example, L45 and a tryptophan at position 103, according to the Kabat numbering. As such, polypeptides belonging to this class show a high amino acid sequence homology to human VH framework regions and said polypeptides might be administered to a human directly without expectation of an unwanted immune response therefrom, and without the burden of further humanization.

[0190] Another exemplary class of humanized Camelid single-domain antibodies has been described in WO 03/035694 and contains hydrophobic FR2 residues typically found in conventional antibodies of human origin or from other species, but compensating this loss in hydrophilicity by the charged arginine residue on position 103 that substitutes the conserved tryptophan residue present in V_H from double-chain antibodies. As such, peptides belonging to these two classes show a high amino acid sequence homology to human V_H framework regions and said peptides might be administered to a human directly without expectation of an unwanted immune response therefrom, and without the burden of further humanization.

Human antibodies

[0191] In some embodiments, the anti-PD-1 sdAb moiety provided herein is a human antibody (known as human domain antibody, or human DAb). Human antibodies can be produced using various techniques known in the art. Human antibodies are described generally in van Dijk and van de Winkel, *Curr. Opin. Pharmacol.* 5: 368-74 (2001), Lonberg, *Curr. Opin. Immunol.* 20:450-459 (2008), and Chen, *Mol. Immunol.* 47(4):912-21 (2010). Transgenic mice or rats capable of producing fully human single-domain antibodies (or DAb) are known in the art. See, *e.g.,* US20090307787A1, U.S. Pat. No. 8,754,287, US20150289489A1, US20100122358A1, and WO2004049794.

[0192] Human antibodies (e.g., human DAbs) may be prepared by administering an immunogen to a transgenic animal that has been modified to produce intact human antibodies or intact antibodies with human variable regions in response to antigenic challenge. Such animals typically contain all or a portion of the human immunoglobulin loci, which replace the endogenous immunoglobulin loci, or which are present extrachromosomally or integrated randomly into the animal's chromosomes. In such transgenic mice, the endogenous immunoglobulin loci have generally been inactivated. For review of methods for obtaining human antibodies from transgenic animals, see Lonberg, *Nat. Biotech.* 23:1117-1125 (2005). *See also, e.g.*, U.S. Patent Nos. 6,075,181 and 6,150,584 describing XENOMOUSE™ technology; U.S. Patent No. 5,770,429 describing HUMAB® technology; U.S. Patent No. 7,041,870 describing K-M MOUSE® technology, and U.S. Patent Application Publication No. US 2007/0061900, describing VELOCIMOUSE® technology). Human variable regions from intact antibodies generated by such animals may be further modified, *e.g.*, by combining with a different human constant region.

[0193] Human antibodies (e.g., human DAbs) can also be made by hybridoma-based methods. Human myeloma and mouse-human heteromyeloma cell lines for the production of human monoclonal antibodies have been described (*See, e.g.*, Kozbor *J. Immunol.*, 133: 3001 (1984); Brodeur et al., *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York, 1987); and Boerner et al., *J. Immunol.*, 147: 86 (1991)). Human antibodies generated via human B-cell hybridoma technology are also described in Li et al., *Proc. Natl. Acad. Sci. USA*, 103:3557-3562 (2006). Additional methods include those described, for example, in U.S. Patent No. 7,189,826 (describing production of monoclonal human IgM antibodies from hybridoma cell lines) and Ni, *Xiandai Mianyixue*, 26(4):265-268 (2006) (describing human-human hybridomas). Human hybridoma technology (Trioma technology) is also described in Vollmers and Brandlein, *Histology and Histopathology*, 20(3):927-937 (2005) and Vollmers and Brandlein, *Methods and Findings in Experimental and Clinical Pharmacology*, 27(3):185-91 (2005).

[0194] Human antibodies (e.g., human DAbs) may also be generated by isolating Fv clone variable domain sequences selected from human-derived phage display libraries. Such variable domain sequences may then be combined with a desired human constant domain. Techniques for selecting human antibodies from antibody libraries are described below.

[0195] One technique for obtaining V_HH sequences directed against a particular antigen or target involves suitably immunizing a transgenic mammal that is capable of expressing heavy chain antibodies (*i.e.* so as to raise an immune response and/or heavy chain antibodies directed against said antigen or target), obtaining a suitable biological sample from said transgenic mammal that contains (nucleic acid sequences encoding) said V_HH sequences (such as a blood sample, serum sample or sample of B-cells),

and then generating V_HH sequences directed against said antigen or target, starting from said sample, using any suitable technique known *per se* (such as any of the methods described herein or a hybridoma technique). For example, for this purpose, the heavy chain antibody-expressing mice and the further methods and techniques described in WO 02/085945, WO 04/049794 and WO 06/008548 and Janssens *et al.*, Proc. Natl. Acad. Sci. USA. 2006 Oct. 10; 103(41):15130-5 can be used. For example, such heavy chain antibody expressing mice can express heavy chain antibodies with any suitable (single) variable domain, such as (single) variable domains from natural sources (*e.g.* human (single) variable domains, Camelid (single) variable domains or shark (single) variable domains), as well as for example synthetic or semi-synthetic (single) variable domains.

Library-derived antibodies

[0196] Antibodies of the present application may be isolated by screening combinatorial libraries for antibodies with the desired activity or activities. For example, a variety of methods are known in the art for generating phage display libraries and screening such libraries for antibodies possessing the desired binding characteristics. Such methods are reviewed, *e.g.*, in Hoogenboom et al. in *Methods in Molecular Biology* 178:1-37 (O'Brien et al., ed., Human Press, Totowa, NJ, 2001) and further described, *e.g.*, in the McCafferty et al., *Nature* 348:552-554; Clackson et al., *Nature* 352: 624-628 (1991); Marks et al., *J. Mol. Biol.* 222: 581-597 (1992); Marks and Bradbury, in *Methods in Molecular Biology* 248:161-175 (Lo, ed., Human Press, Totowa, NJ, 2003); Sidhu et al., *J. Mol. Biol.* 338(2): 299-310 (2004); Lee et al., *J. Mol. Biol.* 340(5): 1073-1093 (2004); Fellouse, *Proc. Natl. Acad. Sci. USA* 101(34): 12467-12472 (2004); and Lee et al., *J. Immunol. Methods* 284(1-2): 119-132(2004). Methods for constructing single-domain antibody libraries have been described, for example, see U.S. Pat. NO. 7371849.

[0197] In certain phage display methods, repertoires of V_H and V_L genes are separately cloned by polymerase chain reaction (PCR) and recombined randomly in phage libraries, which can then be screened for antigen-binding phage as described in Winter et al., *Ann. Rev. Immunol.*, 12: 433-455 (1994). Repertoires of V_HH genes can be similarly cloned by PCR, recombined randomly in phage libraries, and screened for antigen-binding phage. Phage typically display antibody fragments, either as scFv fragments or as Fab fragments. Libraries from immunized sources provide high-affinity antibodies to the immunogen without the requirement of constructing hybridomas. Alternatively, the naive repertoire can be cloned (*e.g.*, from human) to provide a single source of antibodies to a wide range of non-self and also self-antigens without any immunization as described by Griffiths et al., *EMBO J,* 12: 725-734 (1993). Finally, naive libraries can also be made synthetically by cloning unrearranged V-gene segments from stem cells, and using PCR primers containing random sequence to encode the highly variable CDR3 regions and to accomplish rearrangement *in vitro*, as described by Hoogenboom and Winter, *J. Mol. Biol.*,

227: 381-388 (1992). Patent publications describing human antibody phage libraries include, for example: US Patent No. 5,750,373, and US Patent Publication Nos. 2005/0079574, 2005/0119455, 2005/0266000, 2007/0117126, 2007/0160598, 2007/0237764, 2007/0292936, and 2009/0002360.

[0198] Antibodies or antibody fragments isolated from human antibody libraries are considered human antibodies or human antibody fragments herein.

Biological activities

[0199] The biological activity of anti-PD-1 sdAb moiety described herein can be determined by measuring its half maximal effective concentration (EC_{50}), which is a measure of the effectiveness of an antibody in binding to its target, or half maximal inhibitory concentration (IC_{50}), which is a measure of the effectiveness of an antibody in inhibiting a specific biological or biochemical function (such as inhibiting the binding between PD-1 and PD-L1/PD-L2). For example, here EC_{50} can be used to indicate the effective concentration of anti-PD-1 sdAb needed to bind 50% PD-1 on cell surface, IC_{50} can be used to indicate the effective concentration of anti-PD-1 sdAb needed to neutralize 50% of PD-1 bioactivity *in vitro*. EC_{50} also represents the plasma concentration required for obtaining 50% of a maximum effect *in vivo*. EC_{50} or IC_{50} can be measured by assays known in the art, for example, bioassays such as FACS binding analysis, inhibition of ligand binding by FACS analysis (competition binding assay), cell-based cytokine release assay, or amplified luminescent proximity homogeneous assay (AlphaLISA).

[0200] For example, the blockade of ligand binding can be studied using flow cytometry (also *see* Example 1). CHO cells expressing human PD-1 can be dissociated from adherent culture flasks and mixed with varying concentrations of anti-PD-1 sdAb for test, and a constant concentration of labeled-PD-L1 protein or labeled-PD-L2 protein (such as biotin-labeled human PD-L1-Fc protein or biotin-labeled human PD-L2-Fc protein). An anti-PD-1 antibody positive control can be employed, such as Keytruda®. The mixture is equilibrated for 30 minutes at room temperature, washed three times with FACS buffer (PBS containing 1% BSA). Then, an antibody specifically recognizing the labeled PD-L1 or PD-L2 protein of constant concentration (such as PE/Cy5 Streptavidin secondary antibody) is added and incubated for 15 minutes at room temperature. Cells are washed with FACS buffer and analyzed by flow cytometry. Data can be analyzed with Prism (GraphPad Software, San Diego, CA) using non-linear regression to calculate IC_{50}. The results from the competition assay can demonstrate the ability of anti-PD-1 sdAbs in inhibiting the interaction between labeled-PD-L1/PD-L2 and PD-1.

[0201] The biological activity of anti-PD-1 sdAb moiety can also be tested by PD-1-based blockade assay for cytokine release (also see Example 1). PD-1 signaling typically has a greater effect on cytokine production than on cellular proliferation, with significant effects on IFN-γ, TNF-α and IL-2 production.

Thus, blockade of PD-1 pathways by anti-PD-1 antibodies can be studied using a variety of bioassays that monitor T cell proliferation, IFN-γ release, or IL-2 secretion. PD-1 mediated inhibitory signaling also depends on the strength of the TCR signaling, with greater inhibition delivered at low levels of TCR stimulation. This reduction can be overcome by costimulation through CD28 (Freeman *et al.*, J. Exp. Med. 192: 1027-34 (2000)) or the presence of IL-2 (Carter *et al.*, Eur. J. Immunol. 32: 634-43 (2002)). PD-L1 and PD-L2 have been shown to downregulate T cell activation upon binding to PD-1 (Freeman et al., J. Exp. Med. 192: 1027-34 (2000)) or the presence of IL-2 (Carter et al., Eur. J. Immunol. 32: 634-43 (2002)).

[0202] For examples, PD-1 Effector Cells (Jurkat cell stably transfected with human PD-1 protein and NFAT luciferase) and CHO-K1/human CD274 (CHO-K1 stably expressing human PD-L1) are mixed in wells. Anti-PD-1 sdAbs are added into each well at different concentrations. No antibody can be used as a background control. Negative control (such as human IgG4) and positive control (such as Keytruda®) can be employed. After 24-hour incubation in 37°C/5% CO_2 incubator, medium is taken from each testing well for IL-2 secretion measurement (Cisbio). EC_{50} value for each test antibody is measured, which will reflect the ability of the test anti-PD-1 sdAb in blocking the interaction between PD-1 and PD-L1 on Jurkat cells (PD-1/PD-L1 interaction inhibits T-cell IL-2 production).

[0203] The biological activity of anti-PD-1 sdAb moiety can also be tested by PD-1-based blockade assay for luciferase reporter activity (also see Example 1). The effector cells contain a luciferase construct that is induced upon disruption of the PD-1/PD-L1 receptor-ligand interaction. For example, PD-1 Effector Cells (Jurkat cell stably transfected with human PD-1 protein and NFAT luciferase) can be plated overnight and then incubated with a serial dilution of anti-PD-1 construct comprising anti-PD-1 sdAb, followed by addition of PD-L1 expressing cells (CHO-K1/human CD274) at a suitable E: T ratio. After 6 hours induction at 37°C, 5% CO_2, Bio-Glo™ Luciferase Assay Reagent can be added and luminescence can be determined. The results can demonstrate the ability of anti-PD-1 sdAbs in inhibiting the interaction between PD-L1 and PD-1.

[0204] In some embodiments, the anti-PD-1 sdAb moiety blocks or antagonizes signals transduced by the PD-1 receptor. In some embodiments, the anti-PD-1 sdAb moiety can bind to an epitope on PD-1 so as to inhibit PD-1 from interacting with PD-L1/PD-L2. In some embodiments, the anti-PD-1 sdAb moiety can reduce the binding of PD-1 to PD-L1/PD-L2 by at least about any of 5%, 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 90%, 95%, 99% or 99.9% under conditions in which the ratio of antibody combining site to PD-1 ligand binding site is greater than 1:1 and the concentration of antibody is greater than 10^{-8} M.

(II) Construct comprising the anti-PD-1 sdAb moiety

[0205] The anti-PD-1 construct comprising the anti-PD-1 sdAb moiety can be of any possible format.

[0206] In some embodiments, the anti-PD-1 construct comprising the anti-PD-1 sdAb moiety may further comprise additional polypeptide sequences, such as one or more antibody moieties (or antigen binding portions), or Fc fragment of immunoglobulin. Such additional polypeptide sequences may or may not change or otherwise influence the (biological) properties of the anti-PD-1 sdAb, and may or may not add further functionality to the anti-PD-1 sdAb described herein. In some embodiments, the additional polypeptide sequences confer one or more desired properties or functionalities to the anti-PD-1 sdAb of the present invention.

[0207] In some embodiments, the additional polypeptide sequences may comprise a second antibody moiety or second antigen binding portion (such as sdAb, scFv, Fab, full-length antibody, etc.) that specifically recognizes a second epitope. In some embodiments, the second epitope is from PD-1. In some embodiments, the second epitope is not from PD-1. In some embodiments, the second antibody moiety (or second antigen binding portion) specifically recognizes the same epitope on PD-1 as the anti-PD-1 sdAb described herein. In some embodiments, the second antibody moiety (or second antigen binding portion) specifically recognizes a different epitope on PD-1 as the anti-PD-1 sdAb described herein. In some embodiments, the anti-PD-1 construct comprises two or more anti-PD-1-sdAb moieties described herein linked together via optional linkers (such as peptide linkers, *e.g.*, any of those disclosed in the "Peptide linkers" section below). The two or more anti-PD-1-sdAb moieties linked together can be the same or different. In some embodiments, the optional peptide linker comprises the amino acid sequence of any one of SEQ ID NOs: 367-376.

[0208] In some embodiments, the additional polypeptide sequences may comprise a second antibody moiety or second antigen binding portion (such as sdAb, scFv, Fab, full-length antibody, etc.) that specifically recognizes CTLA-4. In some embodiments, the anti-PD-1 construct comprises one or more anti-PD-1-sdAb moieties described herein and one or more anti-CTLA-4 sdAb linked together via optional linkers (such as peptide linkers, *e.g.*, any of those disclosed in the "peptide linkers" section below). The one or more anti-PD-1-sdAb moieties linked together can be the same or different, the one or more anti-CTLA-4-sdAbs linked together can be the same or different. The anti-CTLA-4 sdAb can be of any sequence, such as any of those disclosed in PCT/CN2017/105506 and PCT/CN2016/101777, which are incorporated herein by reference in their entirety. In some embodiments, the anti-CTLA-4 sdAb comprises the amino acid sequence of A34311VH11, AS07014VH11, or AS07189TKDVH11. The anti-PD-1 construct comprising the anti-PD-1 sdAb moiety described herein and anti-CTLA-4 sdAb can be of any format, for example, from N- to C-terminus: (anti-CTLA-4 sdAb1)-L_1-(anti-CTLA-4 sdAb2)-L_2-

(anti-PD-1 sdAb), (anti-CTLA-4 sdAb1)-L_1-(anti-PD-1 sdAb)-L_2-(anti-CTLA-4 sdAb2), (anti-PD-1 sdAb)-L_1-(anti-CTLA-4 sdAb1)-L_2-(anti-CTLA-4 sdAb2), (anti-PD-1 sdAb1)-L_1-(anti-PD-1 sdAb2)-L_2-(anti-CTLA-4 sdAb), (anti-PD-1 sdAb1)-L_1-(anti-CTLA-4 sdAb)-L_2-(anti-PD-1 sdAb2), (anti-CTLA-4 sdAb)-L_1-(anti-PD-1 sdAb1)-L_2-(anti-PD-1 sdAb2), *etc.* (L_1 and L_2 can be the same or different optional linker, such as optional peptide linker). In some embodiments, the optional peptide linker comprises the amino acid sequence of any one of SEQ ID NOs: 367-376.

[0209] In some embodiments, the additional polypeptide sequences may increase the antibody construct half-life, solubility, or absorption, reduce immunogenicity or toxicity, eliminate or attenuate undesirable side effects, and/or confer other advantageous properties to and/or reduce undesired properties of the anti-PD-1 construct of the invention, compared to the anti-PD-1 sdAb described herein *per se*. Some non-limiting examples of such additional polypeptide sequences are serum proteins, such as human serum albumin (HSA; *see e.g.* WO 00/27435) or haptenic molecules (*e.g.* haptens that are recognized by circulating antibodies, *see e.g.* WO 98/22141). It was shown that linking fragments of immunoglobulins (such as V_H domains) to serum albumin or fragments thereof may increase antibody half-life (*see e.g.* WO 00/27435 and WO 01/077137). Thus, in some embodiments, the anti-PD-1 construct of the present invention may comprise an anti-PD-1 sdAb moiety described herein linked to serum albumin (or to a suitable fragment thereof), optionally via a suitable linker (such as peptide linker). In some embodiments, the anti-PD-1 sdAb moiety described herein can be linked to a fragment of serum albumin at least comprising serum albumin domain III (*see* PCT/EP2007/002817). The anti-PD-1 sdAb-HSA fusion protein can be of any format, such as $(sdAb)_n$-HSA (n is an integer of at least 1), sdAb-HSA-sdAb, *etc.* In some embodiments, the optional peptide linker comprises the amino acid sequence of any one of SEQ ID NOs: 367-376.

Anti-PD-1 heavy chain-only antibody (HCAb)

[0210] In some embodiments, the isolated anti-PD-1 construct is a heavy chain-only antibody (HCAb) comprising the anti-PD-1 sdAb moiety described herein. In some embodiments, the anti-PD-1 sdAb moiety described herein can be linked to one or more (preferably human) C_H2 and/or C_H3 domains, *e.g.*, an Fc fragment, optionally via a linker sequence, to increase its half-life *in vivo*.

[0211] Thus in some embodiments, the isolated anti-PD-1 construct is an anti-PD-1 HCAb comprising an anti-PD-1 sdAb moiety described herein fused to an Fc fragment of an immunoglobulin, such as IgA, IgD, IgE, IgG, and IgM. In some embodiments, the anti-PD-1 HCAb comprises an Fc fragment of IgG, such as any of IgG1, IgG2, IgG3, or IgG4. In some embodiments, the Fc fragment is a human Fc, such as human IgG1 (hIgG1) Fc, hIgG2 Fc, or hIgG4 Fc. In some embodiments, the Fc fragment is effectorless, with reduced, minimized, or eliminated antibody effector functions such as ADCC, CDC, and/or ADCP

(antibody-dependent cellular phagocytosis). For example, in some embodiments, the effectorless Fc comprises an N297A or DANA mutation (D265A+N297A) in the C_H2 region. In some embodiments, the effectorless Fc comprises K322A and L234A/L235A (LALA) mutations. In some embodiments, the Fc fragment is an effectorless (inert) IgG1 Fc, such as effectorless hIgG1 Fc. In some embodiments, the Fc fragment is a human IgG4 Fc (S228P). In some embodiments, the Fc fragment comprises the amino acid sequence of any one of SEQ ID NOs: 363-365. In some embodiments, the anti-PD-1 HCAb is monomeric. In some embodiments, the anti-PD-1 HCAb is dimeric. In some embodiments, the anti-PD-1 HCAb is multispecific and multivalent (such as bispecific and bivalent), *e.g.*, comprising two or more different anti-PD-1 sdAb moieties described herein (exemplified as FIG. 26B). In some embodiments, the anti-PD-1 HCAb is monospecific and multivalent (*e.g.*, bivalent; exemplified as FIG. 26A).

[0212] In some embodiments, the anti-PD-1 sdAb moiety and the Fc fragment are optionally connected by a peptide linker. In some embodiments, the peptide linker is a human IgG1 hinge (SEQ ID NO: 369). In some embodiments, the peptide linker is a mutated human IgG1 hinge (SEQ ID NO: 368). In some embodiments, the peptide linker is a human IgG4 hinge (SEQ ID NO: 367). In some embodiments, the peptide linker is a hIgG2 hinge. In some embodiments, the peptide linker comprises the amino acid sequence of any one of SEQ ID NOs: 367-376, such as SEQ ID NO: 371, 375, or 376.

[0213] Thus in some embodiments, there is provided an isolated anti-PD-1 HCAb comprising an sdAb moiety specifically recognizing PD-1, wherein the anti-PD-1 sdAb moiety comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 37-72, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 109-144, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 181-216, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and wherein the anti-PD-1 sdAb moiety is fused to an Fc fragment of an immunoglobulin via an optional linker. In some embodiments, there is provided an isolated anti-PD-1 HCAb comprising an sdAb moiety specifically recognizing PD-1, wherein the sdAb moiety comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 37-72; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 109-144; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 181-216; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions, and wherein the anti-PD-1 sdAb moiety is fused to an Fc fragment of an immunoglobulin via an optional linker. In some embodiments, the amino acid substitutions are in CDR1 and/or CDR2. In some embodiments, there is provided an anti-PD-1 HCAb comprising an sdAb moiety specifically recognizing PD-1, wherein the sdAb moiety comprises a CDR1

comprising the amino acid sequence of any one of SEQ ID NOs: 37-72; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 109-144; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 181-216, and wherein the anti-PD-1 sdAb moiety is fused to an Fc fragment of an immunoglobulin via an optional linker. In some embodiments, there is provided an isolated anti-PD-1 HCAb comprising an sdAb moiety specifically recognizing PD-1, wherein the sdAb moiety comprises a V_HH domain comprising the amino acid sequence of any one of SEQ ID NOs: 289-324, or a variant thereof having at least about 80% (such as at least about any of 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) sequence identify to any one of SEQ ID NOs: 289-324, and wherein the anti-PD-1 sdAb moiety is fused to an Fc fragment of an immunoglobulin via an optional linker. In some embodiments, there is provided an isolated anti-PD-1 HCAb comprising a sdAb moiety specifically recognizing PD-1, wherein the sdAb moiety comprises a V_HH domain comprising the amino acid sequence of any one of SEQ ID NOs: 289-324, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the V_HH domain, and wherein the anti-PD-1 sdAb moiety is fused to an Fc fragment of an immunoglobulin via an optional linker. In some embodiments, the amino acid substitutions in the V_HH domain are in CDRs, such as the CDR1, and/or the CDR2, and/or the CDR3 of any one of SEQ ID NOs: 289-324. In some embodiments, the amino acid substitutions in the V_HH domain are in FRs, such as the FR1, and/or the FR2, and/or the FR3, and/or the FR4 of any one of SEQ ID NOs: 289-324. In some embodiments, the amino acid substitutions are in both CDRs and FRs of any one of SEQ ID NOs: 289-324. In some embodiments, there is provided an isolated anti-PD-1 HCAb comprising an sdAb moiety specifically recognizing PD-1, wherein the sdAb moiety comprises a V_HH domain comprising the amino acid sequence of any one of SEQ ID NOs: 289-324, and wherein the anti-PD-1 sdAb moiety is fused to an Fc fragment of an immunoglobulin via an optional linker. In some embodiments, there is provided an isolated anti-PD-1 HCAb comprising an sdAb moiety specifically recognizing PD-1, wherein the sdAb moiety comprises CDR1, CDR2, and CDR3 of any one of SEQ ID NOs: 289-324, and wherein the anti-PD-1 sdAb moiety is fused to an Fc fragment of an immunoglobulin via an optional linker. In some embodiments, there is provided an isolated anti-PD-1 HCAb comprising two sdAb moieties specifically recognizing PD-1, wherein each anti-PD-1 sdAb moiety comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 37-72, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 109-144, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 181-216, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and wherein the C-terminus of each anti-PD-1 sdAb is fused to the N-terminus of an Fc fragment of an immunoglobulin via an optional linker. In some

embodiments, the two anti-PD-1 sdAb moieties are the same (exemplified as FIG. 26A). In some embodiments, the two anti-PD-1 sdAb moieties are different (exemplified as FIG. 26B). In some embodiments, the Fc fragment is a human IgG1 Fc, effectorless human IgG1 Fc, hIgG2 Fc, human IgG4 Fc, or hIgG4 Fc (S228P). In some embodiments, the Fc fragment comprises the amino acid sequence of any one of SEQ ID NOs: 363-365. In some embodiments, the anti-PD-1 HCAb is monomeric. In some embodiments, the anti-PD-1 HCAb is dimeric. In some embodiments, the anti-PD-1 sdAb moiety and the Fc fragment are optionally connected by a peptide linker. In some embodiments, the peptide linker comprises the amino acid sequence of any one of SEQ ID NOs: 367-376. In some embodiments, the K_d of the binding between the anti-PD-1 sdAb moiety and PD-1 is about 10^{-5} M to about 10^{-12} M (such as about 10^{-7} M to about 10^{-12} M, or about 10^{-8} M to about 10^{-12} M). In some embodiments, the anti-PD-1 sdAb moiety is camelid, chimeric, human, partially humanized, or fully humanized.

[0214] In some embodiments, there is provided an isolated anti-PD-1 HCAb comprising the amino acid sequence of any one of SEQ ID NOs: 325-360.

[0215] In some embodiments, there is also provided an isolated anti-PD-1 HCAb (hereinafter referred to as "competing anti-PD-1 HCAb") that specifically binds to PD-1 competitively with any one of the isolated anti-PD-1 HCAbs, anti-PD-1 sdAbs, or anti-PD-1 constructs comprising the anti-PD-1 sdAb moiety described herein. Competitive binding may be determined using an ELISA assay. For example, in some embodiments, there is provided an isolated anti-PD-1 HCAb that specifically binds to PD-1 competitively with an isolated anti-PD-1 HCAb comprising the amino acid sequence of any one of SEQ ID NOs: 325-360. In some embodiments, there is provided an isolated anti-PD-1 HCAb that specifically binds to PD-1 competitively with an anti-PD-1 HCAb comprising an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 37-72; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 109-144; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 181-216. In some embodiments, there is provided an isolated anti-PD-1 HCAb that specifically binds to PD-1 competitively with an anti-PD-1 sdAb moiety (or an anti-PD-1 construct comprising an anti-PD-1 sdAb moiety) comprising a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 37-72; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 109-144; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 181-216. In some embodiments, the Fc fragment of the competing anti-PD-1 HCAb comprises the amino acid sequence of any one of SEQ ID NOs: 363-365. In some embodiments, the K_d of the binding between the competing anti-PD-1 HCAb and PD-1 is about 10^{-5} M to about 10^{-12} M (such as about 10^{-7} M to about 10^{-12} M, or about 10^{-8} M to about 10^{-12} M). In some embodiments, the competing anti-PD-1 HCAb is camelid, chimeric, human, partially humanized, or fully humanized.

Multivalent and/or multispecific antibodies

[0216] In some embodiments, the isolated anti-PD-1 construct comprises an anti-PD-1 sdAb moiety described herein fused to one or more other antibody moiety or antigen binding portion (such as an antibody moiety that specifically recognizes another epitope). The one or more other antibody moiety can be of any antibody or antibody fragment format, such as a full-length antibody, a Fab, a Fab', a $(Fab')_2$, an Fv, an scFv, an scFv-scFv, a minibody, a diabody, or an sdAb. In some embodiments, the one or more antibody moiety (or antigen binding portion) comprises a heavy chain variable domain (V_H) and a light chain variable domain (V_L). For a review of certain antibody fragments, *see* Hudson *et al. Nat. Med.* 9:129-134 (2003). For a review of scFv fragments, *see*, e.g., Pluckthün, in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenburg and Moore *eds.*, (Springer-Verlag, New York), pp. 269-315 (1994); see also WO 93/16185; and U.S. Patent Nos. 5,571,894 and 5,587,458. For discussion of Fab and $F(ab')_2$ fragments comprising salvage receptor binding epitope residues and having increased *in vivo* half-life, *see* U.S. Patent No. 5,869,046. For a review of multispecific antibodies, *see* Weidle *et al.*, Cancer Genomics Proteomics, 10(1):1-18, 2013; Geering and Fussenegger, Trends Biotechnol., 33(2):65-79, 2015; Stamova *et al.*, Antibodies, 1(2):172-198, 2012. Diabodies are antibody fragments with two antigen-binding sites that may be bivalent or bispecific. *See*, for example, EP 404,097; WO 1993/01161; Hudson et al., *Nat. Med.* 9:129-134 (2003); and Hollinger et al., *Proc. Natl. Acad. Sci. USA* 90: 6444-6448 (1993). Triabodies and tetrabodies are also described in Hudson *et al.*, *Nat. Med.* 9:129-134 (2003). Antibody fragments can be made by various techniques, including but not limited to proteolytic digestion of an intact antibody as well as production by recombinant host cells (*e.g. E. coli* or phage), as described herein.

[0217] Techniques for making multispecific antibodies include, but are not limited to, recombinant co-expression of two immunoglobulin heavy chain-light chain pairs having different specificities (see Milstein and Cuello, *Nature* 305: 537 (1983)), WO 93/08829, and Traunecker et al., *EMBO J.* 10: 3655 (1991)), and "knob-in-hole" engineering (*see, e.g.*, U.S. Patent No. 5,731,168). Multi-specific antibodies may also be made by engineering electrostatic steering effects for making antibody Fc-heterodimeric molecules (WO 2009/089004A1); cross-linking two or more antibodies or fragments (*see, e.g.*, US Patent No. 4,676,980, and Brennan et al., *Science*, 229: 81 (1985)); using leucine zippers to produce bi-specific antibodies (*see, e.g.*, Kostelny et al., *J. Immunol.*, 148(5):1547-1553 (1992)); using "diabody" technology for making bispecific antibody fragments (see, e.g., Hollinger et al., *Proc. Natl. Acad. Sci. USA*, 90:6444-6448 (1993)); and using single-chain Fv (sFv) dimers (*see*, *e.g.*, Gruber et al., *J. Immunol.*, 152:5368 (1994)); and preparing trispecific antibodies as described, *e.g.*, in Tutt et al. *J. Immunol.* 147: 60 (1991); and creating polypeptides comprising tandem single-domain antibodies (*see,* e.g., U.S. Patent Application

No. 20110028695; and Conrath et al. J. Biol. Chem., 2001; 276(10):7346-50). Engineered antibodies with three or more functional antigen binding sites, including "Octopus antibodies," are also included herein (*see, e.g.,* US 2006/0025576A1).

Peptide linkers

[0218] In some embodiments, the anti-PD-1 sdAb and the other one or more antibody moieties (such as a full-length antibody, sdAb, or an antigen binding portion comprising a V_H and a V_L) within the anti-PD-1 construct can be optionally connected by a peptide linker. The length, the degree of flexibility and/or other properties of the peptide linker(s) used in the anti-PD-1 construct may have some influence on properties, including but not limited to the affinity, specificity or avidity for one or more particular antigens or epitopes. For example, longer peptide linkers may be selected to ensure that two adjacent domains do not sterically interfere with one another. In some embodiment, a peptide linker comprises flexible residues (such as glycine and serine) so that the adjacent domains are free to move relative to each other. For example, a glycine-serine doublet can be a suitable peptide linker.

[0219] The peptide linker can be of any suitable length. In some embodiments, the peptide linker is at least about any of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 50, 75, 100 or more amino acids long. In some embodiments, the peptide linker is no more than about any of 100, 75, 50, 40, 35, 30, 25, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5 or fewer amino acids long. In some embodiments, the length of the peptide linker is any of about 1 amino acid to about 10 amino acids, about 1 amino acid to about 20 amino acids, about 1 amino acid to about 30 amino acids, about 5 amino acids to about 15 amino acids, about 10 amino acids to about 25 amino acids, about 5 amino acids to about 30 amino acids, about 10 amino acids to about 30 amino acids long, about 30 amino acids to about 50 amino acids, about 50 amino acids to about 100 amino acids, or about 1 amino acid to about 100 amino acids.

[0220] The peptide linker may have a naturally occurring sequence, or a non-naturally occurring sequence. For example, a sequence derived from the hinge region of heavy chain only antibodies may be used as the linker. *See,* for example, WO1996/34103. In some embodiments, the peptide linker is a human IgG1 hinge (SEQ ID NO: 369). In some embodiments, the peptide linker is a mutated human IgG1 hinge (SEQ ID NO: 368). In some embodiments, the peptide linker is a human IgG4 hinge (SEQ ID NO: 367). In some embodiments, the peptide linker is a hIgG2 hinge. In some embodiments, the peptide linker is a flexible linker. Exemplary flexible linkers include glycine polymers $(G)_n$ (SEQ ID NO: 372), glycine-serine polymers (including, for example, $(GS)_n$ (SEQ ID NO: 373), $(GSGGS)_n$ (SEQ ID NO: 374), $(GGGS)_n$ (SEQ ID NO: 375), and $(GGGGS)_n$ (SEQ ID NO: 376), where n is an integer of at least one), glycine-alanine polymers, alanine-serine polymers, and other flexible linkers known in the art. In some

embodiments, the peptide linker comprises the amino acid sequence of SEQ ID NO: 370 (GGGGSGGGS) or 371 (GGGGSGGGGSGGGGS).

[0221] In some embodiments, the anti-PD-1 construct comprising an anti-PD-1 sdAb moiety described herein and one or more other antibody moiety (such as a full-length antibody, sdAb, or an antigen binding portion comprising a V_H and a V_L) is monospecific. In some embodiments, the anti-PD-1 construct comprising an anti-PD-1 sdAb moiety described herein and one or more other antibody moiety (such as a full-length antibody, sdAb, or an antigen binding portion comprising a V_H and a V_L) is multispecific (such as bispecific). Multispecific molecules are molecules that have binding specificities for at least two different epitopes *(e.g.,* bispecific antibodies have binding specificities for two epitopes). Multispecific molecules with more than two valencies and/or specificities are also contemplated. For example, trispecific antibodies can be prepared. Tutt *et al. J. Immunol.* 147: 60 (1991). It is to be appreciated that one of skill in the art could select appropriate features of individual multispecific molecules described herein to combine with one another to form a multispecific anti-PD-1 construct of the invention.

[0222] In some embodiments, the anti-PD-1 construct is multivalent but monospecific, *i.e.*, the anti-PD-1 construct comprises an anti-PD-1 sdAb moiety described herein and at least a second antibody moiety (such as a full-length antibody, sdAb, or an antigen binding portion comprising a V_H and a V_L) specifically recognizing the same PD-1 epitope as the anti-PD-1 sdAb moiety described herein. In some embodiments, the one or more antibody moiety that specifically recognizes the same PD-1 epitope as the anti-PD-1 sdAb moiety described herein may comprise the same CDRs and/or the same V_HH amino acid sequence as the anti-PD-1 sdAb moiety. For example, the anti-PD-1 construct may comprise two or more anti-PD-1 sdAb moieties described herein, wherein the two or more anti-PD-1 sdAb moieties are the same, and are optionally connected by peptide linker(s). In some embodiments, the peptide linker comprises the amino acid sequence of any one of SEQ ID NOs: 367-376.

[0223] In some embodiments, the anti-PD-1 construct is multivalent and multispecific (*e.g.*, bispecific), *i.e.*, the anti-PD-1 construct comprises an anti-PD-1 sdAb moiety described herein and at least a second antibody moiety (such as a full-length antibody, sdAb, or an antigen binding portion comprising a V_H and a V_L) specifically recognizing a second antigen other than PD-1, or a different PD-1 epitope recognized by the anti-PD-1 sdAb moiety described herein. In some embodiments, the second antibody moiety is an sdAb, such as anti-PD-1 sdAb, or anti-CTLA-4 sdAb (such as any of those disclosed in PCT/CN2017/105506 and PCT/CN2016/101777, which are incorporated herein by reference in their entirety). In some embodiments, the second antibody moiety specifically recognizes human serum albumin (HSA). In some embodiments, the anti-PD-1 sdAb moiety described herein is fused to the N-terminus and/or C-terminus of the second antibody moiety. In some embodiments, the anti-PD-1

construct is trivalent and bispecific. In some embodiments, the anti-PD-1 construct comprises two anti-PD-1 sdAb moieties described herein and a second antibody moiety (such as an anti-HSA sdAb, anti-CTLA-4 sdAb), wherein the second antibody moiety is between the two anti-PD-1 sdAb moieties. In some embodiments, the antibody moieties are optionally connected by peptide linker(s). In some embodiments, the peptide linker comprises the amino acid sequence of any one of SEQ ID NOs: 367-376.

[0224] The monospecific or multispecific anti-PD-1 construct comprising two or more anti-PD-1 sdAb moieties may have increase avidity compared to that of a single anti-PD-1 sdAb moiety described herein.

Bispecific antibodies comprising an anti-PD-1 sdAb moiety fused to a full-length antibody

[0225] In some embodiments, the anti-PD-1 construct comprises an anti-PD-1 sdAb moiety described herein fused to a second antibody moiety, wherein the second antibody moiety is a full-length antibody consisting of two heavy chains and two light chains (such as full-length antibody specifically recognizing TIGIT, LAG-3, TIM-3, CTLA-4, or PD-1 (*e.g.*, a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein)). In some embodiments, the anti-PD-1 sdAb moiety and the full-length antibody are connected via an optional linker, such as a peptide linker.

[0226] Thus in some embodiments, there is provided an isolated anti-PD-1 construct comprising an sdAb moiety specifically recognizing PD-1 and a full-length antibody (such as full-length antibody specifically recognizing TIGIT, LAG-3, TIM-3, CTLA-4, or PD-1 (*e.g.*, a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein)), wherein the anti-PD-1 sdAb moiety comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 37-72, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 109-144, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 181-216, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, there is provided an isolated anti-PD-1 construct comprising an sdAb moiety specifically recognizing PD-1 and a full-length antibody (such as full-length antibody specifically recognizing TIGIT, LAG-3, TIM-3, CTLA-4, or PD-1 (*e.g.*, a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein)), wherein the anti-PD-1 sdAb moiety comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 37-72, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 109-144, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 181-216, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid

substitutions; and wherein the N-terminus of the anti-PD-1 sdAb moiety is fused to the C-terminus of at least one of the heavy chains of the full-length antibody (exemplified as FIG. 17). In some embodiments, there is provided an isolated anti-PD-1 construct comprising an sdAb moiety specifically recognizing PD-1 and a full-length antibody (such as full-length antibody specifically recognizing TIGIT, LAG-3, TIM-3, CTLA-4, or PD-1 (*e.g.*, a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein)), wherein the anti-PD-1 sdAb moiety comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 37-72, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 109-144, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 181-216, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and wherein the C-terminus of the anti-PD-1 sdAb moiety is fused to the N-terminus of at least one of the heavy chains of the full-length antibody (exemplified as FIG. 16). In some embodiments, there is provided an isolated anti-PD-1 construct comprising an sdAb moiety specifically recognizing PD-1 and a full-length antibody (such as full-length antibody specifically recognizing TIGIT, LAG-3, TIM-3, CTLA-4, or PD-1 (*e.g.*, a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein)), wherein the anti-PD-1 sdAb moiety comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 37-72, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 109-144, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 181-216, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and wherein the N-terminus of the anti-PD-1 sdAb moiety is fused to the C-terminus of at least one of the light chains of the full-length antibody (exemplified as FIG. 19). In some embodiments, there is provided an isolated anti-PD-1 construct comprising an sdAb moiety specifically recognizing PD-1 and a full-length antibody (such as full-length antibody specifically recognizing TIGIT, LAG-3, TIM-3, CTLA-4, or PD-1 (*e.g.*, a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein)), wherein the anti-PD-1 sdAb moiety comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 37-72, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 109-144, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 181-216, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and wherein the C-terminus of the anti-PD-1 sdAb moiety is fused to the N-terminus of at

least one of the light chains of the full-length antibody (exemplified as FIG. 18). In some embodiments, there is provided an isolated anti-PD-1 construct comprising four sdAb moieties specifically recognizing PD-1 and a full-length antibody (such as full-length antibody specifically recognizing TIGIT, LAG-3, TIM-3, CTLA-4, or PD-1 (*e.g.*, a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein)), wherein each anti-PD-1 sdAb moiety comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 37-72, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 109-144, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 181-216, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and wherein the C-terminus of the anti-PD-1 sdAb moiety is fused to the N-terminus of both heavy and light chains of the full-length antibody (exemplified as FIG. 20). In some embodiments, there is provided an isolated anti-PD-1 construct comprising four sdAb moieties specifically recognizing PD-1 and a full-length antibody (such as full-length antibody specifically recognizing TIGIT, LAG-3, TIM-3, CTLA-4, or PD-1 (*e.g.*, a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein)), wherein each anti-PD-1 sdAb moiety comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 37-72, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 109-144, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 181-216, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; wherein two anti-PD-1 sdAb moieties are fused together via a first optional linker, and the other two anti-PD-1 sdAb moieties are fused together via a second optional linker, and wherein the C-terminus of each set of the two anti-PD-1 sdAb fusion is fused to the N-terminus of each heavy chain of the full-length antibody via a third and fourth optional linkers (exemplified as FIG. 21). In some embodiments, the four anti-PD-1 sdAb moieties are identical. In some embodiments, the four anti-PD-1 sdAb moieties are different. In some embodiments, the anti-PD-1 sdAb moiety comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 37-72; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 109-144; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 181-216. In some embodiments, the anti-PD-1 sdAb moiety comprises a V_HH domain comprising the amino acid sequence of any one of SEQ ID NOs: 289-324. In some embodiments, the anti-PD-1 sdAb moiety comprises CDR1, CDR2, and CDR3 of any one of SEQ ID NOs: 289-324. In some embodiments, the Fc fragment of the full-length antibody is hIgG1 Fc, effectorless hIgG1 Fc, hIgG2 Fc, hIgG4 Fc, or hIgG4 Fc (S228P). In some embodiments, the Fc fragment of the full-length

antibody comprises the amino acid sequence of any one of SEQ ID NOs: 363-365. In some embodiments, the full-length antibody is an activator of a stimulatory immune checkpoint molecule. In some embodiments, the full-length antibody is an immune checkpoint inhibitor, such as an inhibitor of TIGIT, LAG-3, TIM-3, CTLA-4, or PD-1 (*e.g.*, an antibody that specifically recognizes a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein). In some embodiments, the full-length antibody is an anti-TIGIT antibody. In some embodiments, the anti-TIGIT antibody comprises a V_H comprising HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 377, and a V_L comprising LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 378. In some embodiments, the anti-TIGIT antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 377, and a light chain comprising the amino acid sequence of SEQ ID NO: 378. In some embodiments, the anti-TIGIT antibody is tiragolumab. In some embodiments, the full-length antibody is an anti-LAG-3 antibody. In some embodiments, the anti-LAG-3 antibody comprises a V_H comprising HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 379, and a V_L comprising LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 380. In some embodiments, the anti-LAG-3 antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 379, and a light chain comprising the amino acid sequence of SEQ ID NO: 380. In some embodiments, the anti-LAG-3 antibody is relatlimab. In some embodiments, the full-length antibody is an anti-TIM-3 antibody. In some embodiments, the anti-TIM-3 antibody comprises a V_H comprising HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 381, and a V_L comprising LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 382. In some embodiments, the anti-TIM-3 antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 381, and a light chain comprising the amino acid sequence of SEQ ID NO: 382. In some embodiments, the anti-TIM-3 antibody is MBG453. In some embodiments, the full-length antibody is an anti-CTLA-4 antibody. In some embodiments, the anti-CTLA-4 antibody comprises a V_H comprising HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 383, and a V_L comprising LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 384. In some embodiments, the anti-CTLA-4 antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 383, and a light chain comprising the amino acid sequence of SEQ ID NO: 384. In some embodiments, the anti-CTLA-4 antibody is ipilimumab (*e.g.*, Yervoy®). In some embodiments, the full-length antibody is an anti-PD-1 antibody (e.g., specifically recognizes a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein). In some embodiments, the anti-PD-1 full-length antibody comprises a V_H comprising HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 385, and a V_L comprising LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 386. In some embodiments, the anti-PD-1 full-length antibody

comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 385, and a light chain comprising the amino acid sequence of SEQ ID NO: 386. In some embodiments, the anti-PD-1 full-length antibody is pembrolizumab (*e.g.*, Keytruda®) or nivolumab (*e.g.*, Opdivo®). In some embodiments, the anti-PD-1 sdAb moiety and the full-length antibody are optionally connected by a peptide linker. In some embodiments, the peptide linker comprises the amino acid sequence of any one of SEQ ID NOs: 367-376. In some embodiments, the K_d of the binding between the anti-PD-1 sdAb moiety and PD-1 is about 10^{-5} M to about 10^{-12} M (such as about 10^{-7} M to about 10^{-12} M, or about 10^{-8} M to about 10^{-12} M). In some embodiments, the anti-PD-1 sdAb moiety is camelid, chimeric, human, partially humanized, or fully humanized.

TIGIT

[0227] T cell immunoreceptor with Ig and ITIM domains (TIGIT, also known as Vstm3 or WUCAM) is an immune receptor belonging to the CD28 family. TIGIT exerts its inhibitory immune checkpoint function via several mechanisms. First, upon binding to its major ligand CD155 (PVR), the subsequent phosphorylation of TIGIT in its ITIM domain transduces inhibitory signals to downregulate IFN-γ expression in T cells and NK cells via NF-κB pathway. Second, as TIGIT interacts with PVR at higher affinity than with CD226, it competes with CD226 and attenuates the stimulatory signal transduced by CD226. Third, PVR binding to TIGIT on dendritic cells may lead to upregulation of IL-10 expression and downregulation of IL-12 expression, therefore impairing the anti-tumor immune response of dendritic cells. Lastly, recent research indicated that TIGIT can directly bind to CD226 *in cis* to inhibit CD226 dimerization, which is required for T cell activation. Therefore, TIGIT acts as an important negative regulator in immune responses in infection and cancer, and blockade of TIGIT signaling has been proposed as an approach to enhance T cell and NK cell immunity for cancer treatment. Exemplary anti-TIGIT antibodies that can be applied in the present application include, but are not limited to, tiragolumab.

[0228] The construct comprising bispecificity against TIGIT and PD-1 will be hereinafter referred to as "anti-TIGIT/PD-1 antibody", "anti-TIGIT/PD-1 construct", "PD-1×TIGIT antibody", or "PD-1×TIGIT BABP".

LAG-3

[0229] LAG-3 (Lymphocyte Activating Gene-3, CD223) works to suppress an immune response by action to Tregs, as well as direct effects on CD8+ T cells (Huang *et al.*, 2004, *Immunity*. 21(4):503-13; Grosso *et al.*, 2007, *J Clin Invest*. 117(11):3383-92). Exemplary anti-LAG-3 antibodies that can be applied in the present application include, but are not limited to, relatlimab (BMS-986016).

[0230] The construct comprising bispecificity against LAG-3 and PD-1 will be hereinafter referred to as "anti-LAG-3/PD-1 antibody", "anti-LAG-3/PD-1 construct", "PD-1×LAG-3 antibody", or "PD-1×LAG-3 BABP".

TIM-3

[0231] T-cell immunoglobulin and mucin-domain containing-3 (TIM-3) is also known as Hepatitis A virus cellular receptor 2 (HAVCR2). It is an immune checkpoint molecule which has been associated with the inhibition of lymphocyte activity and in some cases induction of lymphocyte anergy (Pardoll D. Nature Reviews 2012 April Vol. 12: 252). TIM-3 is a receptor for galectin 9 (GAL9), which is up-regulated in various types of cancers, including breast cancers. TIM-3 has been identified as another important inhibitory receptor expressed by exhausted CD8+ T cells. In mouse models of cancer, it has been shown that the most dysfunctional tumor-infiltrating CD8+ T cells actually co-express PD-1 and TIM-3. Exemplary anti-TIM-3 antibodies that can be applied in the present application include, but are not limited to, MBG453.

[0232] The construct comprising bispecificity against TIM-3 and PD-1 will be hereinafter referred to as "anti-TIM-3/PD-1 antibody", "anti-TIM-3/PD-1 construct", "PD-1×TIM-3 antibody", or "PD-1×TIM-3 BABP".

CTLA-4

[0233] Cytotoxic T-Lymphocyte-Associated protein 4 (CTLA-4, or CD152) is a homolog of CD28, and is known as an inhibitory immune checkpoint molecule up-regulated on activated T-cells. CTLA-4 also binds to B7-1 and B7-2, but with greater affinity than CD28. The interaction between B7 and CTLA-4 dampens T cell activation, which constitutes an important mechanism of tumor immune escape. Anti-CTLA-4 antibody therapy has shown promise in a number of cancers, such as melanoma. Exemplary anti-CTLA-4 antibodies that can be applied in the present application include, but are not limited to, ipilimumab (*e.g.*, Yervoy®).

[0234] The construct comprising bispecificity against CTLA-4 and PD-1 will be hereinafter referred to as "anti-CTLA-4/PD-1 antibody", "anti-CTLA-4/PD-1 construct", "PD-1×CTLA-4 antibody", or "PD-1×CTLA-4 BABP".

PD-1

[0235] In some embodiments, the second antibody moiety is a full-length antibody consisting of two heavy chains and two light chains that specifically recognizes another epitope of PD-1, different from the PD-1 epitope recognized by the anti-PD-1 sdAb moiety described herein. In some embodiments, fusing

an anti-PD-1 sdAb moiety described herein with an anti-PD-1 full-length antibody that specifically recognizes a different PD-1 epitope will increase antibody potency. Exemplary anti-PD-1 antibodies that can be applied in the present application include, but are not limited to, pembrolizumab (*e.g.*, Keytruda®) and nivolumab (*e.g.*, Opdivo®).

[0236] The construct comprising bispecificity against PD-1 will be hereinafter referred to as "anti-PD-1/PD-1 antibody", "anti-PD-1/PD-1 construct", "PD-1×PD-1 antibody", or "PD-1×PD-1 BABP".

[0237] In some embodiments, there is also provided an anti-PD-1 construct comprising an sdAb moiety specifically recognizing PD-1 (hereinafter referred to as "competing anti-PD-1 construct") that specifically binds to PD-1 competitively with any one of the anti-PD-1 constructs described herein (such as anti-PD-1 sdAb moiety, anti-PD-1 sdAb-Fc fusion protein (*e.g.*, anti-PD-1 HCAb), multispecific or monospecific anti-PD-1 construct comprising an anti-PD-1 sdAb moiety descried herein, *e.g.*, PD-1×TIGIT BABP, PD-1×LAG-3 BABP, PD-1×TIM-3 BABP, PD-1×CTLA-4 BABP, or PD-1×PD-1 BABP described herein).

Anti-PD-1 multispecific antigen binding proteins (MABPs)

[0238] In some embodiments, there is provided an isolated anti-PD-1 construct comprising an anti-PD-1 sdAb moiety described herein fused to a full-length antibody or antigen binding fragment that comprises a V_H and a V_L, wherein the anti-PD-1 construct is multispecific (hereinafter referred to as "multispecific anti-PD-1 construct" or "anti-PD-1 multispecific antigen binding protein (MABP)"). In some embodiments, the anti-PD-1 MABP is bispecific (hereinafter referred to as "bispecific anti-PD-1 construct" or "anti-PD-1 bispecific antigen binding protein (BABP)"). The anti-PD-1 sdAb moiety specifically binds PD-1 that is distinct from the target(s) recognized by the full-length antibody or antigen binding fragment comprising a V_H and a V_L, thereby conferring a broadened targeting capability. Due to the small size of the sdAb, in some embodiments the anti-PD-1 MABPs (*e.g.*, anti-PD-1 BABPs) described herein can have similar molecular weight and pharmacokinetic properties compared to those of the full-length antibody or antigen binding fragment component. For example, an anti-PD-1 MABP can be designed by fusing one or more anti-PD-1 sdAb moieties to a monoclonal antibody with proven clinical efficacy and safety to provide increased clinical benefits and desirable pharmacokinetic properties without impeding the expressibility of the multispecific construct. In some embodiments, the one or more anti-PD-1 sdAb moiety described herein is fused to the full-length antibody or antigen binding fragment by an optional peptide linker. The anti-PD-1 MABPs (*e.g.*, anti-PD-1 BABPs) described herein can be adopted to target a variety of disease-related epitope or antigen combinations besides PD-1, such as PD-1 with the combination of immune checkpoint molecules, cell surface antigens (such as tumor antigens), or pro-inflammatory molecules, thereby providing agents that are useful for treating a variety of diseases and

conditions, such as cancer, inflammation, and autoimmune diseases. The anti-PD-1 MABP (*e.g.*, anti-PD-1 BABPs) can be of any format, such as those disclosed in PCT/CN2017/093644, which is incorporated herein by reference in their entirety.

Exemplary anti-PD-1 MABPs and BABPs

[0239] In some embodiments, the anti-PD-1 MABP (*e.g.*, BABP) comprises (a) a first antigen binding portion comprising an sdAb moiety specifically recognizing PD-1 described herein, and (b) a second antigen binding portion comprising a V_H and a V_L, wherein the V_H and V_L together form an antigen-binding site that specifically binds TIGIT, wherein the first antigen binding portion and the second antigen binding portion are fused to each other (herein after referred to as "PD-1×TIGIT MABP" or "PD-1×TIGIT BABP"). In some embodiments, the anti-PD-1 MABP (*e.g.*, BABP) comprises (a) a first antigen binding portion comprising an sdAb moiety specifically recognizing PD-1 described herein, and (b) a second antigen binding portion comprising a V_H and a V_L, wherein the V_H and V_L together form an antigen-binding site that specifically binds LAG-3, wherein the first antigen binding portion and the second antigen binding portion are fused to each other (herein after referred to as "PD-1×LAG-3 MABP" or "PD-1×LAG-3 BABP"). In some embodiments, the anti-PD-1 MABP (*e.g.*, BABP) comprises (a) a first antigen binding portion comprising an sdAb moiety specifically recognizing PD-1 described herein, and (b) a second antigen binding portion comprising a V_H and a V_L, wherein the V_H and V_L together form an antigen-binding site that specifically binds TIM-3, wherein the first antigen binding portion and the second antigen binding portion are fused to each other (herein after referred to as "PD-1×TIM-3 MABP" or "PD-1×TIM-3 BABP"). In some embodiments, the anti-PD-1 MABP (*e.g.*, BABP) comprises (a) a first antigen binding portion comprising an sdAb moiety specifically recognizing PD-1 described herein, and (b) a second antigen binding portion comprising a V_H and a V_L, wherein the V_H and V_L together form an antigen-binding site that specifically binds CTLA-4, wherein the first antigen binding portion and the second antigen binding portion are fused to each other (herein after referred to as "PD-1×CTLA-4 MABP" or "PD-1×CTLA-4 BABP"). In some embodiments, the anti-PD-1 MABP (*e.g.*, BABP) comprises (a) a first antigen binding portion comprising an sdAb moiety specifically recognizing PD-1 described herein, and (b) a second antigen binding portion comprising a V_H and a V_L, wherein the V_H and V_L together form an antigen-binding site that specifically binds a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein, wherein the first antigen binding portion and the second antigen binding portion are fused to each other (herein after referred to as "PD-1×PD-1 MABP" or "PD-1×PD-1 BABP").

[0240] In some embodiments, there is provided an anti-PD-1 BABP comprising: (a) a first polypeptide comprising from N-terminus to C-terminus: V_H-C_H1-C_H2-C_H3-anti-PD-1 sdAb moiety; and (b) a second

polypeptide comprising from N-terminus to C-terminus: V_L-C_L, wherein V_H and V_L forms an antigen binding site that specifically binds a second epitope (*e.g.*, TIGIT, LAG-3, TIM-3, CTLA-4, PD-1 (such as a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein)), and wherein the anti-PD-1 sdAb moiety comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 37-72, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 109-144, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 181-216, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, the anti-PD-1 sdAb comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 37-72; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 109-144; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 181-216. In some embodiments, the anti-PD-1 sdAb moiety comprises a V_HH domain comprising the amino acid sequence of any one of SEQ ID NO: 289-324. In some embodiments, V_H and V_L form an antigen binding site that specifically binds TIGIT. In some embodiments, the V_H and V_L domains are derived from tiragolumab. In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 377, and V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 378. In some embodiments, V_H and V_L form an antigen binding site that specifically binds LAG-3. In some embodiments, the V_H and V_L domains are derived from relatlimab. In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 379, and V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 380. In some embodiments, V_H and V_L form an antigen binding site that specifically binds TIM-3. In some embodiments, the V_H and V_L domains are derived from MBG453. In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 381 and V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 382. In some embodiments, V_H and V_L form an antigen binding site that specifically binds CTLA-4. In some embodiments, the V_H and V_L domains are derived from ipilimumab (*e.g.*, Yervoy®). In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 383 and V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 384. In some embodiments, V_H and V_L form an antigen binding site that specifically binds PD-1 (such as a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein). In some embodiments, the V_H and V_L domains are derived from pembrolizumab (*e.g.*, Keytruda®) or nivolumab (*e.g.*, Opdivo®). In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 385 and V_L comprises LC-CDR1, LC-

CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 386. In some embodiments, the C_H3 and anti-PD-1 sdAb moiety are fused to each other optionally via a peptide linker, such as a peptide linker comprising the amino acid sequence of any one of SEQ ID NOs: 367-376. In some embodiments, the C_H2 and C_H3 domains are derived from IgG1 Fc, effectorless IgG1 Fc, IgG2 Fc, IgG4 Fc, or IgG4 Fc (S228P), e.g., any of SEQ ID NOs: 363-365. In some embodiments, the anti-PD-1 BABP comprises two identical copies of the first polypeptide and two identical copies of the second polypeptide. In some embodiments, the K_d of the binding between the anti-PD-1 sdAb moiety and PD-1 is about 10^{-5} M to about 10^{-12} M (such as about 10^{-7} M to about 10^{-12} M, or about 10^{-8} M to about 10^{-12} M). In some embodiments, the anti-PD-1 sdAb moiety is camelid, chimeric, human, partially humanized, or fully humanized. In some embodiments, the PD-1 BABP has the structure as shown in FIG. 17.

[0241] In some embodiments, there is provided an anti-PD-1 BABP comprising: (a) a first polypeptide comprising from N-terminus to C-terminus: anti-PD-1 sdAb moeity-V_H-C_H1-C_H2-C_H3; and (b) a second polypeptide comprising from N-terminus to C-terminus: V_L-C_L, wherein V_H and V_L forms an antigen binding site that specifically binds a second epitope (*e.g.*, TIGIT, LAG-3, TIM-3, CTLA-4, PD-1 (such as a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein)), and wherein the anti-PD-1 sdAb moiety comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 37-72, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 109-144, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 181-216, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, the anti-PD-1 sdAb moiety comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 37-72; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 109-144; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 181-216. In some embodiments, the anti-PD-1 sdAb moiety comprises a V_HH domain comprising the amino acid sequence of any one of SEQ ID NO: 289-324. In some embodiments, V_H and V_L form an antigen binding site that specifically binds TIGIT. In some embodiments, the V_H and V_L domains are derived from tiragolumab. In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 377, and V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 378. In some embodiments, V_H and V_L form an antigen binding site that specifically binds LAG-3. In some embodiments, the V_H and V_L domains are derived from relatlimab. In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 379, and V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 380. In some embodiments, V_H and V_L form an antigen binding site that

specifically binds TIM-3. In some embodiments, the V_H and V_L domains are derived from MBG453. In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 381 and V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 382. In some embodiments, V_H and V_L form an antigen binding site that specifically binds CTLA-4. In some embodiments, the V_H and V_L domains are derived from ipilimumab (*e.g.*, Yervoy®). In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 383 and V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 384. In some embodiments, V_H and V_L form an antigen binding site that specifically binds PD-1 (such as a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein). In some embodiments, the V_H and V_L domains are derived from pembrolizumab (*e.g.*, Keytruda®) or nivolumab (*e.g.*, Opdivo®). In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 385 and V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 386. In some embodiments, the V_H and the anti-PD-1 sdAb moiety are fused to each other optionally via a peptide linker, such as a peptide linker comprising the amino acid sequence of any one of SEQ ID NOs: 367-376. In some embodiments, the C_H2 and C_H3 domains are derived from IgG1 Fc, effectorless IgG1 Fc, IgG2 Fc, IgG4 Fc, or IgG4 Fc (S228P), e.g., any of SEQ ID NOs: 363-365. In some embodiments, the anti-PD-1 BABP comprises two identical copies of the first polypeptide and two identical copies of the second polypeptide. In some embodiments, the K_d of the binding between the anti-PD-1 sdAb moiety and PD-1 is about 10^{-5} M to about 10^{-12} M (such as about 10^{-7} M to about 10^{-12} M, or about 10^{-8} M to about 10^{-12} M). In some embodiments, the anti-PD-1 sdAb moiety is camelid, chimeric, human, partially humanized, or fully humanized. In some embodiments, the PD-1 BABP has the structure as shown in FIG. 16.

[0242] In some embodiments, there is provided an anti-PD-1 BABP comprising: (a) a first polypeptide comprising from N-terminus to C-terminus: V_H-C_H1-C_H2-C_H3; and (b) a second polypeptide comprising from N-terminus to C-terminus: V_L-C_L-anti-PD-1 sdAb moiety, wherein V_H and V_L forms an antigen binding site that specifically binds a second epitope (*e.g.*, TIGIT, LAG-3, TIM-3, CTLA-4, PD-1 (such as a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein)), and wherein the anti-PD-1 sdAb moiety comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 37-72, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 109-144, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 181-216, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In

some embodiments, the anti-PD-1 sdAb moiety comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 37-72; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 109-144; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 181-216. In some embodiments, the anti-PD-1 sdAb moiety comprises a V_HH domain comprising the amino acid sequence of any one of SEQ ID NO: 289-324. In some embodiments, V_H and V_L form an antigen binding site that specifically binds TIGIT. In some embodiments, the V_H and V_L domains are derived from tiragolumab. In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 377, and V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 378. In some embodiments, V_H and V_L form an antigen binding site that specifically binds LAG-3. In some embodiments, the V_H and V_L domains are derived from relatlimab. In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 379, and V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 380. In some embodiments, V_H and V_L form an antigen binding site that specifically binds TIM-3. In some embodiments, the V_H and V_L domains are derived from MBG453. In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 381 and V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 382. In some embodiments, V_H and V_L form an antigen binding site that specifically binds CTLA-4. In some embodiments, the V_H and V_L domains are derived from ipilimumab (*e.g.*, Yervoy®). In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 383 and V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 384. In some embodiments, V_H and V_L form an antigen binding site that specifically binds PD-1 (such as a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein). In some embodiments, the V_H and V_L domains are derived from pembrolizumab (*e.g.*, Keytruda®) or nivolumab (*e.g.*, Opdivo®). In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 385 and V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 386. In some embodiments, the C_L and the anti-PD-1 sdAb moiety are fused to each other optionally via a peptide linker, such as a peptide linker comprising the amino acid sequence of any one of SEQ ID NOs: 367-376. In some embodiments, the C_H2 and C_H3 domains are derived from IgG1 Fc, effectorless IgG1 Fc, IgG2 Fc, IgG4 Fc, or IgG4 Fc (S228P), e.g., any of SEQ ID NOs: 363-365. In some embodiments, the anti-PD-1 BABP comprises two identical copies of the first polypeptide and two identical copies of the second polypeptide. In some embodiments, the K_d of the binding between the anti-PD-1 sdAb moiety and PD-1 is about 10^{-5} M to about 10^{-12} M (such as about 10^{-7} M to about 10^{-12} M, or about 10^{-8} M to about 10^{-12} M).

In some embodiments, the anti-PD-1 sdAb moiety is camelid, chimeric, human, partially humanized, or fully humanized. In some embodiments, the PD-1 BABP has the structure as shown in FIG. 19.

[0243] In some embodiments, there is provided an anti-PD-1 BABP comprising: (a) a first polypeptide comprising from N-terminus to C-terminus: V_H-C_H1-C_H2-C_H3; and (b) a second polypeptide comprising from N-terminus to C-terminus: anti-PD-1 sdAb moiety-V_L-C_L, wherein V_H and V_L forms an antigen binding site that specifically binds a second epitope (*e.g.*, TIGIT, LAG-3, TIM-3, CTLA-4, PD-1 (such as a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein)), and wherein the anti-PD-1 sdAb moiety comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 37-72, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 109-144, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 181-216, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, the anti-PD-1 sdAb moiety comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 37-72; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 109-144; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 181-216. In some embodiments, the anti-PD-1 sdAb moiety comprises a V_HH domain comprising the amino acid sequence of any one of SEQ ID NO: 289-324. In some embodiments, V_H and V_L form an antigen binding site that specifically binds TIGIT. In some embodiments, the V_H and V_L domains are derived from tiragolumab. In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 377, and V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 378. In some embodiments, V_H and V_L form an antigen binding site that specifically binds LAG-3. In some embodiments, the V_H and V_L domains are derived from relatlimab. In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 379, and V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 380. In some embodiments, V_H and V_L form an antigen binding site that specifically binds TIM-3. In some embodiments, the V_H and V_L domains are derived from MBG453. In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 381 and V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 382. In some embodiments, V_H and V_L form an antigen binding site that specifically binds CTLA-4. In some embodiments, the V_H and V_L domains are derived from ipilimumab (*e.g.*, Yervoy®). In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 383 and V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 384. In some embodiments, V_H and V_L form an antigen binding site that

specifically binds PD-1 (such as a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein). In some embodiments, the V_H and V_L domains are derived from pembrolizumab (*e.g.*, Keytruda®) or nivolumab (*e.g.*, Opdivo®). In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 385 and V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 386. In some embodiments, the V_L and the anti-PD-1 sdAb moiety are fused to each other optionally via a peptide linker, such as a peptide linker comprising the amino acid sequence of any one of SEQ ID NOs: 367-376. In some embodiments, the C_H2 and C_H3 domains are derived from IgG1 Fc, effectorless IgG1 Fc, IgG2 Fc, IgG4 Fc, or IgG4 Fc (S228P), e.g., any of SEQ ID NOs: 363-365. In some embodiments, the anti-PD-1 BABP comprises two identical copies of the first polypeptide and two identical copies of the second polypeptide. In some embodiments, the K_d of the binding between the anti-PD-1 sdAb moiety and PD-1 is about 10^{-5} M to about 10^{-12} M (such as about 10^{-7} M to about 10^{-12} M, or about 10^{-8} M to about 10^{-12} M). In some embodiments, the anti-PD-1 sdAb moiety is camelid, chimeric, human, partially humanized, or fully humanized. In some embodiments, the PD-1 BABP has the structure as shown in FIG. 18.

[0244] In some embodiments, there is provided an anti-PD-1 MABP (*e.g.*, BABP) comprising: (a) a first polypeptide comprising from N-terminus to C-terminus: anti-PD-1 sdAb1 moiety-V_H-C_H1-C_H2-C_H3; and (b) a second polypeptide comprising from N-terminus to C-terminus: anti-PD-1 sdAb2 moiety-V_L-C_L, wherein V_H and V_L forms an antigen binding site that specifically binds a second epitope (*e.g.*, TIGIT, LAG-3, TIM-3, CTLA-4, PD-1 (such as a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein)), and wherein the anti-PD-1 sdAb1 moiety and the anti-PD-1 sdAb2 moiety each comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 37-72, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 109-144, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 181-216, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, the anti-PD-1 sdAb1 moiety and the anti-PD-1 sdAb2 moiety each comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 37-72; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 109-144; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 181-216. In some embodiments, the anti-PD-1 sdAb1 moiety and the anti-PD-1 sdAb2 moiety each comprises a V_HH domain comprising the amino acid sequence of any one of SEQ ID NO: 289-324. In some embodiments, the anti-PD-1 sdAb1 moiety and the anti-PD-1 sdAb2 moiety are the same. In some embodiments, the anti-PD-1 sdAb1 moiety and the anti-PD-1 sdAb2 moiety are different. In some embodiments, V_H and V_L form an antigen binding site that specifically binds TIGIT. In some

embodiments, the V_H and V_L domains are derived from tiragolumab. In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 377, and V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 378. In some embodiments, V_H and V_L form an antigen binding site that specifically binds LAG-3. In some embodiments, the V_H and V_L domains are derived from relatlimab. In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 379, and V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 380. In some embodiments, V_H and V_L form an antigen binding site that specifically binds TIM-3. In some embodiments, the V_H and V_L domains are derived from MBG453. In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 381 and V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 382. In some embodiments, V_H and V_L form an antigen binding site that specifically binds CTLA-4. In some embodiments, the V_H and V_L domains are derived from ipilimumab (*e.g.*, Yervoy®). In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 383 and V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 384. In some embodiments, V_H and V_L form an antigen binding site that specifically binds PD-1 (such as a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein). In some embodiments, the V_H and V_L domains are derived from pembrolizumab (*e.g.*, Keytruda®) or nivolumab (*e.g.*, Opdivo®). In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 385 and V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 386. In some embodiments, the V_L and anti-PD-1 sdAb2 moiety are fused to each other optionally via a peptide linker, such as a peptide linker comprising the amino acid sequence of any one of SEQ ID NOs: 367-376. In some embodiments, the V_H and anti-PD-1 sdAb1 moiety are fused to each other optionally via a peptide linker, such as a peptide linker comprising the amino acid sequence of any one of SEQ ID NOs: 367-376. In some embodiments, the C_H2 and C_H3 domains are derived from IgG1 Fc, effectorless IgG1 Fc, IgG2 Fc, IgG4 Fc, or IgG4 Fc (S228P), e.g., any of SEQ ID NOs: 363-365. In some embodiments, the anti-PD-1 MABP (*e.g.*, BABP) comprises two identical copies of the first polypeptide and two identical copies of the second polypeptide. In some embodiments, the K_d of the binding between the anti-PD-1 sdAb moiety and PD-1 is about 10^{-5} M to about 10^{-12} M (such as about 10^{-7} M to about 10^{-12} M, or about 10^{-8} M to about 10^{-12} M). In some embodiments, the anti-PD-1 sdAb moiety is camelid, chimeric, human, partially humanized, or fully humanized. In some embodiments, the PD-1 MABP (*e.g.*, BABP) has the structure as shown in FIG. 20.

[0245] In some embodiments, there is provided an anti-PD-1 MABP (*e.g.*, BABP) comprising: (a) a first polypeptide comprising from N-terminus to C-terminus: anti-PD-1 sdAb1 moiety-anti-PD-1 sdAb2

moiety-V_H-C_H1-C_H2-C_H3; and (b) a second polypeptide comprising from N-terminus to C-terminus: V_L-C_L, wherein V_H and V_L forms an antigen binding site that specifically binds a second epitope (*e.g.*, TIGIT, LAG-3, TIM-3, CTLA-4, PD-1 (such as a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein)), and wherein the anti-PD-1 sdAb1 moiety and the anti-PD-1 sdAb2 moiety each comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 37-72, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 109-144, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 181-216, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, the anti-PD-1 sdAb1 moiety and the anti-PD-1 sdAb2 moiety each comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 37-72; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 109-144; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 181-216. In some embodiments, the anti-PD-1 sdAb1 moiety and the anti-PD-1 sdAb2 moiety each comprises a V_HH domain comprising the amino acid sequence of any one of SEQ ID NOs: 289-324. In some embodiments, the anti-PD-1 sdAb1 moiety and the anti-PD-1 sdAb2 moiety are the same. In some embodiments, the anti-PD-1 sdAb1 moiety and the anti-PD-1 sdAb2 moiety are different. In some embodiments, V_H and V_L form an antigen binding site that specifically binds TIGIT. In some embodiments, the V_H and V_L domains are derived from tiragolumab. In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 377, and V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 378. In some embodiments, V_H and V_L form an antigen binding site that specifically binds LAG-3. In some embodiments, the V_H and V_L domains are derived from relatlimab. In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 379, and V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 380. In some embodiments, V_H and V_L form an antigen binding site that specifically binds TIM-3. In some embodiments, the V_H and V_L domains are derived from MBG453. In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 381 and V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 382. In some embodiments, V_H and V_L form an antigen binding site that specifically binds CTLA-4. In some embodiments, the V_H and V_L domains are derived from ipilimumab (*e.g.*, Yervoy®). In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 383 and V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 384. In some embodiments, V_H and V_L form an antigen binding site that specifically binds

PD-1 (such as a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein). In some embodiments, the V_H and V_L domains are derived from pembrolizumab (*e.g.*, Keytruda®) or nivolumab (*e.g.*, Opdivo®). In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 385 and V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 386. In some embodiments, the anti-PD-1 sdAb1 moiety and the anti-PD-1 sdAb2 moiety, and/or the V_H and anti-PD-1 sdAb2 moiety, are fused to each other optionally via a peptide linker, such as a peptide linker comprising the amino acid sequence of any one of SEQ ID NOs: 367-376. In some embodiments, the C_H2 and C_H3 domains are derived from IgG1 Fc, effectorless IgG1 Fc, IgG2 Fc, IgG4 Fc, or IgG4 Fc (S228P), e.g., any of SEQ ID NOs: 363-365. In some embodiments, the anti-PD-1 MABP (e.g., BABP) comprises two identical copies of the first polypeptide and two identical copies of the second polypeptide. In some embodiments, the K_d of the binding between the anti-PD-1 sdAb moiety and PD-1 is about 10^{-5} M to about 10^{-12} M (such as about 10^{-7} M to about 10^{-12} M, or about 10^{-8} M to about 10^{-12} M). In some embodiments, the anti-PD-1 sdAb moiety is camelid, chimeric, human, partially humanized, or fully humanized. In some embodiments, the PD-1 MABP (*e.g.*, BABP) has the structure as shown in FIG. 21.

[0246] In some embodiments, there is provided an anti-PD-1 BABP comprising: (a) a first polypeptide comprising from N-terminus to C-terminus: V_H-C_H1-anti-PD-1 sdAb moiety-C_H2-C_H3; and (b) a second polypeptide comprising from N-terminus to C-terminus: V_L-C_L, wherein V_H and V_L forms an antigen binding site that specifically binds a second epitope (*e.g.*, TIGIT, LAG-3, TIM-3, CTLA-4, PD-1 (such as a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein)), and wherein the anti-PD-1 sdAb moiety comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 37-72, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 109-144, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 181-216, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, the anti-PD-1 sdAb moiety comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 37-72; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 109-144; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 181-216. In some embodiments, the anti-PD-1 sdAb moiety comprises a V_HH domain comprising the amino acid sequence of any one of SEQ ID NO: 289-324. In some embodiments, V_H and V_L form an antigen binding site that specifically binds TIGIT. In some embodiments, the V_H and V_L domains are derived from tiragolumab. In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 377, and V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the

amino acid sequence of SEQ ID NO: 378. In some embodiments, V_H and V_L form an antigen binding site that specifically binds LAG-3. In some embodiments, the V_H and V_L domains are derived from relatlimab. In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 379, and V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 380. In some embodiments, V_H and V_L form an antigen binding site that specifically binds TIM-3. In some embodiments, the V_H and V_L domains are derived from MBG453. In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 381 and V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 382. In some embodiments, V_H and V_L form an antigen binding site that specifically binds CTLA-4. In some embodiments, the V_H and V_L domains are derived from ipilimumab (*e.g.*, Yervoy®). In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 383 and V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 384. In some embodiments, V_H and V_L form an antigen binding site that specifically binds PD-1 (such as a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein). In some embodiments, the V_H and V_L domains are derived from pembrolizumab (*e.g.*, Keytruda®) or nivolumab (*e.g.*, Opdivo®). In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 385 and V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 386. In some embodiments, the C_H1 and the anti-PD-1 sdAb moiety are fused to each other optionally via a peptide linker, such as a peptide linker comprising the amino acid sequence of any one of SEQ ID NOs: 367-376. In some embodiments, the C_H2 and C_H3 domains are derived from IgG1 Fc, effectorless IgG1 Fc, IgG2 Fc, IgG4 Fc, or IgG4 Fc (S228P), e.g., any of SEQ ID NOs: 363-365. In some embodiments, the anti-PD-1 BABP comprises two identical copies of the first polypeptide and two identical copies of the second polypeptide. In some embodiments, the K_d of the binding between the anti-PD-1 sdAb moiety and PD-1 is about 10^{-5} M to about 10^{-12} M (such as about 10^{-7} M to about 10^{-12} M, or about 10^{-8} M to about 10^{-12} M). In some embodiments, the anti-PD-1 sdAb moiety is camelid, chimeric, human, partially humanized, or fully humanized. In some embodiments, the PD-1 BABP has the structure as shown in FIG. 22.

[0247] In some embodiments, there is provided an anti-PD-1 BABP comprising a polypeptide comprising from N-terminus to C-terminus: V_L-V_H-anti-PD-1 sdAb moiety-C_H2-C_H3, wherein the V_L and V_H together forms an scFv that specifically binds a second epitope (*e.g.*, TIGIT, LAG-3, TIM-3, CTLA-4, PD-1 (such as a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein)), and wherein the anti-PD-1 sdAb moiety comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 37-72, or a variant thereof comprising up to about 3 (such as about

any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 109-144, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 181-216, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, there is provided an anti-PD-1 BABP comprising a polypeptide comprising from N-terminus to C-terminus: V_H-V_L-anti-PD-1 sdAb moiety-C_H2-C_H3, wherein the V_L and V_H together forms an scFv that specifically binds a second epitope (*e.g.*, TIGIT, LAG-3, TIM-3, CTLA-4, PD-1 (such as a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein)), and wherein the anti-PD-1 sdAb moiety comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 37-72, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 109-144, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 181-216, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, the anti-PD-1 sdAb moiety comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 37-72; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 109-144; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 181-216. In some embodiments, the anti-PD-1 sdAb moiety comprises a V_HH domain comprising the amino acid sequence of any one of SEQ ID NO: 289-324. In some embodiments, the scFv (or the V_L and V_H that form the scFv) specifically binds TIGIT. In some embodiments, the scFv (or the V_L and V_H that form the scFv) is derived from tiragolumab. In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 377, and V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 378. In some embodiments, the scFv (or the V_L and V_H that form the scFv) specifically binds LAG-3. In some embodiments, the scFv (or the V_L and V_H that form the scFv) is derived from relatlimab. In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 379, and V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 380. In some embodiments, the scFv (or the V_L and V_H that form the scFv) specifically binds TIM-3. In some embodiments, the scFv (or the V_L and V_H that form the scFv) is derived from MBG453. In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 381 and V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 382. In some embodiments, the scFv (or the V_L and V_H that form the scFv) specifically binds CTLA-4. In some embodiments, the scFv (or the V_L and V_H that form the scFv) is derived from ipilimumab (*e.g.*, Yervoy®). In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-

CDR3 of the amino acid sequence of SEQ ID NO: 383 and V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 384. In some embodiments, the scFv (or the V_L and V_H that form the scFv) specifically binds PD-1 (such as a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein). In some embodiments, the scFv (or the V_L and V_H that form the scFv) is derived from pembrolizumab (*e.g.*, Keytruda®) or nivolumab (*e.g.*, Opdivo®). In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 385 and V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 386. In some embodiments, the V_H and V_L that forms the scFv, and/or the scFv and the anti-PD-1 sdAb moiety, are fused to each other optionally via a peptide linker, such as a peptide linker comprising the amino acid sequence of any one of SEQ ID NOs: 367-376. In some embodiments, the C_H2 and C_H3 domains are derived from IgG1 Fc, effectorless IgG1 Fc, IgG2 Fc, IgG4 Fc, or IgG4 Fc (S228P), e.g., any of SEQ ID NOs: 363-365. In some embodiments, the anti-PD-1 BABP comprises two identical copies of the polypeptide. In some embodiments, the K_d of the binding between the anti-PD-1 sdAb moiety and PD-1 is about 10^{-5} M to about 10^{-12} M (such as about 10^{-7} M to about 10^{-12} M, or about 10^{-8} M to about 10^{-12} M). In some embodiments, the anti-PD-1 sdAb moiety is camelid, chimeric, human, partially humanized, or fully humanized. In some embodiments, the PD-1 BABP has the structure as shown in FIG. 23.

[0248] In some embodiments, there is provided an anti-PD-1 MABP (*e.g.*, BABP) comprising: (a) a first polypeptide comprising from N-terminus to C-terminus: V_H-C_H1-anti-PD-1 sdAb1 moiety-C_H1-C_H2-C_H3; and (b) a second polypeptide comprising from N-terminus to C-terminus: V_L-C_L-anti-PD-1 sdAb2 moiety-C_L, wherein V_H and V_L forms an antigen binding site that specifically binds a second epitope (*e.g.*, TIGIT, LAG-3, TIM-3, CTLA-4, PD-1 (such as a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein)), and wherein the anti-PD-1 sdAb1 moiety and the anti-PD-1 sdAb2 moiety each comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 37-72, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 109-144, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 181-216, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, the anti-PD-1 sdAb1 moiety and the anti-PD-1 sdAb2 moiety each comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 37-72; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 109-144; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 181-216. In some embodiments, the anti-PD-1 sdAb1 moiety and the anti-PD-1 sdAb2 moiety each comprises a V_HH domain comprising the amino acid sequence of any one of SEQ ID NOs: 289-324.

In some embodiments, the anti-PD-1 sdAb1 moiety and the anti-PD-1 sdAb2 moiety are the same. In some embodiments, the anti-PD-1 sdAb1 moiety and the anti-PD-1 sdAb2 moiety are different. In some embodiments, V_H and V_L form an antigen binding site that specifically binds TIGIT. In some embodiments, the V_H and V_L domains are derived from tiragolumab. In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 377, and V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 378. In some embodiments, V_H and V_L form an antigen binding site that specifically binds LAG-3. In some embodiments, the V_H and V_L domains are derived from relatlimab. In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 379, and V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 380. In some embodiments, V_H and V_L form an antigen binding site that specifically binds TIM-3. In some embodiments, the V_H and V_L domains are derived from MBG453. In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 381 and V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 382. In some embodiments, V_H and V_L form an antigen binding site that specifically binds CTLA-4. In some embodiments, the V_H and V_L domains are derived from ipilimumab (*e.g.*, Yervoy®). In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 383 and V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 384. In some embodiments, V_H and V_L form an antigen binding site that specifically binds PD-1 (such as a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein). In some embodiments, the V_H and V_L domains are derived from pembrolizumab (*e.g.*, Keytruda®) or nivolumab (*e.g.*, Opdivo®). In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 385 and V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 386. In some embodiments, the C_H1 and the anti-PD-1 sdAb1 moiety, and/or C_L and the anti-PD-1 sdAb2 moiety, are fused to each other optionally via a peptide linker, such as a peptide linker comprising the amino acid sequence of any one of SEQ ID NOs: 367-376. In some embodiments, the C_H2 and C_H3 domains are derived from IgG1 Fc, effectorless IgG1 Fc, IgG2 Fc, IgG4 Fc, or IgG4 Fc (S228P), e.g., any of SEQ ID NOs: 363-365. In some embodiments, the anti-PD-1 MABP (e.g., BABP) comprises two identical copies of the first polypeptide and two identical copies of the second polypeptide. In some embodiments, the K_d of the binding between the anti-PD-1 sdAb moiety and PD-1 is about 10^{-5} M to about 10^{-12} M (such as about 10^{-7} M to about 10^{-12} M, or about 10^{-8} M to about 10^{-12} M). In some embodiments, the anti-PD-1 sdAb moiety is camelid, chimeric, human, partially humanized, or fully humanized. In some embodiments, the PD-1 MABP (*e.g.*, BABP) has the structure as shown in FIG. 24.

[0249] In some embodiments, there is provided an anti-PD-1 MABP (*e.g.*, BABP) comprising: (a) a first polypeptide comprising from N-terminus to C-terminus: V_L-V_H-anti-PD-1 sdAb1 moiety-C_H2-C_H3; and (b) a second polypeptide comprising from N-terminus to C-terminus: anti-PD-1 sdAb2 moiety-C_L, wherein the V_L and V_H that forms the scFv specifically binds a second epitope (*e.g.*, TIGIT, LAG-3, TIM-3, CTLA-4, PD-1 (such as a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein)), and wherein the anti-PD-1 sdAb1 moiety and the anti-PD-1 sdAb2 moiety each comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 37-72, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 109-144, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 181-216, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, there is provided an anti-PD-1 MABP (*e.g.*, BABP) comprising: (a) a first polypeptide comprising from N-terminus to C-terminus: V_H-V_L-anti-PD-1 sdAb1 moiety-C_H2-C_H3; and (b) a second polypeptide comprising from N-terminus to C-terminus: anti-PD-1 sdAb2 moiety-C_L, wherein the V_L and V_H that forms the scFv specifically binds a second epitope (*e.g.*, TIGIT, LAG-3, TIM-3, CTLA-4, PD-1 (such as a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein)), and wherein the anti-PD-1 sdAb1 moiety and the anti-PD-1 sdAb2 moiety each comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 37-72, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 109-144, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 181-216, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, the anti-PD-1 sdAb1 moiety and the anti-PD-1 sdAb2 moiety each comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 37-72; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 109-144; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 181-216. In some embodiments, the anti-PD-1 sdAb1 moiety and the anti-PD-1 sdAb2 moiety each comprises a V_HH domain comprising the amino acid sequence of any one of SEQ ID NOs: 289-324. In some embodiments, the anti-PD-1 sdAb1 moiety and the anti-PD-1 sdAb2 moiety are the same. In some embodiments, the anti-PD-1 sdAb1 moiety and the anti-PD-1 sdAb2 moiety are different. In some embodiments, the scFv (or the V_L and V_H that form the scFv) specifically binds TIGIT. In some embodiments, the scFv (or the V_L and V_H that form the scFv) is derived from tiragolumab. In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 377, and V_L comprises LC-CDR1, LC-CDR2, and LC-

CDR3 of the amino acid sequence of SEQ ID NO: 378. In some embodiments, the scFv (or the V_L and V_H that form the scFv) specifically binds LAG-3. In some embodiments, the scFv (or the V_L and V_H that form the scFv) is derived from relatlimab. In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 379, and V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 380. In some embodiments, the scFv (or the V_L and V_H that form the scFv) specifically binds TIM-3. In some embodiments, the scFv (or the V_L and V_H that form the scFv) is derived from MBG453. In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 381 and V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 382. In some embodiments, the scFv (or the V_L and V_H that form the scFv) specifically binds CTLA-4. In some embodiments, the scFv (or the V_L and V_H that form the scFv) is derived from ipilimumab (*e.g.*, Yervoy®). In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 383 and V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 384. In some embodiments, the scFv (or the V_L and V_H that form the scFv) specifically binds PD-1 (such as a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein). In some embodiments, the scFv (or the V_L and V_H that form the scFv) is derived from pembrolizumab (*e.g.*, Keytruda®) or nivolumab (*e.g.*, Opdivo®). In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 385 and V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 386. In some embodiments, the V_H and V_L that forms the scFv, and/or the scFv and the anti-PD-1 sdAb1 moiety, are fused to each other optionally via a peptide linker, such as a peptide linker comprising the amino acid sequence of any one of SEQ ID NOs: 367-376. In some embodiments, the C_H2 and C_H3 domains are derived from IgG1 Fc, effectorless IgG1 Fc, IgG2 Fc, IgG4 Fc, or IgG4 Fc (S228P), e.g., any of SEQ ID NOs: 363-365. In some embodiments, the anti-PD-1 MABP (*e.g.*, BABP) comprises two identical copies of the first polypeptide and two identical copies of the second polypeptide. In some embodiments, the K_d of the binding between the anti-PD-1 sdAb moiety and PD-1 is about 10^{-5} M to about 10^{-12} M (such as about 10^{-7} M to about 10^{-12} M, or about 10^{-8} M to about 10^{-12} M). In some embodiments, the anti-PD-1 sdAb moiety is camelid, chimeric, human, partially humanized, or fully humanized. In some embodiments, the PD-1 MABP (*e.g.*, BABP) has the structure as shown in FIG. 25.

[0250] In some embodiments, there is also provided an anti-PD-1 MABP (*e.g.*, BABP) comprising an sdAb moiety specifically recognizing PD-1 (hereinafter referred to as "competing anti-PD-1 construct", "competing anti-PD-1 MABP", or "competing anti-PD-1 BABP") that specifically binds to PD-1 competitively with any one of the anti-PD-1 construct described herein (such as anti-PD-1 sdAb moiety, anti-PD-1 sdAb-Fc fusion protein (e.g., HCAb), multispecific (e.g., bispecific) or monospecific anti-PD-1

construct comprising an anti-PD-1 sdAb moiety descried herein, e.g., anti-PD-1/TIGIT, anti-PD-1/LAG-3, anti-PD-1/TIM-3, anti-PD-1/CTLA-4, or anti-PD-1/PD-1 constructs (*e.g.*, MABP or BABP) described herein).

(III) Anti-PD-1 construct antibody variants

[0251] In some embodiments, amino acid sequence variants of the anti-PD-1 construct (e.g., anti-PD-1 sdAb moiety, anti-PD-1 sdAb-Fc fusion protein (e.g., HCAb), anti-PD-1 MABP/BABP) provided herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. Amino acid sequence variants of an antibody may be prepared by introducing appropriate modifications into the nucleic acid sequence encoding the antibody, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics, *e.g.*, antigen-binding.

a) Substitution, insertion, deletion and variants

[0252] In some embodiments, antibody variants having one or more amino acid substitutions are provided. Sites of interest for substitutional mutagenesis include the HVRs (or CDRs) and FRs. Conservative substitutions are shown in Table 2 under the heading of "Preferred substitutions." More substantial changes are provided in Table 2 under the heading of "exemplary substitutions," and as further described below in reference to amino acid side chain classes. Amino acid substitutions may be introduced into an antibody of interest and the products screened for a desired activity, *e.g.*, retained/improved antigen binding, decreased immunogenicity, or improved ADCC or CDC.

Table 2. Amino acid substitutions

Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala (A)	Val; Leu; Ile	Val
Arg (R)	Lys; Gln; Asn	Lys
Asn (N)	Gln; His; Asp, Lys; Arg	Gln
Asp (D)	Glu; Asn	Glu
Cys (C)	Ser; Ala	Ser
Gln (Q)	Asn; Glu	Asn
Glu (E)	Asp; Gln	Asp
Gly (G)	Ala	Ala
His (H)	Asn; Gln; Lys; Arg	Arg
Ile (I)	Leu; Val; Met; Ala; Phe; Norleucine	Leu
Leu (L)	Norleucine; Ile; Val; Met; Ala; Phe	Ile

Lys (K)	Arg; Gln; Asn	Arg
Met (M)	Leu; Phe; Ile	Leu
Phe (F)	Trp; Leu; Val; Ile; Ala; Tyr	Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Val; Ser	Ser
Trp (W)	Tyr; Phe	Tyr
Tyr (Y)	Trp; Phe; Thr; Ser	Phe
Val (V)	Ile; Leu; Met; Phe; Ala; Norleucine	Leu

[0253] Amino acids may be grouped according to common side-chain properties: (1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile; (2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln; (3) acidic: Asp, Glu; (4) basic: His, Lys, Arg; (5) residues that influence chain orientation: Gly, Pro; and (6) aromatic: Trp, Tyr, Phe.

[0254] Non-conservative substitutions will entail exchanging a member of one of these classes for another class.

[0255] One type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody (*e.g.*, a humanized or human antibody). Generally, the resulting variant(s) selected for further study will have modifications (*e.g.,* improvements) in certain biological properties (*e.g.*, increased affinity, reduced immunogenicity) relative to the parent antibody and/or will have substantially retained certain biological properties of the parent antibody. An exemplary substitutional variant is an affinity matured antibody, which may be conveniently generated, *e.g.*, using phage display-based affinity maturation techniques such as those described herein. Briefly, one or more HVR residues are mutated and the variant antibodies displayed on phage and screened for a particular biological activity (*e.g.* binding affinity).

[0256] Alterations (*e.g.*, substitutions) may be made in HVRs, *e.g.*, to improve antibody affinity. Such alterations may be made in HVR "hotspots," *i.e.*, residues encoded by codons that undergo mutation at high frequency during the somatic maturation process (*see, e.g.*, Chowdhury, *Methods Mol. Biol.* 207:179-196 (2008)), and/or SDRs (a-CDRs), with the resulting variant VH or VL being tested for binding affinity. Affinity maturation by constructing and reselecting from secondary libraries has been described, *e.g.*, in Hoogenboom et al. in *Methods in Molecular Biology* 178:1-37 (O'Brien et al., ed., Human Press, Totowa, NJ, (2001)). In some embodiments of affinity maturation, diversity is introduced into the variable genes chosen for maturation by any of a variety of methods (*e.g.*, error-prone PCR, chain shuffling, or oligonucleotide-directed mutagenesis). A secondary library is then created. The library is then screened to identify any antibody variants with the desired affinity. Another method to introduce

diversity involves HVR-directed approaches, in which several HVR residues (*e.g.*, 4-6 residues at a time) are randomized. HVR residues involved in antigen binding may be specifically identified, e.g., using alanine scanning mutagenesis or modeling. CDR-H3 and CDR-L3 in particular are often targeted.

[0257] In some embodiments, substitutions, insertions, or deletions may occur within one or more HVRs so long as such alterations do not substantially reduce the ability of the antibody to bind antigen. For example, conservative alterations (*e.g.*, conservative substitutions as provided herein) that do not substantially reduce binding affinity may be made in HVRs. Such alterations may be outside of HVR "hotspots" or CDRs. In some embodiments of the variant V_HH sequences provided above, each HVR either is unaltered, or contains no more than one, two or three amino acid substitutions.

[0258] A useful method for identification of residues or regions of an antibody that may be targeted for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells (1989) *Science*, 244:1081-1085. In this method, a residue or group of target residues (*e.g.*, charged residues such as Arg, Asp, His, Lys, and Glu) are identified and replaced by a neutral or negatively charged amino acid (*e.g.*, alanine or polyalanine) to determine whether the interaction of the antibody with antigen is affected. Further substitutions may be introduced at the amino acid locations demonstrating functional sensitivity to the initial substitutions. Alternatively, or additionally, a crystal structure of an antigen-antibody complex to identify contact points between the antibody and antigen. Such contact residues and neighboring residues may be targeted or eliminated as candidates for substitution. Variants may be screened to determine whether they contain the desired properties.

[0259] Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue. Other insertional variants of the antibody molecule include the fusion to the N- or C-terminus of the antibody to an enzyme (*e.g.*, for ADEPT) or a polypeptide which increases the serum half-life of the antibody.

b) Glycosylation variants

[0260] In some embodiments, an isolated anti-PD-1 construct provided herein is altered to increase or decrease the extent to which the construct is glycosylated. Addition or deletion of glycosylation sites to an antibody may be conveniently accomplished by altering the amino acid sequence such that one or more glycosylation sites is created or removed.

[0261] Where the anti-PD-1 construct comprises an Fc region (*e.g.*, anti-PD-1 sdAb-Fc fusion protein (*e.g.*, HCAb), PD-1×TIGIT MABP, PD-1×LAG-3 MABP, PD-1×TIM-3 MABP, PD-1×CTLA-4 MABP,

or PD-1×PD-1 MABP), the carbohydrate attached thereto may be altered. Native antibodies produced by mammalian cells typically comprise a branched, biantennary oligosaccharide that is generally attached by an N-linkage to Asn297 of the C_H2 domain of the Fc region. *See, e.g.*, Wright et al. *TIBTECH* 15:26-32 (1997). The oligosaccharide may include various carbohydrates, *e.g.*, mannose, N-acetyl glucosamine (GlcNAc), galactose, and sialic acid, as well as a fucose attached to a GlcNAc in the "stem" of the biantennary oligosaccharide structure. In some embodiments, modifications of the oligosaccharide in an anti-PD-1 construct of the present application may be made in order to create antibody variants with certain improved properties.

[0262] In some embodiments, anti-PD-1 construct antibody variants are provided having a carbohydrate structure that lacks fucose attached (directly or indirectly) to an Fc region. For example, the amount of fucose in such antibody may be from 1% to 80%, from 1% to 65%, from 5% to 65% or from 20% to 40%. The amount of fucose is determined by calculating the average amount of fucose within the sugar chain at Asn297, relative to the sum of all glycostructures attached to Asn 297 (*e.g.*, complex, hybrid and high mannose structures) as measured by MALDI-TOF mass spectrometry, as described in WO 2008/077546, for example. Asn297 refers to the asparagine residue located at about position 297 in the Fc region (EU numbering of Fc region residues); however, Asn297 may also be located about ± 3 amino acids upstream or downstream of position 297, *i.e.,* between positions 294 and 300, due to minor sequence variations in antibodies. Such fucosylation variants may have improved ADCC function. *See, e.g.,* US Patent Publication Nos. US 2003/0157108 (Presta, L.); US 2004/0093621 (Kyowa Hakko Kogyo Co., Ltd). Examples of publications related to "defucosylated" or "fucose-deficient" antibody variants include: US 2003/0157108; WO 2000/61739; WO 2001/29246; US 2003/0115614; US 2002/0164328; US 2004/0093621; US 2004/0132140; US 2004/0110704; US 2004/0110282; US 2004/0109865; WO 2003/085119; WO 2003/084570; WO 2005/035586; WO 2005/035778; WO2005/053742; WO2002/031140; Okazaki et al. *J. Mol. Biol.* 336:1239-1249 (2004); Yamane-Ohnuki et al. *Biotech. Bioeng.* 87: 614 (2004). Examples of cell lines capable of producing defucosylated antibodies include Lec13 CHO cells deficient in protein fucosylation (Ripka et al. *Arch. Biochem. Biophys.* 249:533-545 (1986); US Patent Application No. US 2003/0157108 A1, Presta, L; and WO 2004/056312 A1, Adams *et al.*, especially at Example 11), and knockout cell lines, such as alpha-1,6-fucosyltransferase gene, *FUT8,* knockout CHO cells (*see, e.g.*, Yamane-Ohnuki et al. *Biotech. Bioeng.* 87: 614 (2004); Kanda, Y. et al., *Biotechnol. Bioeng.*, 94(4):680-688 (2006); and WO2003/085107).

[0263] Anti-PD-1 construct variants are further provided with bisected oligosaccharides, *e.g.*, in which a biantennary oligosaccharide attached to the Fc region of the antibody is bisected by GlcNAc. Such antibody variants may have reduced fucosylation and/or improved ADCC function. Examples of such

antibody variants are described, *e.g.*, in WO 2003/011878 (Jean-Mairet et al.); US Patent No. 6,602,684 (Umana et al.); and US 2005/0123546 (Umana *et al.*). Antibody variants with at least one galactose residue in the oligosaccharide attached to the Fc region are also provided. Such antibody variants may have improved CDC function. Such antibody variants are described, *e.g.*, in WO 1997/30087 (Patel et al.); WO 1998/58964 (Raju, S.); and WO 1999/22764 (Raju, S.).

c) Fc region variants

[0264] In some embodiments, one or more amino acid modifications may be introduced into the Fc region of the anti-PD-1 construct provided herein (*e.g.*, anti-PD-1 sdAb-Fc fusion protein (*e.g.*, HCAb), PD-1×TIGIT MABP, PD-1×LAG-3 MABP, PD-1×TIM-3 MABP, PD-1×CTLA-4 MABP, or PD-1×PD-1 MABP), thereby generating an Fc region variant. The Fc region variant may comprise a human Fc region sequence (*e.g.*, a human IgG1, IgG2, IgG3 or IgG4 Fc region) comprising an amino acid modification (*e.g.* a substitution) at one or more amino acid positions.

[0265] In some embodiments, the present application contemplates an anti-PD-1 construct (*e.g.*, anti-PD-1 sdAb-Fc fusion protein (*e.g.*, HCAb), PD-1×TIGIT MABP, PD-1×LAG-3 MABP, PD-1×TIM-3 MABP, PD-1×CTLA-4 MABP, or PD-1×PD-1 MABP) variant that possesses some but not all effector functions, which make it a desirable candidate for applications in which the half-life of the anti-PD-1 construct *in vivo* is important yet certain effector functions (such as complement and ADCC) are unnecessary or deleterious. *In vitro* and/or *in vivo* cytotoxicity assays can be conducted to confirm the reduction/depletion of CDC and/or ADCC activities. For example, Fc receptor (FcR) binding assays can be conducted to ensure that the antibody lacks FcγR binding (hence likely lacking ADCC activity), but retains FcRn binding ability. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. FcR expression on hematopoietic cells is summarized in Table 2 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol.* 9:457-492 (1991). Non-limiting examples of *in vitro* assays to assess ADCC activity of a molecule of interest is described in U.S. Patent No. 5,500,362 (*see, e.g.* Hellstrom, I. et al. *Proc. Nat'l Acad. Sci. USA* 83:7059-7063 (1986)) and Hellstrom, I et al., *Proc. Nat'l Acad. Sci. USA* 82:1499-1502 (1985); 5,821,337 (*see* Bruggemann, M. et al., *J. Exp. Med.* 166:1351-1361 (1987)). Alternatively, non-radioactive assays methods may be employed (*see*, for example, ACTI™ non-radioactive cytotoxicity assay for flow cytometry (CellTechnology, Inc. Mountain View, CA; and CytoTox 96® non-radioactive cytotoxicity assay (Promega, Madison, WI). Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo*, *e.g.*, in an animal model such as that disclosed in Clynes et al. *Proc. Nat'l Acad. Sci. USA* 95:652-656 (1998). C1q binding assays may also be carried out to confirm that the

antibody is unable to bind C1q and hence lacks CDC activity. *See, e.g.*, C1q and C3c binding ELISA in WO 2006/029879 and WO 2005/100402. To assess complement activation, a CDC assay may be performed (*see*, for example, Gazzano-Santoro *et al.*, *J. Immunol. Methods* 202:163 (1996); Cragg, M.S. et al., *Blood* 101:1045-1052 (2003); and Cragg, M.S. and M.J. Glennie, *Blood* 103:2738-2743 (2004)). FcRn binding and *in vivo* clearance/half-life determinations can also be performed using methods known in the art (*see, e.g.*, Petkova, S.B. et al., *Int'l. Immunol.* 18(12):1759-1769 (2006)).

[0266] Antibodies with reduced effector function include those with substitution of one or more of Fc region residues 238, 265, 269, 270, 297, 327 and 329 (U.S. Patent No. 6,737,056). Such Fc mutants include Fc mutants with substitutions at two or more of amino acid positions 265, 269, 270, 297 and 327, including the so-called "DANA" Fc mutant with substitution of residues 265 and 297 to alanine (US Patent No. 7,332,581).

[0267] Certain antibody variants with improved or diminished binding to FcRs are described. (*See, e.g.*, U.S. Patent No. 6,737,056; WO 2004/056312, and Shields et al., *J. Biol. Chem.* 9(2): 6591-6604 (2001).)

[0268] In some embodiments, an anti-PD-1 construct variant comprises an Fc region with one or more amino acid substitutions which improve ADCC, *e.g.*, substitutions at positions 298, 333, and/or 334 of the Fc region (EU numbering of residues).

[0269] Anti-PD-1 constructs (such as anti-PD-1 sdAb-Fc fusion protein (e.g., HCAb), anti-PD-1 sdAb fused to a full-length antibody, or anti-PD-1 MABP/BABP described herein) comprising any of the Fc variants described herein, or combinations thereof, are contemplated.

d) Cysteine engineered antibody variants

[0270] In some embodiments, it may be desirable to create cysteine engineered anti-PD-1 constructs, *e.g.*, "thioMAbs," in which one or more residues of an antibody are substituted with cysteine residues. In particular embodiments, the substituted residues occur at accessible sites of the antibody. By substituting those residues with cysteine, reactive thiol groups are thereby positioned at accessible sites of the antibody and may be used to conjugate the antibody to other moieties, such as drug moieties or linker-drug moieties, to create an immunoconjugate, as described further herein. In some embodiments, any one or more of the following residues may be substituted with cysteine: A118 (EU numbering) of the heavy chain; and S400 (EU numbering) of the heavy chain Fc region. Cysteine engineered anti-PD-1 constructs may be generated as described, *e.g.*, in U.S. Patent No. 7,521,541.

III. Pharmaceutical compositions

[0271] Further provided by the present application are pharmaceutical compositions comprising any one of the anti-PD-1 constructs comprising a sdAb specifically recognizing PD-1 as described herein (such as anti-PD-1 sdAb, anti-PD-1 sdAb-Fc fusion protein (e.g., HCAb), anti-PD-1/TIGIT bispecific antibody (e.g., PD-1×TIGIT BABP), anti-PD-1/LAG-3 bispecific antibody (e.g., PD-1×LAG-3 BABP), anti-PD-1/TIM-3 bispecific antibody (e.g., PD-1×TIM-3 BABP), anti-PD-1/CTLA-4 bispecific antibody (e.g., PD-1×CTLA-4 BABP), or anti-PD-1/PD-1 bispecific antibody (e.g., PD-1×PD-1 BABP)), and optionally a pharmaceutically acceptable carrier. Pharmaceutical compositions can be prepared by mixing an anti-PD-1 construct described herein having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions.

[0272] The pharmaceutical composition is preferably to be stable, in which the anti-PD-1 construct comprising anti-PD-1 sdAb moiety described here essentially retains its physical and chemical stability and integrity upon storage. Various analytical techniques for measuring protein stability are available in the art and are reviewed in *Peptide and Protein Drug Delivery,* 247-301, Vincent Lee Ed., Marcel Dekker, Inc., New York, N.Y., Pubs. (1991) and Jones, A. *Adv. Drug Delivery Rev.* 10: 29-90 (1993).

[0273] In order for the pharmaceutical compositions to be used for *in vivo* administration, they must be sterile. The pharmaceutical composition may be rendered sterile by filtration through sterile filtration membranes. The pharmaceutical compositions herein generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

[0274] The route of administration is in accordance with known and accepted methods, such as by single or multiple bolus or infusion over a long period of time in a suitable manner, *e.g.*, injection or infusion by subcutaneous, intravenous, intraperitoneal, intramuscular, intra-arterial, intralesional or intraarticular routes, topical administration, inhalation or by sustained release or extended-release means. In some embodiments, the pharmaceutical composition is administered locally, such as intratumorally.

[0275] The pharmaceutical compositions herein may also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. Alternatively, or in addition, the composition may comprise a cytotoxic agent, chemotherapeutic agent, cytokine, immunosuppressive agent, or growth inhibitory agent. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

IV. Methods of treating PD-1-related diseases

[0276] The anti-PD-1 construct comprising an sdAb moiety specifically recognizing PD-1 as described herein (such as anti-PD-1 sdAb, anti-PD-1 sdAb-Fc fusion protein (e.g., HCAb), anti-PD-1/TIGIT bispecific antibody (e.g., PD-1×TIGIT BABP), anti-PD-1/LAG-3 bispecific antibody (e.g., PD-1×LAG-3 BABP), anti-PD-1/TIM-3 bispecific antibody (e.g., PD-1×TIM-3 BABP), anti-PD-1/CTLA-4 bispecific antibody (e.g., PD-1×CTLA-4 BABP), or anti-PD-1/PD-1 bispecific antibody (e.g., PD-1×PD-1 BABP)), and the compositions (such as pharmaceutical compositions) thereof are useful for a variety of applications, such as in diagnosis, molecular assays, and therapy.

[0277] One aspect of the invention provides a method of treating a PD-1 related disease or a condition in an individual in need thereof, comprising administering to the individual an effective amount of a pharmaceutical composition comprising the anti-PD-1 construct described herein. In some embodiments, the PD-1 related disease is cancer, such as solid tumor (e.g., colon cancer). In some embodiments, the PD-1-related disease is pathogenic infection, such as viral infection. In some embodiments, the PD-1-related disease is an immune-related disease. In some embodiments, immune-related disease is associated with a T cell dysfunctional disorder. In some embodiments, the T cell dysfunctional disorder is characterized by T cell anergy or decreased ability to secrete cytokines, proliferate or execute cytolytic activity. In some embodiments, the T cell dysfunctional disorder is characterized by T cell exhaustion. In some embodiments, the T cells are CD4+ and CD8+ T cells. In some embodiments, the immune-related disease is selected from the group consisting of unresolved acute infection, chronic infection, and tumor immunity. In some embodiments, an anti-PD-1 construct described herein may be for use in increasing, enhancing, or stimulating an immune response or function in a subject in need thereof. In some embodiments, the PD-1-related disease (e.g., cancer, immune-related disease) is partially resistant to immune checkpoint molecule mono-blockade (e.g., partially resistant to anti-TIGIT, LAG-3, TIM-3, CTLA-4, or PD-1 antibody monotherapy treatment).

[0278] The present invention contemplates, in part, anti-PD-1 protein constructs (such as anti-PD-1 sdAb, anti-PD-1 sdAb-Fc fusion protein (e.g., HCAb), anti-PD-1/TIGIT bispecific antibody (e.g., PD-1×TIGIT BABP), anti-PD-1/LAG-3 bispecific antibody (e.g., PD-1×LAG-3 BABP), anti-PD-1/TIM-3 bispecific antibody (e.g., PD-1×TIM-3 BABP), anti-PD-1/CTLA-4 bispecific antibody (e.g., PD-1×CTLA-4 BABP), or anti-PD-1/PD-1 bispecific antibody (e.g., PD-1×PD-1 BABP)), nucleic acid molecules or vectors encoding thereof, host cells comprising nucleic acid molecules or vectors encoding thereof, that can be administered either alone or in any combination with another therapy, and in at least some aspects, together with a pharmaceutically acceptable carrier or excipient. In some embodiments, prior to administration of the anti-PD-1 construct, they may be combined with suitable pharmaceutical

carriers and excipients that are well known in the art. The compositions prepared according to the disclosure can be used for the treatment or delaying of worsening of cancer, or increasing, enhancing, or stimulating an immune response or function in a subject in need thereof.

[0279] In some embodiments, there is provided a method of treating a PD-1-related disease (e.g., cancer, immune-related disease such as that associated with a T cell dysfunctional disorder) comprising administering to the individual an effective amount of a pharmaceutical composition comprising an isolated anti-PD-1 construct comprising an sdAb moiety specifically recognizing PD-1 (such as anti-PD-1 sdAb, anti-PD-1 sdAb-Fc fusion protein (e.g., HCAb), anti-PD-1/TIGIT bispecific antibody (e.g., PD-1×TIGIT BABP), anti-PD-1/LAG-3 bispecific antibody (e.g., PD-1×LAG-3 BABP), anti-PD-1/TIM-3 bispecific antibody (e.g., PD-1×TIM-3 BABP), anti-PD-1/CTLA-4 bispecific antibody (e.g., PD-1×CTLA-4 BABP), or anti-PD-1/PD-1 bispecific antibody (e.g., PD-1×PD-1 BABP)), wherein the anti-PD-1 sdAb moiety comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 37-72, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 109-144, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 181-216, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and optionally a pharmaceutical acceptable carrier. In some embodiments, the K_d of the binding between the anti-PD-1 sdAb moiety and PD-1 is about 10^{-5} M to about 10^{-12} M (such as about 10^{-7} M to about 10^{-12} M, or about 10^{-8} M to about 10^{-12} M). In some embodiments, the anti-PD-1 sdAb moiety is camelid, chimeric, human, partially humanized, or fully humanized. In some embodiments, the anti-PD-1 sdAb moiety comprises a V_HH domain comprising the amino acid sequence of any one of SEQ ID NOs: 289-324. In some embodiments, the PD-1-related disease is cancer. In some embodiments, the cancer is a solid tumor (such as colon cancer). In some embodiments, the PD-1-related disease is an immune-related disease. In some embodiments, immune-related disease is associated with a T cell dysfunctional disorder. In some embodiments, the T cell dysfunctional disorder is characterized by T cell anergy or decreased ability to secrete cytokines, proliferate or execute cytolytic activity. In some embodiments, the T cell dysfunctional disorder is characterized by T cell exhaustion. In some embodiments, the immune-related disease is selected from the group consisting of unresolved acute infection, chronic infection, and tumor immunity. In some embodiments, the PD-1-related disease (e.g., cancer, immune-related disease) is partially resistant to immune checkpoint inhibitor monotherapy (e.g., partially resistant to anti-TIGIT, LAG-3, TIM-3, CTLA-4, or PD-1 antibody monotherapy treatment). In some embodiments, the method further comprises administering to the individual an additional therapy (e.g., cancer therapy, such as surgery, radiation, chemotherapy, immunotherapy, hormone therapy, or a combination thereof). In some

embodiments, the additional therapy is immunotherapy, e.g., by administering to the individual an effective amount of a second pharmaceutical composition comprising an immunomodulator. In some embodiments, the immunomodulator is an immune checkpoint inhibitor, e.g., anti-TIGIT, anti-LAG-3, anti-TIM-3, anti-CTLA-4, anti-PD-1, or anti-PD-L1 antibody. In some embodiments, the pharmaceutical composition is administered systemically (such as intravenously or intraperitoneally). In some embodiments, the pharmaceutical composition is administered locally (such as intratumorally). In some embodiments, the individual is a human. In some embodiments, the method of treating cancer has one or more of the following biological activities: (1) killing cancer cells (including bystander killing); (2) inhibiting proliferation of cancer cells; (3) inducing immune response in a tumor; (4) reducing tumor size; (5) alleviating one or more symptoms in an individual having cancer; (6) inhibiting tumor metastasis; (7) prolonging survival; (8) prolonging time to cancer progression; and (9) preventing, inhibiting, or reducing the likelihood of the recurrence of a cancer. In some embodiments, the method of killing cancer cells mediated by the pharmaceutical composition described herein can achieve a tumor cell death rate of at least about any of 40%, 50%, 60%, 70%, 80%, 90%, 95%, or more. In some embodiments, the method of killing cancer cells mediated by the pharmaceutical composition described herein can achieve a bystander tumor cell (e.g., uninfected by oncolytic VV encoding the anti-PD-1 construct) death rate of at least about any of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or more. In some embodiments, the method of reducing tumor size mediated by the pharmaceutical composition described herein can reduce at least about 10% (including for example at least about any of 20%, 30%, 40%, 60%, 70%, 80%, 90%, or 100%) of the tumor size. In some embodiments, the method of inhibiting tumor metastasis mediated by the pharmaceutical composition described herein can inhibit at least about 10% (including for example at least about any of 20%, 30%, 40%, 60%, 70%, 80%, 90%, or 100%) of the metastasis. In some embodiments, the method of prolonging survival of an individual (such as a human) mediated by the pharmaceutical composition described herein can prolongs the survival of the individual by at least any of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 18, or 24 months. In some embodiments, the method of prolonging time to cancer progression mediated by the pharmaceutical composition described herein can prolongs the time to cancer progression by at least any of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 weeks. In some embodiments, the method of treating immune-related disease can increase, enhance, or stimulate an immune response or function in a subject. In some embodiments, the immune response or function is increased, enhanced, and/or stimulated by activating effector cells (e.g., T cells, e.g., CD8+ and/or CD4+ T cells), expanding (increasing) an effector cell population, and/or killing target cells (e.g., target tumor cells) in the subject.

[0280] In some embodiments, the method is suitable for treating cancers with aberrant PD-1 or PD-L1/PD-L2 expression, activity and/or signaling include, by way of non-limiting example, hematological cancer and/or solid tumors. Some cancers whose growth may be inhibited using the antibodies of the

invention include cancers typically responsive to immunotherapy. Non-limiting examples of cancers for treatment include melanoma (*e.g.*, metastatic malignant melanoma), renal cancer (*e.g.* clear cell carcinoma), prostate cancer (*e.g.* hormone refractory prostate adenocarcinoma), breast cancer, colon cancer and lung cancer (*e.g.* non-small cell lung cancer), gastric cancer, ovarian cancer, and glioblastoma. Additionally, the invention includes refractory or recurrent malignancies whose growth may be inhibited using the antibodies of the invention. Examples of other cancers that may be treated using the antibodies of the invention include bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular malignant melanoma, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, testicular cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, non-Hodgkin's lymphoma, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, chronic or acute leukemias including acute myeloid leukemia, chronic myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, solid tumors of childhood, lymphocytic lymphoma, cancer of the bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, T-cell lymphoma, environmentally induced cancers including those induced by asbestos, and combinations of said cancers. The present invention is also useful for treatment of metastatic cancers, especially metastatic cancers that express PD-1 or PD-L1/PD-L2. In some embodiments, the cancer with aberrant PD-1 or PD-L1/PD-L2 expression, activity and/or signaling is partially resistant to PD-1 mono-blockade (e.g., partially resistant to anti-PD-1 antibody monotherapy treatment), or partially resistant to immune checkpoint inhibitor monotherapy (e.g., anti-TIGIT, LAG-3, TIM-3, CTLA-4 antibody monotherapy treatment). In such case, anti-PD-1 MABPs (e.g., BABPs) such as PD-1×TIGIT, PD-1×LAG-3, PD-1×TIM-3, PD-1×CTLA-4, or PD-1×PD-1 MABPs (e.g., BABPs) described herein can be used.

[0281] The methods described herein are suitable for treating a variety of cancers, including both solid cancer and liquid cancer. The methods are applicable to cancers of all stages, including early stage cancer, non-metastatic cancer, primary cancer, advanced cancer, locally advanced cancer, metastatic cancer, or cancer in remission. The methods described herein may be used as a first therapy, second therapy, third therapy, or combination therapy with other types of cancer therapies known in the art, such as chemotherapy, surgery, hormone therapy, radiation, gene therapy, immunotherapy (such as T-cell therapy or administering immunomodulators), bone marrow transplantation, stem cell transplantation, targeted therapy, cryotherapy, ultrasound therapy, photodynamic therapy, radio-frequency ablation or the like, in

an adjuvant setting or a neoadjuvant setting (i.e., the method may be carried out before the primary/definitive therapy). In some embodiments, the method is used to treat an individual who has previously been treated. In some embodiments, the cancer has been refractory to prior therapy. In some embodiments, the method is used to treat an individual who has not previously been treated. In some embodiments, the cancer is partially resistant to immune checkpoint inhibitor monotherapy (e.g., partially resistant to anti-TIGIT, LAG-3, TIM-3, CTLA-4, or PD-1 antibody monotherapy treatment).

[0282] Thus in some embodiments, there is provided a method of treating cancer (such as carcinoma or adenocarcinoma, such as cancers with aberrant PD-1 expression, activity and/or signaling, and/or cancers with aberrant TIGIT/LAG-3/TIM-3/CTLA-4 expression, activity and/or signaling), comprising administering to the individual an effective amount of a pharmaceutical composition comprising an anti-PD-1 construct (e.g., PD-1×TIGIT, PD-1×LAG-3, PD-1×TIM-3, PD-1×CTLA-4, PD-1×PD-1 MABP or BABP corresponding to the aberrant TIGIT/LAG-3/TIM-3/CTLA-4/PD-1 expression, activity and/or signaling) comprising: (a) a first antigen binding portion comprising an anti-PD-1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 37-72, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 109-144, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 181-216, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and (b) a second antigen binding portion comprising a V_H and a V_L, wherein the V_H and V_L together form an antigen-binding site that specifically binds a second immune checkpoint molecule (e.g., TIGIT, LAG-3, TIM-3, CTLA-4, PD-1 (such as a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein)), wherein the first antigen binding portion is fused to the second antigen binding portion; and optionally a pharmaceutical acceptable carrier. In some embodiments the second antigen binding portion comprises a heavy chain comprising a V_H and light chain comprising a V_L. In some embodiments, the first antigen binding portion is fused to the second antigen binding portion at the N-terminus of the heavy chain, the N-terminus of the light chain, the N-terminus of the Fc region, the C-terminus of the heavy chain, or the C-terminus of the light chain. In some embodiments, the second antigen binding portion comprises a full-length 4-chain antibody or antigen binding fragment thereof (e.g., Fab or scFv). In some embodiments, the first antigen binding portion is fused to the C-terminus of the second antigen binding portion comprising a Fab or an scFv. In some embodiments, the first and second antigen binding portions are fused optionally via a peptide linker, such as a peptide linker comprising the amino acid sequence of any one of SEQ ID NOs: 367-376. In some embodiments, the second antigen binding portion comprises an Fc fragment (e.g. derived from IgG4, IgG2, or IgG1). In some embodiments, the Fc fragment of comprises

the amino acid sequence of any one of SEQ ID NOs: 363-365. In some embodiments, there is provided a method of treating a cancer (such as carcinoma or adenocarcinoma, such as cancers with aberrant PD-1 expression, activity and/or signaling, and/or cancers with aberrant TIGIT/LAG-3/TIM-3/CTLA-4 expression, activity and/or signaling), comprising administering to the individual an effective amount of a pharmaceutical composition comprising an isolated anti-PD-1 construct comprising an sdAb moiety specifically recognizing PD-1 fused to a full-length antibody (e.g., antibody specifically recognizing TIGIT, LAG-3, TIM-3, CTLA-4, or PD-1 (such as a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein), corresponding to the aberrant TIGIT/LAG-3/TIM-3/CTLA-4/PD-1 expression), wherein the anti-PD-1 sdAb moiety comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 37-72, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 109-144, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 181-216, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and optionally a pharmaceutical acceptable carrier. In some embodiments, the V_H and V_L domains are derived from tiragolumab. In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 377, and V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 378. In some embodiments, the full-length antibody is an anti-TIGIT antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 377, and a light chain comprising the amino acid sequence of SEQ ID NO: 378. In some embodiments, the V_H and V_L domains are derived from relatlimab. In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 379, and V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 380. In some embodiments, the full-length antibody is an anti-LAG-3 antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 379, and a light chain comprising the amino acid sequence of SEQ ID NO: 380. In some embodiments, the V_H and V_L domains are derived from MBG453. In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 381, and V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 382. In some embodiments, the full-length antibody is an anti-TIM-3 antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 381, and a light chain comprising the amino acid sequence of SEQ ID NO: 382. In some embodiments, the V_H and V_L domains are derived from ipilimumab (*e.g.*, Yervoy®). In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 383, and V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 384. In some embodiments, the

full-length antibody is an anti-CTLA-4 antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 383, and a light chain comprising the amino acid sequence of SEQ ID NO: 384. In some embodiments, the V_H and V_L domains are derived from pembrolizumab (*e.g.*, Keytruda®) or nivolumab (*e.g.*, Opdivo®). In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 385, and V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 386. In some embodiments, the full-length antibody is an anti-PD-1 antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 385, and a light chain comprising the amino acid sequence of SEQ ID NO: 386. In some embodiments, the K_d of the binding between the anti-PD-1 sdAb moiety and PD-1 is about 10^{-5} M to about 10^{-12} M (such as about 10^{-7} M to about 10^{-12} M, or about 10^{-8} M to about 10^{-12} M). In some embodiments, the anti-PD-1 sdAb moiety is camelid, chimeric, human, partially humanized, or fully humanized. In some embodiments, the N-terminus of the anti-PD-1 sdAb moiety is fused to the C-terminus of at least one of the heavy chains of the full-length antibody. In some embodiments, the C-terminus of the anti-PD-1 sdAb moiety is fused to the N-terminus of at least one of the heavy chains of the full-length antibody. In some embodiments, the N-terminus of the anti-PD-1 sdAb moiety is fused to the C-terminus of at least one of the light chains of the full-length antibody. In some embodiments, the C-terminus of the anti-PD-1 sdAb moiety is fused to the N-terminus of at least one of the light chains of the full-length antibody. In some embodiments, the anti-PD-1 construct comprises four anti-PD-1 sdAb moieties, wherein the C-terminus of each anti-PD-1 sdAb moiety is fused to the N-terminus of each chain of the full-length antibody. In some embodiments, the anti-PD-1 construct comprises four anti-PD-1 sdAb moieties, wherein two anti-PD-1 sdAb moieties are fused together, which is further fused to the N-terminus of each heavy chain of the full-length antibody. In some embodiments, the anti-PD-1 sdAb moiety comprises a V_HH domain comprising the amino acid sequence of any one of SEQ ID NO: 289-324. In some embodiments, the anti-PD-1 sdAb moiety and the full length antibody are optionally connected by a peptide linker (such as peptide linker comprising the amino acid sequence of any one of SEQ ID NOs: 367-376). In some embodiments, the cancer is a solid tumor (such as colon cancer). In some embodiments, the pharmaceutical composition is administered systemically (such as intravenously or intraperitoneally). In some embodiments, the pharmaceutical composition is administered locally (such as intratumorally). In some embodiments, the method further comprises administering to the individual an additional cancer therapy (such as surgery, radiation, chemotherapy, immunotherapy, hormone therapy, or a combination thereof). In some embodiments, the individual is a human. In some embodiments, the cancer is partially resistant to immune checkpoint molecule mono-blockade (e.g., partially resistant to anti-PD-1 antibody, anti-TIGIT antibody, anti-LAG-3 antibody, anti-TIM-3 antibody, or anti-CTLA-4 antibody monotherapy treatment).

[0283] Dosages and desired drug concentrations of pharmaceutical compositions of the present application may vary depending on the particular use envisioned. The determination of the appropriate dosage or route of administration is well within the skill of an ordinary artisan. Animal experiments provide reliable guidance for the determination of effective doses for human therapy. Interspecies scaling of effective doses can be performed following the principles laid down by Mordenti, J. and Chappell, W. "The Use of Interspecies Scaling in Toxicokinetics," In *Toxicokinetics and New Drug Development*, Yacobi *et al.*, Eds, Pergamon Press, New York 1989, pp. 42-46.

[0284] The pharmaceutical compositions of the present application, including but not limited to reconstituted and liquid formulations, are administered to an individual in need of treatment with the anti-PD-1 construct described herein (such as anti-PD-1 sdAb, anti-PD-1 sdAb-Fc fusion protein (e.g., HCAb), anti-PD-1/TIGIT bispecific antibody (e.g., PD-1×TIGIT BABP), anti-PD-1/LAG-3 bispecific antibody (e.g., PD-1×LAG-3 BABP), anti-PD-1/TIM-3 bispecific antibody (e.g., PD-1×TIM-3 BABP), anti-PD-1/CTLA-4 bispecific antibody (e.g., PD-1×CTLA-4 BABP), or anti-PD-1/PD-1 bispecific antibody (e.g., PD-1×PD-1 BABP)), preferably a human, in accord with known methods, such as intravenous administration as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerobrospinal, subcutaneous, intravenous (i.v.), intra-articular, intrasynovial, intrathecal, oral, topical, or inhalation routes. A reconstituted formulation can be prepared by dissolving a lyophilized anti-PD-1 construct described herein in a diluent such that the protein is dispersed throughout. Exemplary pharmaceutically acceptable (safe and non-toxic for administration to a human) diluents suitable for use in the present application include, but are not limited to, sterile water, bacteriostatic water for injection (BWFI), a pH buffered solution (*e.g.* phosphate-buffered saline), sterile saline solution, Ringer's solution or dextrose solution, or aqueous solutions of salts and/or buffers.

[0285] In some embodiments, the pharmaceutical compositions are administered to the individual by subcutaneous (*i.e.* beneath the skin) administration. For such purposes, the pharmaceutical compositions may be injected using a syringe. However, other devices for administration of the pharmaceutical compositions are available such as injection devices; injector pens; auto-injector devices, needleless devices; and subcutaneous patch delivery systems. In some embodiments, the pharmaceutical compositions are administered to the individual intravenously. In some embodiments, the pharmaceutical composition is administered to an individual by infusion, such as intravenous infusion. Infusion techniques for immunotherapy are known in the art (see, *e.g.*, Rosenberg *et al.*, New Eng. J. of Med. 319: 1676 (1988)).

V. Methods of preparation

[0286] The anti-PD-1 construct described herein (such as anti-PD-1 sdAb, anti-PD-1 sdAb-Fc fusion protein (e.g., HCAb), anti-PD-1/TIGIT bispecific antibody (e.g., PD-1×TIGIT BABP), anti-PD-1/LAG-3 bispecific antibody (e.g., PD-1×LAG-3 BABP), anti-PD-1/TIM-3 bispecific antibody (e.g., PD-1×TIM-3 BABP), anti-PD-1/CTLA-4 bispecific antibody (e.g., PD-1×CTLA-4 BABP), or anti-PD-1/PD-1 bispecific antibody (e.g., PD-1×PD-1 BABP) may be prepared using any methods known in the art or as described herein. Also *see* Examples 1-3. In some embodiments, there is provided a method of producing an anti-PD-1 construct, comprising: (a) culturing a host cell comprising an isolated nucleic acid or vector encoding the anti-PD-1 construct described herein under conditions effective to express the encoded anti-PD-1 construct; and (b) obtaining the expressed anti-PD-1 construct from said host cell. In some embodiments, the method of step (a) further comprises producing a host cell comprising the isolated nucleic acid or vector encoding the anti-PD-1 construct described herein.

[0287] Methods of preparing sdAbs have been described. *See*, for example, Els Pardon et al., *Nature Protocol*, 2014; 9(3): 674. sdAbs (such as V_HHs) may be obtained using methods known in the art such as by immunizing a *Camelid* species (such as camel or llama) and obtaining hybridomas therefrom, or by cloning a library of single-domain antibodies using molecular biology techniques known in the art and subsequent selection by ELISA with individual clones of unselected libraries or by using phage display.

[0288] For recombinant production of the sdAbs, the nucleic acids encoding the single-domain antibodies are isolated and inserted into a replicable vector for further cloning (amplification of the DNA) or for expression. DNA encoding the single-domain antibody is readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the antibody). Many vectors are available. The choice of vector depends in part on the host cell to be used. Generally, preferred host cells are of either prokaryotic or eukaryotic (generally mammalian) origin. In some embodiments, the isolated nucleic acid encoding the anti-PD-1 construct described herein comprises the nucleic acid sequence of any one of SEQ ID NOs: 253-288.

1. Recombinant production in eukaryotic cells

[0289] For eukaryotic expression, the vector components generally include, but are not limited to, one or more of the following, a signal sequence, an origin of replication, one or more marker genes, and enhancer element, a promoter, and a transcription termination sequence.

a) Signal sequence component

[0290] A vector for use in a eukaryotic host may also an insert that encodes a signal sequence or other polypeptide having a specific cleavage site at the N-terminus of the mature protein or polypeptide. The heterologous signal sequence selected preferably is one that is recognized and processed (*i.e.*, cleaved by a signal peptidase) by the host cell. In mammalian cell expression, mammalian signal sequences as well as viral secretory leaders, for example, the herpes simplex gD signal, are available.

[0291] The DNA for such precursor region is ligated in reading frame to DNA encoding the antibodies of the present application.

b) Origin of replication

[0292] Generally, the origin of replication component is not needed for mammalian expression vectors (the SV40 origin may typically be used only because it contains the early promoter).

c) Selection gene component

[0293] Expression and cloning vectors may contain a selection gene, also termed a selectable marker. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins, *e.g.*, ampicillin, neomycin, methotrexate, or tetracycline, (b) complement auxotrophic deficiencies, or (c) supply critical nutrients not available from complex media, *e.g.*, the gene encoding D-alanine racemase for Bacilli.

[0294] One example of a selection scheme utilizes a drug to arrest growth of a host cell. Those cells that are successfully transformed with a heterologous gene produce a protein conferring drug resistance and thus survive the selection regimen. Examples of such dominant selection use the drugs neomycin, mycophenolic acid and hygromycin.

[0295] Another example of suitable selectable markers for mammalian cells are those that enable the identification of cells competent to take up nucleic acid encoding the antibodies of the present application, such as DHFR, thymidine kinase, metallothionein-I and -II, preferably primate metallothionein genes, adenosine deaminase, ornithine decarboxylase, etc.

[0296] For example, cells transformed with the DHFR selection gene are first identified by culturing all of the transformants in a culture medium that contains methotrexate (Mtx), a competitive antagonist of DHFR. An appropriate host cell when wild-type DHFR is employed is the Chinese hamster ovary (CHO) cell line deficient in DHFR activity (*e.g.*, ATCC CRL-9096).

[0297] Alternatively, host cells (particularly wild-type hosts that contain endogenous DHFR) transformed or co-transformed with the polypeptide encoding-DNA sequences, wild-type DHFR protein,

and another selectable marker such as aminoglycoside 3′ -phosphotransferase (APH) can be selected by cell growth in medium containing a selection agent for the selectable marker such as an aminoglycosidic antibiotic, *e.g.*, kanamycin, neomycin, or G418. *See* U.S. Pat. No. 4,965,199.

d) Promoter component

[0298] Expression and cloning vectors usually contain a promoter that is recognized by the host organism and is operably linked to the nucleic acid encoding the desired polypeptide sequences. Virtually all eukaryotic genes have an AT-rich region located approximately 25 to 30 based upstream from the site where transcription is initiated. Another sequence found 70 to 80 bases upstream from the start of the transcription of many genes is a CNCAAT region where N may be any nucleotide. At the 3′ end of most eukaryotic is an AATAAA sequence that may be the signal for addition of the poly A tail to the 3′ end of the coding sequence. All of these sequences may be inserted into eukaryotic expression vectors.

[0299] Other promoters suitable for use with prokaryotic hosts include the phoA promoter, -lactamase and lactose promoter systems, alkaline phosphatase promoter, a tryptophan (trp) promoter system, and hybrid promoters such as the tac promoter. However, other known bacterial promoters are suitable. Promoters for use in bacterial systems also will contain a Shine-Dalgarno (S.D.) sequence operably linked to the DNA encoding the antibodies.

[0300] Polypeptide transcription from vectors in mammalian host cells is controlled, for example, by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus, adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus and most preferably Simian Virus 40 (SV40), from heterologous mammalian promoters, *e.g.*, the actin promoter or an immunoglobulin promoter, from heat-shock promoters, provided such promoters are compatible with the host cell systems.

[0301] The early and late promoters of the SV40 virus are conveniently obtained as an SV40 restriction fragment that also contains the SV40 viral origin of replication. The immediate early promoter of the human cytomegalovirus is conveniently obtained as a HindIII E restriction fragment. A system for expressing DNA in mammalian hosts using the bovine papilloma virus as a vector is disclosed in U.S. Pat. No. 4,419,446. A modification of this system is described in U.S. Pat. No. 4,601,978. See also Reyes *et al.*, *Nature* 297:598-601 (1982) on expression of human-interferon cDNA in mouse cells under the control of a thymidine kinase promoter from herpes simplex virus. Alternatively, the Rous Sarcoma Virus long terminal repeat can be used as the promoter.

e) Enhancer element component

[0302] Transcription of a DNA encoding the antibodies of the present application by higher eukaryotes is often increased by inserting an enhancer sequence into the vector. Many enhancer sequences are now known from mammalian genes (globin, elastase, albumin, α-fetoprotein, and insulin). Typically, however, one will use an enhancer from a eukaryotic cell virus. Examples include the SV40 enhancer on the late side of the replication origin (100-270 bp), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. See also Yaniv, *Nature* 297:17-18 (1982) on enhancing elements for activation of eukaryotic promoters. The enhancer may be spliced into the vector at a position 5′ or 3′ to the polypeptide encoding sequence, but is preferably located at a site 5′ from the promoter.

f) Transcription termination component

[0303] Expression vectors used in eukaryotic host cells (yeast, fungi, insect, plant, animal, human, or nucleated cells from other multicellular organisms) will also contain sequences necessary for the termination of transcription and for stabilizing the mRNA. Such sequences are commonly available from the 5′ and, occasionally 3′, untranslated regions of eukaryotic or viral DNAs or cDNAs. These regions contain nucleotide segments transcribed as polyadenylated fragments in the untranslated portion of the polypeptide-encoding mRNA. One useful transcription termination component is the bovine growth hormone polyadenylation region. See WO94/11026 and the expression vector disclosed therein.

g) Selection and transformation of host cells

[0304] Suitable host cells for cloning or expressing the DNA in the vectors herein include higher eukaryote cells described herein, including vertebrate host cells. Propagation of vertebrate cells in culture (tissue culture) has become a routine procedure. Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham *et al.*, *J. Gen Virol.* 36:59 (1977)); baby hamster kidney cells (BHK, ATCC CCL 10); Chinese hamster ovary cells/−DHFR (CHO, Urlaub *et al.*, *Proc. Natl. Acad. Sci. USA* 77:4216 (1980)); mouse sertoli cells (TM4, Mather, *Biol. Reprod.* 23:243-251 (1980)); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TR1 cells (Mather *et al.*, *Annals N.Y. Acad. Sci.* 383:44-68 (1982)); MRC 5 cells; FS4 cells; and a human hepatoma line (Hep G2).

[0305] Host cells are transformed with the above-described expression or cloning vectors for antibodies production and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

h) Culturing the host cells

[0306] The host cells used to produce the antibodies of the present application may be cultured in a variety of media. Commercially available media such as Ham's F10 (Sigma), Minimal Essential Medium ((MEM), (Sigma), RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium ((DMEM), Sigma) are suitable for culturing the host cells. In addition, any of the media described in Ham *et al.*, *Meth. Enz.* 58:44 (1979), Barnes *et al.*, *Anal. Biochem.* 102:255 (1980), U.S. Pat. No. 4,767,704; 4,657,866; 4,927,762; 4,560,655; or 5,122,469; WO 90/03430; WO 87/00195; or U.S. Pat. Re. 30,985 may be used as culture media for the host cells. Any of these media may be supplemented as necessary with hormones and/or other growth factors (such as insulin, transferrin, or epidermal growth factor), salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as HEPES), nucleotides (such as adenosine and thymidine), antibiotics (such as GENTAMYCIN™ drug), trace elements (defined as inorganic compounds usually present at final concentrations in the micromolar range), and glucose or an equivalent energy source. Any other necessary supplements may also be included at appropriate concentrations that would be known to those skilled in the art. The culture conditions, such as temperature, pH, and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

i) Protein purification

[0307] When using recombinant techniques, the antibodies can be produced intracellularly, in the periplasmic space, or directly secreted into the medium. If the antibody is produced intracellularly, as a first step, the particulate debris, either host cells or lysed fragments, are removed, for example, by centrifugation or ultrafiltration. Carter *et al.*, *Bio/Technology* 10:163-167 (1992) describe a procedure for isolating antibodies which are secreted to the periplasmic space of *E. coli*. Briefly, cell paste is thawed in the presence of sodium acetate (pH 3.5), EDTA, and phenylmethylsulfonylfluoride (PMSF) over about 30 min. Cell debris can be removed by centrifugation. Where the antibody is secreted into the medium, supernatants from such expression systems are generally first concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. A protease inhibitor such as PMSF may be included in any of the foregoing steps to inhibit proteolysis and antibiotics may be included to prevent the growth of adventitious contaminants.

[0308] The protein composition prepared from the cells can be purified using, for example, hydroxylapatite chromatography, gel electrophoresis, dialysis, and affinity chromatography, with affinity chromatography being the preferred purification technique. The suitability of protein A as an affinity ligand depends on the species and isotype of any immunoglobulin Fc domain that is present in the antibody. Protein A can be used to purify the antibodies that are based on human immunoglobulins containing 1, 2, or 4 heavy chains (Lindmark *et al.*, *J. Immunol. Meth.* 62:1-13 (1983)). Protein G is recommended for all mouse isotypes and for human 3 (Guss *et al.*, *EMBO J.* 5:15671575 (1986)). The matrix to which the affinity ligand is attached is most often agarose, but other matrices are available. Mechanically stable matrices such as controlled pore glass or poly(styrene-divinyl)benzene allow for faster flow rates and shorter processing times than can be achieved with agarose. Where the antibody comprises a C_H3 domain, the Bakerbond ABXTMresin (J. T. Baker, Phillipsburg, N.J.) is useful for purification. Other techniques for protein purification such as fractionation on an ion-exchange column, ethanol precipitation, Reverse Phase HPLC, chromatography on silica, chromatography on heparin SEPHAROSE™ chromatography on an anion or cation exchange resin (such as a polyaspartic acid column), chromatofocusing, SDS-PAGE, and ammonium sulfate precipitation are also available depending on the antibody to be recovered.

[0309] Following any preliminary purification step(s), the mixture comprising the antibody of interest and contaminants may be subjected to low pH hydrophobic interaction chromatography using an elution buffer at a pH between about 2.5-4.5, preferably performed at low salt concentrations (*e.g.*, from about 0-0.25M salt).

2. Polyclonal antibodies

[0310] Polyclonal antibodies are generally raised in animals by multiple subcutaneous (s.c.) or intraperitoneal (i.p.) injections of the relevant antigen and an adjuvant. It may be useful to conjugate the relevant antigen to a protein that is immunogenic in the species to be immunized, *e.g.*, keyhole limpet hemocyanin (KLH), serum albumin, bovine thyroglobulin, or soybean trypsin inhibitor, using a bifunctional or derivatizing agent, *e.g.*, maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaraldehyde, succinic anhydride, $SOCl_2$, or $R^1N{=}C{=}NR$, where R and R^1 are independently lower alkyl groups. Examples of adjuvants which may be employed include Freund's complete adjuvant and MPL-TDM adjuvant (monophosphoryl Lipid A, synthetic trehalose dicorynomycolate). The immunization protocol may be selected by one skilled in the art without undue experimentation.

[0311] The animals are immunized against the antigen, immunogenic conjugates, or derivatives by combining, *e.g.*, 100 μg or 5 μg or the protein or conjugate (for rabbits or mice, respectively) with 3

volumes of Freund's complete adjuvant and injecting the solution intradermally at multiple sites. One month later, the animals are boosted with 1/5 to 1/10 the original amount of peptide or conjugate in Freund's complete adjuvant by subcutaneous injection at multiple sites. Seven to fourteen days later, the animals are bled and the serum is assayed for antibody titer. Animals are boosted until the titer plateaus. Conjugates also can be made in recombinant cell culture as protein fusions. Also, aggregating agents such as alum are suitable to enhance the immune response. Also see Example 1 for immunization in Camels.

VI. Articles of manufacture and kits

[0312] Further provided are kits and articles of manufacture comprising any of the isolated anti-PD-1 constructs (such as anti-PD-1 sdAb, anti-PD-1 sdAb-Fc fusion protein (e.g., HCAb), anti-PD-1/TIGIT bispecific antibody (e.g., PD-1×TIGIT BABP), anti-PD-1/LAG-3 bispecific antibody (e.g., PD-1×LAG-3 BABP), anti-PD-1/TIM-3 bispecific antibody (e.g., PD-1×TIM-3 BABP), anti-PD-1/CTLA-4 bispecific antibody (e.g., PD-1×CTLA-4 BABP), or anti-PD-1/PD-1 bispecific antibody (e.g., PD-1×PD-1 BABP)), isolated nucleic acids or vectors encoding thereof, or isolated host cells comprising the isolated nucleic acids or vectors encoding the anti-PD-1 constructs described herein. In some embodiments, a kit is provided which comprises any one of the pharmaceutical compositions described herein and preferably provides instructions for its use.

[0313] The kits of the present application are in suitable packaging. Suitable packaging includes, but is not limited to, vials, bottles, jars, flexible packaging (*e.g.*, sealed Mylar or plastic bags), and the like. Kits may optionally provide additional components such as buffers and interpretative information. The present application thus also provides articles of manufacture, which include vials (such as sealed vials), bottles, jars, flexible packaging, and the like.

[0314] The kits may include multiple unit doses of the pharmaceutical composition and instructions for use, packaged in quantities sufficient for storage and use in pharmacies, for example, hospital pharmacies and compounding pharmacies.

EXAMPLES

[0315] The examples below are intended to be purely exemplary of the invention and should therefore not be considered to limit the invention in any way. The following examples and detailed description are offered by way of illustration and not by way of limitation.

Example 1: Generation of anti-PD-1 sdAbs and anti-PD-1 HCAbs

Immunization

[0316] Two Camels were immunized with recombinant PD-1 extracellular domain (ECD) protein (Accession #NP_005009.2, SEQ ID NO: 362) under all current animal welfare regulations. For immunization, the antigen was formulated as an emulsion with CFA (primary immunization) or IFA (boost immunization). The antigen was administered by double-spot injections intramuscularly at the neck. Each animal received two injections of the emulsion, containing 100 μg of PD-1 ECD and 4 subsequent injections containing 50 μg of antigen at weekly intervals. At different time points during immunization, 10 ml blood samples were collected from the animal and sera were prepared. The induction of an antigen specific humoral immune response was verified using the serum samples in an ELISA-based experiment with immobilized PD-1 ECD protein (FIGs. 1A-1B and FIGs. 2A-2B). Five days after the last immunization, a blood sample of 200 ml was collected. Peripheral blood lymphocytes (PBLs), as the genetic source of the camelid heavy chain immunoglobulins (HCAbs), were isolated from the 200 ml blood sample using a Ficoll-Paque gradient (Amersham Biosciences), yielding 5×10^8 PBLs. The maximal diversity of antibodies is expected to be equal to the number of sampled B-lymphocytes, which is about 20% of the number of PBLs (1×10^8). The fraction of heavy-chain antibodies in camel is up to 20% of the number of B-lymphocytes. Therefore, the maximal diversity of HCAbs in the 200 ml blood sample is calculated as 2×10^7 different molecules.

Library construction and binders selection

[0317] RNA extracted from PBLs and lymph node was used as starting material for RT-PCR to amplify sdAb encoding gene fragments. These fragments were cloned into an in-house phagemid vector. The vector encodes a C-terminal His6 tag in frame with the sdAb coding sequence. The library size is more than 1×10^9. The library phage was prepared according to a standard protocol and stored after filter sterilization at 4°C for further use.

[0318] Selections were carried out with the above libraries using solid-phase panning as well as cell-based panning. Colonies were picked and grown in 96 deep well plates (1 ml volume) and induced by adding IPTG and 0.1% Triton for sdAb expression in the supernatant. The supernatant was analyzed for their ability to bind to PD-1 ECD protein (by ELISA) and PD-1 stable cell line (by FACS). The positive binders were sequenced and the unique clones were selected for further characterization (Table 3).

SdAb production

[0319] The selected His6-tagged sdAbs were purified from periplasmic extracts by ÄKTA. The NTA resin was processed according to the manufacturer's instructions. Periplasmic extracts prepared were

incubated with the resin for 30 min at RT on a rotator. The resin was washed with PBS and transferred to a column. The packed resin was washed with 15 mM Imidazole. SdAbs were eluted from the column using 150 mM Imidazole. The eluted fractions were analyzed by spotting on Hybond Membrane and visualized with Ponceau. Fractions containing protein were pooled and dialyzed against PBS. Dialyzed protein was collected, filter sterilized, concentration determined and stored at -20°C.

[0320] To determine the purity, protein samples were analyzed on a 12% SDS-PAGE gel. 10 µl Laemmli sample buffer was added to 10 µl (2 µg) purified protein, then the sample was heated for 10 minutes at 95°C, cooled and loaded onto a 12% SDS-PAGE gel. The gel was processed according to general procedures and stained with Coomassie Brilliant Blue (CBB). The purification data was summarized in FIG.28.

Affinity measurements of sdAbs by Surface Plasmon Resonance (SPR)

[0321] Binding kinetics of sdAbs were determined by SPR on a BIAcore T200 instrument (GE Healthcare). Briefly, PD-1 ECD was amine-coupled to a CM5 sensor chip at a density of about 50 RU. 5 concentrations of the sdAbs with 3-fold serial dilutions (starting from 2 nM to 162 nM) were injected over the coated sensor chip. Flow rate was 30 µl/min in all experiments. Association phase was 5 min, and dissociation phase was 5, 10 or 15 min. The chip was regenerated using Glycine/HCl pH 1.5. Binding curves at different concentrations of sdAbs were fitted to a 1: 1 Langmuir binding model to calculate the kinetic parameters k_{on}, k_{off} and K_D (see FIGs. 3A-3F for sdAb affinity data). Sensorgram processing and data analysis was performed with Biacore T200 Evaluation Software (GE Healthcare). The affinity parameters were summarized in FIG. 3G.

Binding to PD-1 expressed on cells by FACS analysis

[0322] Binding of sdAb to PD-1 expressed on CHO cells is determined using a fluorescence-activated cell sorting (FACS)-based assay. CHO cells expressing human PD-1 were dissociated from adherent culture flasks and mixed with varying concentrations of antibodies (both in a 96-well plate). The mixture was equilibrated for 30 minutes at room temperature, washed three times with FACS buffer (PBS containing 1% BSA). Secondary antibody fluorescein isothiocyanate (FITC)-conjugated anti-human kappa antibody (Jackson ImmunoResearch) was then added and incubated for 15 minutes at room temperature. Cells were washed again with FACS buffer and analyzed by flow cytometry. Data were analyzed with Prism (GraphPad Software, San Diego, CA) using non-linear regression, and EC_{50} values were calculated. As can be seen from FIGs.4A-4B, the FACS binding assays demonstrated that A31543, AS06962, and AS07424 sdAbs can bind to PD-1 at low concentrations (1-10 µg/ml).

Inhibition of ligand binding by FACS analysis

[0323] Blockade of ligand binding was studied using flow cytometry. For anti-PD-1 sdAbs evaluation, CHO cells expressing human PD-1 were dissociated from adherent culture flasks and mixed with varying concentrations of antibodies and a constant concentration of biotin-labeled human PD-L1/Fc or human PD-L2/Fc protein (both in a 96-well plate). Keytruda® was used as an anti-PD-1 antibody positive control. The mixture was equilibrated for 30 minutes at room temperature, washed three times with FACS buffer (PBS containing 1% BSA). PE/Cy5 Streptavidin secondary antibody was then added and incubated for 15 minutes at room temperature. Cells were washed again with FACS buffer and analyzed by flow cytometry (FIG. 5A). Data were analyzed with Prism (GraphPad Software, San Diego, CA) using non-linear regression, and IC_{50} values were calculated (FIG. 5B).

HCAb construction, production and characterization

[0324] SdAbs with functional activities and slow off-rate from the above studies were selected for HCAb construction and production. DNA sequences of selected sdAbs were fused with DNA sequences of human IgG4 Fc (S228P) to make HCAb constructs. The HCAb constructs were transfected into CHO cell for HCAb expression. Secreted HCAbs in the condition medium were purified by protein A column. The purification data was summarized in FIG. 29.

Affinity measurements of HCAbs by Surface Plasmon Resonance (SPR)

[0325] Binding kinetics of anti-PD-1 camelid HCAbs were determined using a SPR biosensor, Biacore T200 (GE Healthcare). Antibody was immobilized on the sensor chip through Fc capture method at a density of about 50 RU. 5 concentrations of human PD-1-His antigen with 3-fold serial dilutions (starting from 3 nM to 243 nM) were injected over the coated sensor chip. Flow rate was 30 μl/min in all experiments. The chip was regenerated using Glycine/HCl pH 1.5. Binding curves at different concentrations of PD-1-His antigen were fitted to a 1: 1 Langmuir binding model to calculate the kinetic parameters k_{on}, k_{off} and K_D (see FIGs. 6B-6K for HCAb affinity data). Keytruda® was used as an experimental control (FIG. 6L). Sensorgram processing and data analysis was performed with Biacore T200 Evaluation Software (GE Healthcare). The affinity parameters were summarized in FIG. 6A.

[0326] As can be seen in FIGs. 6A-6L, the selected HCAbs (AS15140_HCAb, AS15152_HCAb, AS15156_HCAb, AS15193_HCAb, AS06962_HCAb, AS15881_HCAb, AS15883_HCAb, AS15892_HCAb, AS15899_HCAb, and AS25170_HCAb) show comparable binding affinities to Keytruda®.

Binding of HCAbs to PD-1 expressed on cells by FACS analysis

[0327] 22 purified anti-PD-1 HCAbs were tested for their binding abilities to PD-1 expressed on CHO cells as described above (FIGs. 7A-7V). Keytruda® was used as an experimental control (FIG. 7W). EC_{50} were summarized in FIG. 7X. As can be seen from FIGs.7A-7X, the FACS binding assays demonstrated all 22 HCAbs exhibited good binding to PD-1 on cell surface.

Inhibition of ligand binding by FACS analysis

[0328] 22 purified anti-PD-1 HCAbs were tested for their abilities to inhibit PD-1 and PD-L1 binding by FACS analysis, similarly as described in above (FIGs. 8A-8V). Keytruda® was used as an experimental control (FIG. 8W). IC_{50} were summarized in FIG. 8X. As can be seen from FIGs. 8A-8X, the competition assays demonstrated the ability of anti-PD-1 HCAbs in efficiently inhibiting PD-1/PD-L1 interactions at low concentrations (1-10 μg/ml). And according to IC_{50} of the FACS data, all 22 HCAbs showed good ligand competition activity.

PD-1 based functional blockade assay

[0329] CHO-K1 cells stably expressing PD-L1 and Jurkat effector cells are used to assess PD-1 blockade for anti-PD-1 HCAbs evaluation. The effector cells contain a luciferase construct that is induced upon disruption of the PD-1/PD-L1 receptor-ligand interaction, such as when the PD-L1 expressing cells are mixed with effector cells expressing PD-1. Thus, the efficacy of inhibiting the interaction of PD-L1 on CHO-K1 stable cells with PD-1 on effector cells by anti-PD-1 HCAbs can be assessed by measuring luciferase reporter activity. The assay is performed as follows.

[0330] On day one, PD-L1 expressing CHO-K1 cells were thawed in a 37°C water bath until cells were just thawed (about 3-4 minutes), and 0.5 mL of thawed cells were transferred to 14.5 mL cell recovery medium (10% FBS/F-12). The cell suspension was mixed well by gently inverting the tube 1-2 times. The cell suspension was then transferred to a sterile reagent reservoir, and dispensed into assay plates with 25 μL of cell suspension per well. 100 μL of assay medium was added per well as blank control. 100 μL of cell recovery medium was added per well for wells serving as blank control. The plates were then lidded and incubated overnight in a CO_2 incubator at 37°C.

[0331] Next, PD-1 Jurkat effector cells were thawed in a 37°C water bath until cells were just thawed (about 3-4 minutes). The cell suspension was gently mixed in the vial by pipetting up and down, and 0.5 mL of the cells was added to 5.9 mL assay buffer. The cell suspension was mixed well by gently inverting the tube 1-2 times. The cells were spinned down then transferred to a sterile reagent reservoir in 200 μL assay buffer, and 40 μL of the buffer with cells was dispensed to each well containing the various concentration of anti-PD-1 HCAb or control antibody (starting from 1 μM with 3-fold dilution, total 8

concentrations). Then 160 μL of PD-L1 CHO-K1 cells were added into each well. The plates were lidded and incubated for six hours at 37°C in a CO_2 incubator.

[0332] The Luciferase Assay System was reconstituted by transferring one bottle of Buffer to the bottle containing Substrate. The system was stored at room temperature and shielded from light for same day use. After 6 hours induction, assay plates were removed from the CO_2 incubator and equilibrated at ambient temperature for 5-10 min. 80 μL of reagent was added to each well. The plates were incubated for 5-10 min at ambient temperature. Luminescence was measured in GloMax® Discover System (Promega, Madison, WI) or a plate reader with glow-type luminescence reading capabilities.

[0333] Luminescence is expressed as Relative Light Unit (RLU). The RLU values of wells having diluted HCAbs or Keytruda® control was normalized to the RLU of no antibody to provide Fold of Luciferase Induction. Data were graphed as Fold of RLU Induction versus Log10 concentration of HCAb antibody (or Keytruda® control). The data were fitted to a curve and EC_{50} of each HCAb and control anti-PD-1 antibody Keytruda® was determined using curve fitting software such as GraphPad Prism (FIGs. 9A-9G). EC_{50} data were summarized in FIG. 9H.

[0334] PD-1 inhibition by the antibodies can also be studied by determining IL-2 secretion level in mixed lymphocyte reactions (MLR) comprising target cells expressing PD-1 and activated T cells, with anti-PD-1 HCAbs provided at various concentrations.

[0335] Human $CD4^+$ T cells and allogeneic monocytes were purified from PBMC using the isolation kits (Miltenyl Biotec). Monocytes were induced into dendritic cells. Each well contained 10^5 $CD4^+$ T cells and 10^4 allogeneic dendritic cells with a final working volume of 200 μl. Anti-PD-1 HCAbs were added into each well at different concentrations. No antibody was used as a background control. Human IgG4 was used as a negative control (not shown), and Keytruda® was used as the positive anti-PD-1 antibody control. After 72-hour incubation in 37°C/5% CO_2 incubator, 100 μl medium was taken from each testing well for IL-2 secretion measurement (Cisbio). Antibody concentration-dependent secretion of IL-2 in the MLRs was used to extract an EC_{50} value for anti-PD-1 activity of the anti-PD-1 antibodies (FIGs. 10A-10C), and compared with the EC_{50} value of the full-length PD-1 antibody Keytruda® (FIG. 10D). The EC_{50} of MLR assay were summarized in FIG. 10E. Consistent with the FACS-based ligand competition assay results (FIGs. 8A-8X), the functional activities of three selected HCAbs in targeting PD-1 were comparable to their monoclonal antibody Keytruda® by MLR.

Example 2: anti-PD-1 sdAb humanization and characterization

Humanization of anti-PD-1 sdAbs

[0336] Protein sequence of AS15193 sdAb was aligned with the 3 closest human germline sequences sharing the highest degree of homology. The best human germline sequences were selected as human acceptor. Homology model was made. According to the model analysis data, residues potentially critical for antigen binding or antibody scaffold formation were left untouched while the rest were selected for conversion into the human counterpart. Three panels of sequence optimized variants were generated and selected based on binding, stability and functional activity data. Four variants (AS15193VH8, AS15193VH8M1, AS15193VH18 and AS15193VH18M1) were selected based on binding and off-rate ranking data. The camelid parent sdAb, the humanized variants and the human acceptor sequences were aligned in FIG. 11. Humanized sdAbs are indicated with "VH" in their names.

Example 3: Humanized HCAb construction, production and characterization

[0337] 4 sdAbs (AS15193VH8, AS15193VH8M1, AS15193VH18 and AS15193VH18M1) with functional activities and slow off-rate from the above off-rate ranking study using BIAcore T200 in Example 2 (data not shown) were selected for HCAb construction and production. DNA sequences of the selected sdAbs were fused with DNA sequence of human IgG4 Fc (S228P) to make humanized HCAb constructs. The HCAb constructs were transfected into CHO cell for HCAb expression. Secreted HCAbs in the condition medium were purified by protein A column.

Affinity measurements of humanized HCAbs by Surface Plasmon Resonance (SPR)

[0338] Binding kinetics of anti-PD-1 humanized HCAbs were determined using an SPR biosensor, Biacore T200 (GE Healthcare). Antibody was immobilized on the sensor chip through Fc capture method, as mentioned in Example 1. Antigen PD-1-His protein was used as the analyte (4 concentrations of human PD-1-His antigen with 3-fold serial dilutions (starting from 1 nM to 27 nM)). Dissociation (k_d) and association (k_a) rate constants were obtained using Biacore T200 evaluation software (FIGs. 12A-12E). The apparent equilibrium dissociation constants (K_D) were calculated from the ratio of k_d over k_a. Data were summarized in FIG. 12F. According to the data, the binding affinities of humanized HCAbs (AS15193VH8_HCAb, AS15193VH8M1_HCAb, AS15193VH18_HCAb and AS15193VH18M1_HCAb) were close to its parental HCAb (AS15193_HCAb), suggesting that antibody affinity was maintained after humanization.

Binding of humanized HCAbs to PD-1 expressed on cells by FACS analysis

[0339] Purified humanized anti-PD-1 HCAbs were tested for their binding abilities to PD-1 expressed on CHO cells, as described in Example 1. As can be seen from FIGs. 13A-13E, the FACS binding assays demonstrated that various humanized HCAbs exhibited comparable binding ability to parental AS15193_HCAb. FACS binding EC_{50} data were summarized in FIG. 13F. Consistent with the above binding affinity result, the humanized HCAbs (AS15193VH8_HCAb, AS15193VH8M1_HCAb, AS15193VH18_HCAb, and AS15193VH18M1_HCAb) also showed comparable binding ability to its parental AS15193_HCAb.

Inhibition of ligand binding by FACS analysis

[0340] Purified humanized anti-PD-1 HCAbs were tested for their abilities to inhibit PD-1/PD-L1 binding by FACS analysis, as described in Example 1. As can be seen from FIGs. 14A-14E, the competition assays demonstrated the ability of humanized anti-PD-1 HCAbs in efficiently inhibiting PD-1/PD-L1 interactions at low concentrations (1-10 μg/ml). And according to IC_{50} of the FACS data (FIG. 14F), 4 humanized HCAbs showed comparable ligand blocking activity to their parental AS15193_HCAb.

PD-1 based functional blockade assay

[0341] PD-1 inhibition by the 4 humanized HCAbs was studied by IL-2-based luciferase repeater assay, as described in Example 1. Antibody concentration-dependent activation of IL-2 reporter was used to extract an EC_{50} value for anti-PD-1 activity of the humanized anti-PD-1 HCAbs, and compared with the EC_{50} value of the parent AS15193_HCAb (FIGs. 15A- 15E). Consistent with the FACS-based ligand competition assay results, the functional activities of 4 humanized HCAbs were comparable to their parental AS15193_HCAb. Data were summarized in FIG. 15F.

In vivo activity of humanized HCAbs

[0342] In the studies presented here, the efficacy of PD-1 humanized HCAb blockade against murine tumor model was investigated. Inhibition of the PD-1/PD-L1 interaction is proposed to exert a therapeutic effect by restoring anti-tumor CD8+ T cell responses, thus the preclinical efficacy study was conducted in syngeneic murine tumor model in which the immune system of the host is fully intact. The human PD-1 transgenic mice was used.

[0343] In this study, mice were inoculated subcutaneously in the right flank with 1×10^6 human PD-L1 overexpression MC38 colon carcinoma cells. When tumors reached a mean volume of ~100 mm^3, mice were sorted into treatment groups (n=5) (defined as study day 0). 2 humanized anti-PD-1 HCAbs tested in this study: AS15193VH8M1_HCAb and AS15193VH18M1_HCAb. Groups were administered benchmark antibody Keytruda (1 mg/kg) or humanized HCAbs (0.53 mg/kg) intravenously twice a week

for 2 weeks. 2 control groups were treated with 1 ml/kg of PBS or 1 mg/kg of human IgG4 isotype control. Tumors were measured twice weekly for the study duration. All treatment groups demonstrated significant efficacy ($P<0.050$) when compared to the control groups (FIG. 27). These observations support that anti-PD-1 therapy as an effective strategy for driving anti-tumor CD8+ T cell responses.

SEQUENCE LISTING

Table 3. Anti-PD-1 sdAb SEQ ID NOs

	SEQ ID NO:	FR1	SEQ ID NO:	CDR1	SEQ ID NO:	FR2	SEQ ID NO:	CDR2	SEQ ID NO:	FR3	SEQ ID NO:	CDR3	SEQ ID NO:	FR4
A31543	1	QVQLVESGGGKVQPGGSLRLSCAAS	37	GGTLDYYAIG	73	WFRQAPGKEREAVS	109	CISSSDGSTYYADSVKG	145	RFTISRDNAKNTVYLQMNSLKPGDTAVYHCAT	181	DRACGSSWLGAES	217	WAQGTQVTVSS
AS06962	2	QVHLVDSGGGLVQPGGSLRLSCAAS	38	GSITSRNTMG	74	WYRQVPGKQRELVA	110	LIATFVTHYADSVKG	146	RFTISRDNARKMVFLEMNSLQPEDTGAYYCYV	182	DVSPY	218	WGRGTQVTVSS
AS15090	3	QVQLVESGGGSVQAGGSLRLSCAAS	39	GYTYTPNCMA	75	WFRQAPGKEREGVT	111	LIFTGDGTSTYVDSVKG	147	RFTISQDNAKNTVYLQMNSLKPEDTALYYCAA	183	AERCSGSNDRISFWGISY	219	WGQGTQVTVSS
AS15140	4	QVQLVESGGGSVQAGGSLRLSCTAS	40	AYTYSNICLG	76	WLRQAPGGGLEAVA	112	TIYIADQTSYYADSVKG	148	RFRISKDAAKNAVYLQMSSLRPEDTAMYYCAS	184	RYGSTCGEYLADYTS	220	RAQGTQVTVSS
AS15152	5	QMQLVESGGGSVQAGGSLRLSCAVS	41	GYIYNRNFMG	77	WFRQAPGKEREGVA	113	AIYTGGPYTYYTDSVQG	149	RFTISQDNTKNTVYLQMNSLKPEDTAMYYCVS	185	DLSDGTWDQGRWNY	221	WGQGTQVTVSS
AS15156	6	QVQLVESGGGSAQAGGSLRLSCAVS	42	GYIYNRNFMG	78	WFRQVPGKVREGVA	114	AIYTGTERTYYADSVKG	150	RFTISQDNAKNTVYLQMNSLKPEDTAMYYCVA	186	DLRDGTWDTGVWNT	222	WGQGTQVTVSS
AS15193	7	QIQLVESGGGSAQAGGSLRLSCVVS	43	GNIYNRNFMG	79	WFRQAPGKVREGVA	115	AIYTGTSRTYYADSVKG	151	RFTISQDNAKNTVYLQMNSLKPEDTAMYYCAA	187	DLRDGFWDTGVWNT	223	WGQGTQVTVSS
AS15872	8	QVQLVESGGGLVQPGGSLTLSCAAS	44	GFTFSTAAMS	80	WVRQVPEEGLEWVA	116	SIDSSGSRTYYAGSVKG	152	RFTISRDNAKNTLYLQLNSLKAEDTAMYYCAK	188	DHMSWLP	224	RGQGTQVTVSS
AS15881	9	QVQLVESGGGSVQAGGSLRLSCAAS	45	GFTDSSYCGA	81	WFRQVPGKEREGVA	117	IIDRYGGTMYKDSVKG	153	RFTISKDTAKNILYLQMNSLKLEDTAMYYCAA	189	AEYRGSSCDAESGY	225	WGQGTQVTVSS
AS15883	10	QVHLMESGGGSVQAGGSLTLSCAAS	46	VFTDSNYCMA	82	WFRQVPGKEREGVA	118	IIDRYGGTMYKDSVKG	154	RFTISKDTAKNILYLQMNSLKLEDTAMYYCAA	190	AGYRGSSCDADSGY	226	WGQGTQVTVSS
AS15892	11	QIQLVESGGGSVQAGGSLRLSCAAS	47	GFTDSSYCGA	83	WFRQVPGKEREGVA	119	IIDRYGGTMYKDSVKG	155	RFTISKDTAKNILYLQMNSLKLEDTAMYYCAA	191	AEYRGSSCDAESGY	227	WGQGTQVTVSS
AS15899	12	QVQLVESGGGSVQAGGSLRLSCAAS	48	GYTAGSLCMG	84	WFRQAPGKEREGVA	120	AIYTGGGSTYYADSVKG	156	RFTISQDNAKNTVYLQMNSLKPEDTAQYYCGA	192	GSREDYCDRGYIYDH	228	WGQGTQVTVSS

AS17049	13	QVKLVESGGGSVQAGGYLRLSCAAS	49	GDTNNLNFRG	85	WFRQAPGKEREGVA	121	VITHSGSTYYAESVKG	157	RFTISQDLAKNTMYLQMNSLKPEDTAMYYCAA	193	ADVWRISWSFVPELFSY	229	WGQGTQVTVSS
AS17118	14	QVQLVESGGGSVQAGGSLRLSCAGS	50	GFTFNNYAMG	86	WFRQAPGKEREGIA	122	GIWTGGGSTYYADSVKG	158	RFTISEDVAKNTVYLQMDSLKPEDTAMYYCAA	194	ERWDYSDWRRLKRGDYNY	230	WGQGTQVTVSS
AS24984	15	QVHLMESGGGSVPSGGSLRLSCAVS	51	GSGYSYSRGCFA	87	WFQQRPGKEREGVA	123	IINMDGHTRYSDSVQG	159	RFIISQDKAKNTLHLQMNTLRPDDTAMYYCAY	195	DRSQCYVLSDRLRLPGTFSD	231	WGQGTQVTVSS
AS25037	16	EVQLAESGGGSVQAGGSLKLSCLAS	52	QWISSDCGMA	88	WYRQAPGKERELVS	124	RISSDDTTTYADSVKG	160	RFTISQDSAKNTLYLQMNKLKTEDTGVYYCAA	196	EAKSTITSLCYPLNY	232	WGQGTQVTVSS
AS25064	17	QVQLVESGGGSVQAGGSLRLTCAAT	53	GYSWRPDCMG	89	WYRQAAEKEREGVA	125	VIDADGITSYADAAKG	161	RFTISRDNNKITLYLQMLKPDDTGMYVCVV	197	GWRVSSGGNCQFNDY	233	WGQGTQVTVSS
AS25067	18	QVHLMESGGGAVQTGGSLRLSCAVS	54	GISISPDCMG	90	WFRQAPGKKREAVT	126	TIFANTGSARYGDSVKG	162	RFTSSQGNAKNTLYLQMDSVKLDDTGTYYCAA	198	RFTGGDCFDHQPLA	234	WRFWGQGTQVTVSS
AS25071	19	EVQLAESGGGSVQSGGSLRLSCAVS	55	GSGYSYSRGCFA	91	WFQQRPGKEREGVA	127	IINSDGHTAYSDSVQG	163	RFIISQDKAKNTLYLQMNSLKPDDTAMYYCAY	199	DRSQCYVLRDRLRLPDTFTD	235	WGQGTQVTVSS
AS25115	20	QVHLVESGGASVQAGGSLRLSCAAT	56	AYTASNYCMG	92	WFRQSPGKEREAVA	128	SINDDGVTSYADSVKG	164	RFTISQDSAKKTLYLQMNRLKPEDTAMYYCAA	200	TPDGYCYAERLSRWRYEF	236	WGQGTQVTVSS
AS25117	21	EVQLAESGGGSVQAGGSLRLSCVIS	57	GTSISPDCMG	93	WFRQAPGKKREAVM	129	SIFTNTGSTRYGDSVKG	165	RFTSSQGNAKNTLYLQMDSLKLDDTATYYCAA	201	RYTGGDCFNLEPLAWRF	237	WGQGTQVTVSS
AS25119	22	QVQLVESGGGSVQAGGPLRLTCAAT	58	GYSWRPDCMG	94	WYRQAAEKEREGVA	130	VIDADGITSYADAAKG	166	RFTISRDNNNITLYLQMLKPDDTGMYVCVI	202	GWRVSSGGNCQFNDY	238	WGQGTQVTVSS
AS25149	23	QVQLVESGGGSVQSGGSLKLSCAVS	59	GSGYSYSRGCFA	95	WFQQRPGKEREGVA	131	IINSDGHTRYSDSVQG	167	RFIISQDKAKNTLYLQMNSLKPDDAAMYYCAY	203	DRNQCYVLLDRLRLPGTFSD	239	WGQGTQVTVSS
AS25156	24	EVQLVESGGGSVQSGGSLRLSCAVS	60	GSGYSYSRGCFA	96	WFQQRPGKEREGVA	132	IINSDGHTRYSDSVQG	168	RFIISQDKAKNTLYLQMNSLKPDDTAMYYCAY	204	DCSQCYVLRDRLRLPDTFTD	240	WGQGTQVTVSS
AS25164	25	EVQLVESGGGSVQSGGSLRLSCAVS	61	GSGYSYNRGCFA	97	WFQQRPGKEREGVA	133	IINSDGHTTYGDSVQG	169	RFIISQDKAKNTLDLQMNSLKPDDTAMYYCAY	205	DRNQCYVLRDRLRLPDTFTD	241	WGQGTQVTVSS
AS25170	26	QVKLVESGGGLVQSGGSLRLSCAVS	62	GSGYSYSRGCFA	98	WFQQRPGKEREGVA	134	IINMDGHTMYSDLAQG	170	RFIISQDKAKNTLYLQMNSLKPDDTAMYYCAY	206	DRDQCYVLRDRLRLPDTFND	242	WGQGTQVTVSS
AS25222	27	QIQLVESGGGSVQAGGSLRLSCAVT	63	GISISPDCMG	99	WFRQAPGKKREAVA	135	TIFTNTASTRYGDSVKG	171	RFTSSQGNGKNTLYLQMDSLNVDDTATYYCAA	207	RYTGGNCFNLEPLAWHF	243	WGQGTQVTVSS
AS25396	28	QVHLMESGGGSVQAGGSLRLSCVVS	64	GISISPDCMG	100	WFRQAPGKKREAVA	136	TIFTNTRRTRYGDSVKG	172	RVTSSQGNAKNTLYLKMDNLRHDDTATYYCAA	208	RYTGGDCFNLDPLSWRF	244	WGQGTQVTVSS
AS25457	29	QVQLVESGGGSVQAGGSLRLSCAVS	65	GISISPDCMG	101	WFRQAPGKKREAVA	137	TIFTNTRSTRYGDSVKG	173	RFTSSQGNAKNTLYLQMDSLKLDDTATYYCAA	209	RYTGGDCFNLEPVAWRF	245	WGQGTQVTVSS

AS25487	30	EVQLVESGGGLVQPGGSLRLSCAAS	66	GFTFSVWSMS	102	WVRQAPGEGLEWVS	138	TITGSGAQTYYASSVRG	174	RFTTSRDNAKNTVYLQMNSLKSDDTAVYYCER	210	GNGQTAMEALINPP	246	ERPGTQVTVSS
AS25095	31	QVQLVESGGGLMQPGGSLRLSCAAS	67	GFTFSSYWMY	103	WVRQAPGKGLEWVS	139	VINRAGDSAWYADSVTG	175	RFTISRDNAKNTVYLQMDSLKPEDTAMYYCAA	211	DSRGYGGDWYKLLSDFNY	247	CGQGTQVTVSS
AS25435	32	QVQLVESGGGSVQAGGSLRLSCAAT	68	AYTASFYCMG	104	WFRQAPGKEREAVA	140	SINDDGVTMYADSVKG	176	QFTISQDSATKTLYLQMNRLKPEDTAMYYCAA	212	TPEGYCYAERLSTWRYTF	248	WGQGTQVTVSS
AS15193VH8	33	EVQLVESGGGLVQPGGSLRLSCAVS	69	GNIYNRNFMG	105	WFRQAPGKGLEGVS	141	AIYTGTSRTYYADSVKG	177	RFTISRDNSKNTVYLQMNSLRAEDTAVYYCAA	213	DLRDGFWDTGVWNT	249	WGQGTLVTVSS
AS15193VH8M1	34	EVQLVESGGGLVQPGGSLRLSCAVS	70	GNIYNRNFMG	106	WFRQAPGKGLEGVS	142	AIYTGTSRTYYADSVKG	178	RFTISRDNSKNTVYLQMNSLRAEDTAVYYCAA	214	DLREGFWDTGVWNT	250	WGQGTLVTVSS
AS15193VH18	35	EVQLVESGGGLVQPGGSLRLSCAVS	71	GNIYNRNFMG	107	WFRQAPGKGREGVS	143	AIYTGTSRTYYADSVKG	179	RFTISRDNAKNTVYLQMNSLRPEDTAVYYCAA	215	DLRDGFWDTGVWNT	251	WGQGTLVTVSS
AS15193VH18M1	36	EVQLVESGGGLVQPGGSLRLSCAVS	72	GNIYNRNFMG	108	WFRQAPGKGREGVS	144	AIYTGTSRTYYADSVKG	180	RFTISRDNAKNTVYLQMNSLRPEDTAVYYCAA	216	DLREGFWDTGVWNT	252	WGQGTLVTVSS

SEQ ID NO: 253 (A31543 sdAb nucleic acid sequence)
CAGGTACAGCTGGTGGAGTCTGGGGGAGGCAAGGTGCAGCCTGGGGGGTCTCTGAGACTCTCCT
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GAGCGCGAGGCCGTGTCATGTATTAGTAGTAGCGATGGTAGCACATACTATGCAGACTCCGTGAA
GGGCCGATTCACCATCTCCAGAGACAATGCCAAGAACACGGTGTATCTTCAAATGAACAGCCTG
AAACCTGGGGACACGGCCGTTTATCACTGTGCGACAGATCGGGCGTGCGGTAGTAGCTGGTTAG
GGGCCGAATCATGGGCCCAGGGGACCCAGGTCACCGTCTCCTCA
SEQ ID NO: 254 (AS06962 sdAb nucleic acid sequence)
CAGGTGCACCTGGTGGATTCTGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTGAGACTCTCCTG
TGCAGCCTCTGGAAGCATCACCAGTAGAAATACCATGGGCTGGTACCGGCAGGTTCCAGGGAAG
CAGCGCGAATTGGTCGCGCTAATTGCGACTTTTGTCACACATTATGCGGACTCCGTGAAGGGCCG
ATTCACCATCTCCAGAGATAACGCCAGGAAGATGGTGTTTCTAGAGATGAACAGCCTGCAACCTG
AGGACACGGGCGCGTATTATTGTTATGTCGATGTCTCGCCCTATTGGGGCCGGGGGACCCAGGTC
ACCGTCTCCTCA
SEQ ID NO: 255 (AS15090 sdAb nucleic acid sequence)
CAGGTGCAACTGGTGGAGTCTGGGGGAGGCTCGGTGCAGGCTGGAGGGTCTCTGAGACTCTCCT
GTGCAGCCTCTGGATACACCTATATTCCCAACTGCATGGCCTGGTTCCGCCAGGCTCCAGGGAAG
GAGCGCGAGGGGGTCACACTTATTTTTACTGGTGATGGTACCTCAACCTATGTCGACTCCGTGAA
GGGCCGATTCACCATCTCCCAAGACAACGCCAAGAACACGGTGTATCTGCAAATGAACAGCCTG
AAACCCGAGGACACTGCCTTGTACTACTGTGCGGCAGCCGAACGTTGTAGTGGTTCAAACGACAG
AATATCCTTTTGGGGAATTAGCTACTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA
SEQ ID NO: 256 (AS15140 sdAb nucleic acid sequence)
CAGGTGCAACTGGTGGAGTCTGGGGGAGGCTCGGTGCAGGCTGGGGGGTCTTTGAGACTCTCCTG
TACAGCCTCTGCATACACCTACAGTAACATCTGTTTGGGCTGGCTCCGCCAGGCTCCAGGGGGGG
GGCTCGAGGCTGTCGCAACGATTTATATTGCGGATCAGACATCATACTATGCCGACTCCGTGAAG
GGCCGATTCCGCATCTCTAAAGACGCCGCCAAGAACGCGGTGTATCTGCAAATGAGCAGCCTGA
GACCTGAGGACACTGCCATGTACTACTGTGCGTCCCGGTACGGTAGTACCTGCGGCGAATATTTA
GCTGACTATACCTCCCGGGCCCAGGGGACCCAGGTCACCGTCTCCTCA
SEQ ID NO: 257 (AS15152 sdAb nucleic acid sequence)

CAGATGCAGCTGGTGGAGTCTGGGGGAGGCTCGGTGCAGGCTGGAGGGTCTCTGAGACTCTCTTG
TGCAGTCTCTGGATACATCTACAATCGCAACTTCATGGGCTGGTTCCGCCAGGCTCCCGGGAAGG
AGCGCGAGGGGGTCGCGGCTATTTATACTGGTGGCCCATACACATACTATACCGACTCCGTGCAG
GGCCGATTCACCATCTCCCAAGACAACACCAAGAACACGGTGTATCTGCAAATGAACAGCCTGA
AACCTGAGGACACTGCCATGTATTACTGTGTGTCAGATCTTTCGGACGGTACTTGGGACCAGGGC
CGATGGAACTACTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA

SEQ ID NO: 258 (AS15156 sdAb nucleic acid sequence)

CAGGTGCAACTGGTGGAGTCTGGGGGAGGCTCGGCGCAGGCTGGAGGGTCTCTGAGACTCTCCT
GTGCAGTCTCTGGATACATTTACAATCGTAACTTCATGGGCTGGTTCCGCCAGGTTCCAGGAAAG
GTGCGCGAGGGGGTCGCAGCAATTTATACTGGTACTGAACGCACGTACTATGCCGACTCCGTGAA
GGGCCGATTCACCATCTCCCAAGACAACGCCAAGAACACGGTGTATCTGCAGATGAATAGTCTG
AAACCTGAGGACACTGCCATGTACTACTGTGTGGCGGATTTGCGGGATGGTACTTGGGATACGGG
CGTATGGAACACCTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA

SEQ ID NO: 259 (AS15193 sdAb nucleic acid sequence)

CAGATTCAGCTGGTGGAGTCTGGGGGAGGCTCGGCGCAGGCTGGAGGGTCTCTGAGACTCTCCTG
TGTAGTCTCTGGAAACATCTACAATCGTAACTTCATGGGCTGGTTCCGCCAGGCTCCAGGGAAGG
TGCGCGAGGGGGTCGCAGCAATTTATACTGGTACTAGTCGCACGTACTATGCCGACTCCGTGAAG
GGCCGATTCACCATCTCCCAAGACAACGCCAAGAATACGGTGTATCTGCAAATGAATAGTCTGAA
ACCTGAGGACACTGCCATGTACTACTGTGCGGCGGATTTGCGGGATGGTTTCTGGGATACGGGCG
TATGGAACACCTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA

SEQ ID NO: 260 (AS15872 sdAb nucleic acid sequence)

CAGGTGCAACTGGTGGAGTCTGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTGACACTCTCCTG
TGCAGCCTCTGGATTCACGTTCAGTACCGCCGCCATGAGCTGGGTCCGCCAGGTTCCAGAGGAGG
GACTCGAGTGGGTCGCATCTATTGATAGTAGTGGTAGTCGCACATACTATGCGGGCTCCGTGAAG
GGCCGATTCACCATCTCCAGAGACAACGCCAAGAACACGCTGTATTTGCAATTGAACAGCCTGAA
AGCTGAGGACACGGCCATGTATTACTGTGCAAAAGATCACATGAGCTGGTTGCCGCGGGGCCAG
GGGACCCAGGTCACCGTCTCCTCA

SEQ ID NO: 261 (AS15881 sdAb nucleic acid sequence)

CAGGTGCAACTGGTGGAGTCTGGGGGAGGATCGGTGCAGGCTGGAGGGTCTCTGAGACTCTCCT
GTGCAGCCTCTGGATTCACCGACAGTTCGTACTGCGGGGCCTGGTTTCGCCAGGTTCCAGGGAAG
GAGCGCGAGGGGGTCGCGATTATCGATAGATATGGTGGGACAATGTACAAAGACTCCGTGAAGG
GCCGATTCACCATCTCCAAAGACACTGCCAAGAATATTCTGTATCTGCAAATGAACAGCCTGAAA
CTTGAGGACACTGCCATGTACTACTGTGCGGCAGCCGAATATCGAGGCTCTTCGTGTGACGCGGA
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SEQ ID NO: 262 (AS15883 sdAb nucleic acid sequence)

CAGGTGCACCTGATGGAGTCTGGGGGAGGTTCGGTGCAGGCTGGAGGGTCCCTGACTCTCTCCTG
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AGCGCGAGGGGGTCGCAATTATCGATAGATATGGTGGTACGATGTACAAAGACTCCGTGAAGGG
CCGATTCACCATCTCCAAAGACACTGCCAAGAATATTCTGTATCTGCAAATGAACAGCCTGAAAC
TTGAGGACACTGCCATGTACTACTGTGCGGCAGCCGGGTATCGAGGCTCTTCGTGTGACGCGGAT
AGTGGCTACTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA

SEQ ID NO: 263 (AS15892 sdAb nucleic acid sequence)

CAGATTCAGCTGGTGGAGTCTGGGGGAGGATCGGTGCAGGCTGGAGGGTCTCTGAGACTCTCCTG
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AGCGCGAGGGGGTCGCGATTATCGATAGATATGGTGGGACAATGTACAAAGACTCCGTGAAGGG
CCGATTCACCATCTCCAAAGACACTGCCAAGAATATTCTGTATCTGCAAATGAACAGCCTGAAAC
TTGAGGACACTGCCATGTACTACTGTGCGGCAGCCGAATATCGAGGCTCTTCGTGTGACGCGGAG
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SEQ ID NO: 264 (AS15899 sdAb nucleic acid sequence)

CAGGTGCAACTGGTGGAGTCTGGGGGAGGCTCGGTGCAGGCTGGAGGGTCTCTGAGACTCTCCT
GTGCAGCCTCTGGATACACCGCCGGTAGCCTCTGCATGGGCTGGTTCCGCCAGGCTCCAGGGAAG
GAGCGCGAGGGGGTCGCAGCTATTTATACTGGTGGTGGTAGCACATACTATGCCGACTCCGTGAA
GGGCCGATTCACCATCTCCCAAGACAACGCCAAGAACACGGTGTATCTGCAAATGAACAGCCTG

AAACCTGAGGACACTGCCCAGTACTACTGCGGGGCGGGTAGTAGGGAAGACTACTGCGACAGGG
GTTACATCTATGATCACTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA
SEQ ID NO: 265 (AS17049 sdAb nucleic acid sequence)
CAGGTGAAGTTAGTGGAGTCTGGGGGAGGCTCGGTGCAGGCTGGAGGGTACCTGAGACTCTCCT
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GGAGCGCGAGGGGGTCGCAGTTATCACTCACTCTGGTAGCACATACTATGCCGAATCCGTGAAG
GGCCGATTCACCATCTCCCAAGACCTCGCCAAGAACACGATGTATCTGCAAATGAACAGTCTGAA
ACCTGAGGACACTGCTATGTACTACTGTGCGGCAGCAGATGTGTGGCGTATTAGCTGGTCCTTTG
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SEQ ID NO: 266 (AS17118 sdAb nucleic acid sequence)
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SEQ ID NO: 267 (AS24984 sdAb nucleic acid sequence)
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SEQ ID NO: 268 (AS25037 sdAb nucleic acid sequence)
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CCGATTCACCATCTCCCAAGACAGTGCCAAGAACACGCTGTATCTGCAAATGAACAAGCTGAAA
ACTGAAGACACGGGCGTGTATTATTGTGCGGCAGAAGCCAAGAGCACTATAACGAGCCTGTGCT
ACCCCTTGAACTACTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA
SEQ ID NO: 269 (AS25064 sdAb nucleic acid sequence)
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GGAGCGCGAGGGGGTCGCAGTTATTGATGCTGATGGTATCACAAGCTACGCAGACGCCGCGAAG
GGCCGATTCACCATCTCCCGAGACAACAACAAGATCACTCTATATCTGCAAATGCTGAAACCTGA
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SEQ ID NO: 270 (AS25067 sdAb nucleic acid sequence)
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AAACTTGATGACACTGGCACGTACTACTGTGCGGCACGGTTTACGGGGGGTGACTGCTTTGATCA
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SEQ ID NO: 271 (AS25071 sdAb nucleic acid sequence)
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TGCAGGGCCGATTCATCATCTCCCAAGACAAGGCCAAGAACACACTATATCTGCAAATGAACAG
CCTGAAACCTGACGACACGGCCATGTATTACTGTGCGTACGATCGCAGTCAGTGTTACGTGCTTC
GCGACCGCTTACGCCTCCCAGATACCTTTACTGACTGGGGCCAGGGGACCCAGGTCACCGTCTCC
TCA

SEQ ID NO: 272 (AS25115 sdAb nucleic acid sequence)
CAGGTGCACCTGGTGGAGTCTGGGGGAGCCTCGGTGCAGGCTGGAGGGTCTCTGAGACTCTCCTG
TGCAGCCACTGCGTACACCGCCAGTAATTATTGCATGGGCTGGTTCCGCCAGTCTCCAGGGAAGG
AGCGCGAGGCAGTCGCAAGTATTAATGATGACGGCGTCACAAGCTACGCAGACTCCGTGAAGGG
CCGATTCACCATCTCCCAAGACAGCGCCAAGAAGACTCTGTATCTCCAAATGAACCGCCTGAAAC
CTGAGGACACTGCCATGTACTACTGTGCGGCCACCCCGGATGGTTACTGCTACGCCGAGAGACTT
TCCCGGTGGAGATATGAGTTCTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA
SEQ ID NO: 273 (AS25117 sdAb nucleic acid sequence)
GAGGTGCAGCTGGCGGAGTCTGGGGGAGGCTCGGTGCAGGCTGGAGGGTCTCTGAGGCTCTCCT
GTGTAATATCAGGAACCTCCATCAGTCCAGACTGCATGGGCTGGTTCCGCCAGGCTCCAGGGAAG
AAGCGCGAGGCAGTCATGAGTATTTTTACAAATACTGGTAGCACGCGCTATGGCGACTCCGTGAA
GGGCCGATTCACCAGCTCCCAAGGCAACGCCAAGAATACGCTGTATCTGCAAATGGACAGCTTG
AAACTTGATGACACTGCCACGTACTACTGTGCGGCCCGGTATACGGGGGGTGACTGCTTTAATCT
TGAACCATTGGCGTGGCGCTTCTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA
SEQ ID NO: 274 (AS25119 sdAb nucleic acid sequence)
CAGGTTCAGCTGGTGGAGTCTGGGGGAGGCTCCGTGCAGGCTGGAGGGCCTCTGAGACTCACCT
GTGCAGCCACTGGATACTCTTGGAGACCCGACTGCATGGGCTGGTACCGCCAGGCTGCAGAGAA
GGAGCGCGAGGGGGTCGCAGTTATTGATGCTGATGGTATCACAAGTTACGCAGACGCCGCGAAG
GGCCGATTCACCATCTCCCGAGACAACAACAACATCACTCTATATCTGCAAATGCTGAAACCTGA
CGACACTGGCATGTACGTCTGTGTGATAGGATGGAGAGTAAGCAGTGGTGGTAACTGCCAATTCA
ATGACTACTGGGGTCAGGGGACCCAGGTCACCGTCTCCTCA
SEQ ID NO: 275 (AS25149 sdAb nucleic acid sequence)
CAGGTTCAGCTGGTGGAGTCTGGGGGAGGGTCGGTGCAGTCTGGAGGGTCTCTGAAACTCTCCTG
TGCAGTCTCTGGATCTGGATACAGCTATAGTCGCGGCTGCTTCGCATGGTTCCAACAGCGTCCAG
GGAAGGAGCGCGAGGGGGTCGCAATTATTAATAGCGATGGACACACAAGATACTCAGACTCCGT
GCAGGGCCGATTCATCATCTCCCAAGACAAGGCCAAGAACACACTATATCTCCAAATGAACAGC
CTGAAACCTGACGACGCGGCCATGTATTACTGTGCGTACGATCGCAATCAGTGTTACGTTCTTCTC
GACCGCTTACGCCTCCCAGGTACCTTTAGTGACTGGGGCCAGGGGACCCAGGTCACCGTCTCCTC
A
SEQ ID NO: 276 (AS25164 sdAb nucleic acid sequence)
GAGGTGCAGCTGGTGGAGTCTGGGGGAGGGTCGGTGCAGTCTGGAGGGTCTCTGAGACTCTCCT
GTGCAGTCTCTGGATCTGGATACAGCTATAATCGCGGCTGCTTCGCGTGGTTCCAGCAGCGTCCA
GGGAAGGAGCGCGAGGGGGTCGCAATTATTAATAGCGATGGGCACACAACGTACGGAGACTCCG
TGCAGGGCCGATTCATCATCTCCCAAGACAAGGCCAAGAACACACTAGATCTGCAAATGAACAG
CCTGAAACCTGACGACACGGCCATGTATTACTGTGCGTACGATCGCAATCAGTGTTACGTGCTTC
GCGACCGCTTACGCCTCCCAGATACCTTTACTGACTGGGGCCAGGGGACCCAGGTCACCGTCTCC
TCA
SEQ ID NO: 277 (AS25170 sdAb nucleic acid sequence)
CAGGTGAAGTTGGTGGAGTCTGGGGGAGGGTTGGTGCAGTCTGGAGGGTCTCTGAGACTCTCCTG
TGCAGTCTCTGGATCTGGATACAGCTATAGTCGCGGCTGCTTCGCATGGTTCCAGCAGCGTCCAG
GGAAGGAGCGCGAGGGGGTCGCAATTATTAATATGGATGGGCACACAATGTACTCAGACTTGGC
GCAGGGCCGATTCATCATCTCCCAAGACAAGGCCAAGAACACACTATATCTGCAAATGAACAGC
CTGAAACCTGACGACACGGCCATGTATTACTGTGCGTACGATCGCGATCAGTGTTACGTACTTCG
GGACCGCTTACGCCTCCCAGATACCTTTAATGACTGGGGCCAGGGGACCCAGGTCACCGTCTCCT
CA
SEQ ID NO: 278 (AS25222 sdAb nucleic acid sequence)
CAGATTCAGCTGGTGGAGTCTGGGGGAGGCTCGGTGCAGGCTGGAGGGTCTCTGAGGCTCTCCTG
TGCAGTGACAGGAATCTCCATCAGTCCAGACTGCATGGGCTGGTTCCGCCAGGCTCCAGGGAAG
AAGCGCGAGGCAGTCGCGACTATTTTTACTAATACTGCGAGCACGCGCTATGGCGACTCCGTGAA
GGGCCGATTCACCAGCTCCCAAGGGAACGGCAAGAATACGCTGTATCTGCAAATGGACAGCTTG
AACGTTGATGACACTGCCACGTACTACTGTGCGGCCCGCTATACGGGGGGTAACTGCTTTAATCT
TGAGCCATTGGCGTGGCACTTCTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA
SEQ ID NO: 279 (AS25396 sdAb nucleic acid sequence)

CAGGTGCACCTGATGGAGTCTGGGGGAGGCTCGGTGCAGGCTGGAGGGTCTCTGAGGCTCTCCTG
TGTAGTATCAGGAATCTCCATCAGTCCAGACTGTATGGGGTGGTTCCGCCAGGCTCCAGGGAAGA
AGCGCGAGGCAGTCGCGACTATTTTTACTAATACTCGTAGGACGCGCTATGGCGACTCCGTGAAG
GGCCGAGTCACCAGCTCCCAAGGCAACGCCAAGAATACGCTGTATCTAAAAATGGACAACTTGA
GGCACGATGACACTGCCACGTACTACTGTGCGGCCCGGTATACGGGGGGTGACTGCTTTAATCTT
GACCCATTGTCCTGGCGCTTCTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA

SEQ ID NO: 280 (AS25457 sdAb nucleic acid sequence)

CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTCGGTGCAGGCTGGAGGGTCTCTGAGGCTCTCCT
GTGCAGTATCAGGAATCTCCATCAGTCCAGACTGCATGGGCTGGTTCCGCCAGGCTCCAGGGAAG
AAGCGCGAGGCAGTCGCGACTATTTTTACTAATACTCGTAGCACGCGCTATGGCGACTCCGTGAA
GGGCCGATTCACCAGCTCCCAAGGCAACGCCAAGAATACGCTGTATCTGCAAATGGACAGCTTG
AAACTTGATGACACTGCCACGTACTACTGTGCGGCCCGGTATACGGGGGGTGACTGCTTTAATCT
TGAGCCTGTGGCGTGGCGCTTCTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA

SEQ ID NO: 281 (AS25487 sdAb nucleic acid sequence)

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTGAGACTCTCCTG
TGCAGCCTCTGGCTTCACCTTCAGTGTTTGGTCGATGTCCTGGGTCCGCCAGGCTCCAGGGGAGG
GACTCGAGTGGGTCTCAACTATCACTGGGAGTGGCGCACAAACATATTATGCAAGCTCAGTGAG
GGGCCGATTCACCACCTCCAGAGACAACGCCAAGAACACGGTATATCTGCAAATGAACAGCCTG
AAATCTGACGACACGGCCGTGTATTATTGTGAGAGAGGAAATGGTCAGACTGCTATGGAGGCTCT
CATTAACCCGCCCGAGCGTCCGGGGACCCAGGTCACCGTCTCCTCA

SEQ ID NO: 282 (AS25095 sdAb nucleic acid sequence)

CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGATGCAGCCTGGGGGGTCTTTGAGACTCTCCTG
TGCAGCCTCTGGATTCACCTTCAGTAGTTACTGGATGTACTGGGTCCGCCAGGCTCCAGGGAAGG
GGCTTGAGTGGGTCTCGGTTATTAATAGAGCTGGTGATTCCGCCTGGTATGCAGACTCAGTGACG
GGCCGATTCACCATCTCCAGAGACAACGCCAAGAACACGGTGTATCTGCAAATGGACAGCCTGA
AACCTGAGGACACGGCCATGTACTACTGTGCGGCAGACTCGAGGGGGTACGGTGGTGACTGGTA
CAAGCTCCTCTCAGACTTTAATTATTGCGGCCAGGGGACCCAGGTCACCGTCTCCTCA

SEQ ID NO: 283 (AS25435 sdAb nucleic acid sequence)

CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTCGGTGCAGGCTGGAGGGTCTCTGAGACTCTCCT
GTGCAGCCACTGCGTACACCGCCAGTTTCTACTGCATGGGCTGGTTCCGCCAGGCTCCAGGGAAG
GAGCGCGAGGCGGTCGCAAGTATTAATGATGACGGCGTCACAATGTACGCAGACTCCGTGAAGG
GCCAATTCACCATCTCCCAAGACAGCGCCACGAAGACTCTGTATCTGCAAATGAACCGCCTGAAA
CCTGAGGACACCGCCATGTACTACTGTGCGGCCACCCCGGAAGGTTACTGCTACGCCGAGAGACT
TTCCACGTGGAGATATACGTTCTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA

SEQ ID NO: 284 (AS25156 sdAb nucleic acid sequence)

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGGTCGGTGCAGTCTGGAGGGTCTCTGAGACTCTCCT
GTGCAGTCTCTGGATCTGGATACAGCTATAGTCGCGGCTGCTTCGCGTGGTTCCAGCAGCGTCCA
GGAAAGGAGCGCGAGGGGGTCGCAATTATTAATAGCGATGGGCACACAAGATACTCAGACTCCG
TGCAGGGCCGATTCATCATCTCCCAAGACAAGGCCAAGAACACACTATATCTGCAAATGAACAG
CCTGAAACCTGACGACACGGCCATGTATTACTGTGCGTACGATTGCAGTCAGTGTTACGTGCTTC
GCGACCGCTTACGCCTCCCAGATACCTTTACTGACTGGGGCCAGGGGACCCAGGTCACCGTCTCC
TCA

SEQ ID NO: 285 (AS15193VH8 sdAb nucleic acid sequence)

GAGGTGCAGCTGGTGGAGAGCGGAGGAGGACTGGTGCAGCCAGGAGGCTCTCTGAGACTGTCCT
GCGCCGTGAGCGGCAACATCTACAACAGAAATTTCATGGGATGGTTTAGGCAGGCTCCTGGCAA
GGGACTGGAGGGCGTGTCCGCCATCTATACCGGCACATCTCGCACCTACTATGCTGACTCCGTGA
AGGGCAGGTTCACCATCTCTCGGGATAACTCCAAGAATACAGTGTACCTGCAGATGAACTCTCTG
AGGGCCGAGGACACAGCCGTGTACTATTGTGCCGCTGACCTGCGGGATGGCTTTTGGGATACCGG
CGTGTGGAATACATGGGGCCAGGGCACCCTGGTGACAGTGTCCAGC

SEQ ID NO: 286 (AS15193VH8M1 sdAb nucleic acid sequence)

GAGGTGCAGCTGGTGGAGAGCGGAGGAGGACTGGTGCAGCCAGGAGGCTCTCTGAGACTGTCCT
GCGCCGTGAGCGGCAACATCTACAACAGAAATTTCATGGGATGGTTTAGGCAGGCTCCTGGCAA
GGGACTGGAGGGCGTGTCCGCCATCTATACCGGCACATCTCGCACCTACTATGCTGACTCCGTGA

AGGGCAGGTTCACCATCTCTCGGGATAACTCCAAGAATACAGTGTACCTGCAGATGAACTCTCTG
AGGGCCGAGGACACAGCCGTGTACTATTGTGCCGCTGACCTGCGGGAGGGCTTTTGGGATACCG
GCGTGTGGAATACATGGGGCCAGGGCACCCTGGTGACAGTGTCCAGC
SEQ ID NO: 287 (AS15193VH18 sdAb nucleic acid sequence)
GAGGTGCAGCTGGTGGAGTCCGGAGGAGGACTGGTGCAGCCAGGAGGCTCTCTGAGGCTGTCCT
GCGCCGTGAGCGGAAACATCTACAACAGAAATTTCATGGGATGGTTTAGGCAGGCTCCTGGCAA
GGGAAGGGAGGGCGTGTCTGCTATCTATACCGGCACATCCAGGACCTACTATGCCGACAGCGTG
AAGGGCAGGTTCACCATCTCTCGGGATAACGCTAAGAATACAGTGTACCTGCAGATGAACTCCCT
GCGGCCAGAGGACACAGCCGTGTACTATTGTGCCGCTGACCTGAGAGATGGCTTTTGGGATACCG
GCGTGTGGAATACATGGGGCCAGGGCACCCTGGTGACAGTGTCCAGC
SEQ ID NO: 288 (AS15193VH18M1 sdAb nucleic acid sequence)
GAGGTGCAGCTGGTGGAGTCCGGAGGAGGACTGGTGCAGCCAGGAGGCTCTCTGAGGCTGTCCT
GCGCCGTGAGCGGAAACATCTACAACAGAAATTTCATGGGATGGTTTAGGCAGGCTCCTGGCAA
GGGAAGGGAGGGCGTGTCTGCTATCTATACCGGCACATCCAGGACCTACTATGCCGACAGCGTG
AAGGGCAGGTTCACCATCTCTCGGGATAACGCTAAGAATACAGTGTACCTGCAGATGAACTCCCT
GCGGCCAGAGGACACAGCCGTGTACTATTGTGCCGCTGACCTGAGAGAGGGCTTTTGGGATACC
GGCGTGTGGAATACATGGGGCCAGGGCACCCTGGTGACAGTGTCCAGC
SEQ ID NO: 289 (A31543 sdAb amino acid sequence; CDRs are underlined)
QVQLVESGGGKVQPGGSLRLSCAASGGTLDYYAIGWFRQAPGKEREAVSCISSSDGSTYYADSVKGR
FTISRDNAKNTVYLQMNSLKPGDTAVYHCATDRACGSSWLGAESWAQGTQVTVSS
SEQ ID NO: 290 (AS06962 sdAb amino acid sequence; CDRs are underlined)
QVHLVDSGGGLVQPGGSLRLSCAASGSITSRNTMGWYRQVPGKQRELVALIATFVTHYADSVKGRFT
ISRDNARKMVFLEMNSLQPEDTGAYYCYVDVSPYWGRGTQVTVSS
SEQ ID NO: 291 (AS15090 sdAb amino acid sequence; CDRs are underlined)
QVQLVESGGGSVQAGGSLRLSCAASGYTYIPNCMAWFRQAPGKEREGVTLIFTGDGTSTYVDSVKGR
FTISQDNAKNTVYLQMNSLKPEDTALYYCAAAERCSGSNDRISFWGISYWGQGTQVTVSS
SEQ ID NO: 292 (AS15140 sdAb amino acid sequence; CDRs are underlined)
QVQLVESGGGSVQAGGSLRLSCTASAYTYSNICLGWLRQAPGGGLEAVATIYIADQTSYYADSVKGR
FRISKDAAKNAVYLQMSSLRPEDTAMYYCASRYGSTCGEYLADYTSRAQGTQVTVSS
SEQ ID NO: 293 (AS15152 sdAb amino acid sequence; CDRs are underlined)
QMQLVESGGGSVQAGGSLRLSCAVSGYIYNRNFMGWFRQAPGKEREGVAAIYTGGPYTYYTDSVQG
RFTISQDNTKNTVYLQMNSLKPEDTAMYYCVSDLSDGTWDQGRWNYWGQGTQVTVSS
SEQ ID NO: 294 (AS15156 sdAb amino acid sequence; CDRs are underlined)
QVQLVESGGGSAQAGGSLRLSCAVSGYIYNRNFMGWFRQVPGKVREGVAAIYTGTERTYYADSVKG
RFTISQDNAKNTVYLQMNSLKPEDTAMYYCVADLRDGTWDTGVWNTWGQGTQVTVSS
SEQ ID NO: 295 (AS15193 sdAb amino acid sequence; CDRs are underlined)
QIQLVESGGGSAQAGGSLRLSCVVSGNIYNRNFMGWFRQAPGKVREGVAAIYTGTSRTYYADSVKG
RFTISQDNAKNTVYLQMNSLKPEDTAMYYCAADLRDGFWDTGVWNTWGQGTQVTVSS
SEQ ID NO: 296 (AS15872 sdAb amino acid sequence; CDRs are underlined)
QVQLVESGGGLVQPGGSLTLSCAASGFTFSTAAMSWVRQVPEEGLEWVASIDSSGSRTYYAGSVKGR
FTISRDNAKNTLYLQLNSLKAEDTAMYYCAKDHMSWLPRGQGTQVTVSS
SEQ ID NO: 297 (AS15881 sdAb amino acid sequence; CDRs are underlined)
QVQLVESGGGSVQAGGSLRLSCAASGFTDSSYCGAWFRQVPGKEREGVAIIDRYGGTMYKDSVKGR
FTISKDTAKNILYLQMNSLKLEDTAMYYCAAAEYRGSSCDAESGYWGQGTQVTVSS
SEQ ID NO: 298 (AS15883 sdAb amino acid sequence; CDRs are underlined)
QVHLMESGGGSVQAGGSLTLSCAASVFTDSNYCMAWFRQVPGKEREGVAIIDRYGGTMYKDSVKG
RFTISKDTAKNILYLQMNSLKLEDTAMYYCAAAGYRGSSCDADSGYWGQGTQVTVSS
SEQ ID NO: 299 (AS15892 sdAb amino acid sequence; CDRs are underlined)
QIQLVESGGGSVQAGGSLRLSCAASGFTDSSYCGAWFRQVPGKEREGVAIIDRYGGTMYKDSVKGRF
TISKDTAKNILYLQMNSLKLEDTAMYYCAAAEYRGSSCDAESGYWGQGTQVTVSS
SEQ ID NO: 300 (AS15899 sdAb amino acid sequence; CDRs are underlined)
QVQLVESGGGSVQAGGSLRLSCAASGYTAGSLCMGWFRQAPGKEREGVAAIYTGGGSTYYADSVK
GRFTISQDNAKNTVYLQMNSLKPEDTAQYYCGAGSREDYCDRGYIYDHWGQGTQVTVSS

SEQ ID NO: 301 (AS17049 sdAb amino acid sequence; CDRs are underlined)
QVKLVESGGGSVQAGGYLRLSCAASGDTNNLNFRGWFRQAPGKEREGVAVITHSGSTYYAESVKGR
FTISQDLAKNTMYLQMNSLKPEDTAMYYCAAADVWRISWSFVPELFSYWGQGTQVTVSS
SEQ ID NO: 302 (AS17118 sdAb amino acid sequence; CDRs are underlined)
QVQLVESGGGSVQAGGSLRLSCAGSGFTFNNYAMGWFRQAPGKEREGIAGIWTGGGSTYYADSVKG
RFTISEDVAKNTVYLQMDSLKPEDTAMYYCAAERWDYSDWRRLKRGDYNYWGQGTQVTVSS
SEQ ID NO: 303 (AS24984 sdAb amino acid sequence; CDRs are underlined)
QVHLMESGGGSVPSGGSLRLSCAVSGSGYSYSRGCFAWFQQRPGKEREGVAIINMDGHTRYSDSVQG
RFIISQDKAKNTLHLQMNTLRPDDTAMYYCAYDRSQCYVLSDRLRLPGTFSDWGQGTQVTVSS
SEQ ID NO: 304 (AS25037 sdAb amino acid sequence; CDRs are underlined)
EVQLAESGGGSVQAGGSLKLSCLASQWISSDCGMAWYRQAPGKERELVSRISSDDTTTYADSVKGRF
TISQDSAKNTLYLQMNKLKTEDTGVYYCAAEAKSTITSLCYPLNYWGQGTQVTVSS
SEQ ID NO: 305 (AS25064 sdAb amino acid sequence; CDRs are underlined)
QVQLVESGGGSVQAGGSLRLTCAATGYSWRPDCMGWYRQAAEKEREGVAVIDADGITSYADAAKG
RFTISRDNNKITLYLQMLKPDDTGMYVCVVGWRVSSGGNCQFNDYWGQGTQVTVSS
SEQ ID NO: 306 (AS25067 sdAb amino acid sequence; CDRs are underlined)
QVHLMESGGGAVQTGGSLRLSCAVSGISISPDCMGWFRQAPGKKREAVTTIFANTGSARYGDSVKGR
FTSSQGNAKNTLYLQMDSVKLDDTGTYYCAARFTGGDCFDHQPLAWRFWGQGTQVTVSS
SEQ ID NO: 307 (AS25071 sdAb amino acid sequence; CDRs are underlined)
EVQLAESGGGSVQSGGSLRLSCAVSGSGYSYSRGCFAWFQQRPGKEREGVAIINSDGHTAYSDSVQG
RFIISQDKAKNTLYLQMNSLKPDDTAMYYCAYDRSQCYVLRDRLRLPDTFTDWGQGTQVTVSS
SEQ ID NO: 308 (AS25115 sdAb amino acid sequence; CDRs are underlined)
QVHLVESGGASVQAGGSLRLSCAATAYTASNYCMGWFRQSPGKEREAVASINDDGVTSYADSVKGR
FTISQDSAKKTLYLQMNRLKPEDTAMYYCAATPDGYCYAERLSRWRYEFWGQGTQVTVSS
SEQ ID NO: 309 (AS25117 sdAb amino acid sequence; CDRs are underlined)
EVQLAESGGGSVQAGGSLRLSCVISGTSISPDCMGWFRQAPGKKREAVMSIFTNTGSTRYGDSVKGRF
TSSQGNAKNTLYLQMDSLKLDDTATYYCAARYTGGDCFNLEPLAWRFWGQGTQVTVSS
SEQ ID NO: 310 (AS25119 sdAb amino acid sequence; CDRs are underlined)
QVQLVESGGGSVQAGGPLRLTCAATGYSWRPDCMGWYRQAAEKEREGVAVIDADGITSYADAAKG
RFTISRDNNNITLYLQMLKPDDTGMYVCVIGWRVSSGGNCQFNDYWGQGTQVTVSS
SEQ ID NO: 311 (AS25149 sdAb amino acid sequence; CDRs are underlined)
QVQLVESGGGSVQSGGSLKLSCAVSGSGYSYSRGCFAWFQQRPGKEREGVAIINSDGHTRYSDSVQG
RFIISQDKAKNTLYLQMNSLKPDDAAMYYCAYDRNQCYVLLDRLRLPGTFSDWGQGTQVTVSS
SEQ ID NO: 312 (AS25156 sdAb amino acid sequence; CDRs are underlined)
EVQLVESGGGSVQSGGSLRLSCAVSGSGYSYSRGCFAWFQQRPGKEREGVAIINSDGHTRYSDSVQG
RFIISQDKAKNTLYLQMNSLKPDDTAMYYCAYDCSQCYVLRDRLRLPDTFTDWGQGTQVTVSS
SEQ ID NO: 313 (AS25164 sdAb amino acid sequence; CDRs are underlined)
EVQLVESGGGSVQSGGSLRLSCAVSGSGYSYNRGCFAWFQQRPGKEREGVAIINSDGHTTYGDSVQG
RFIISQDKAKNTLDLQMNSLKPDDTAMYYCAYDRNQCYVLRDRLRLPDTFTDWGQGTQVTVSS
SEQ ID NO: 314 (AS25170 sdAb amino acid sequence; CDRs are underlined)
QVKLVESGGGLVQSGGSLRLSCAVSGSGYSYSRGCFAWFQQRPGKEREGVAIINMDGHTMYSDLAQ
GRFIISQDKAKNTLYLQMNSLKPDDTAMYYCAYDRDQCYVLRDRLRLPDTFNDWGQGTQVTVSS
SEQ ID NO: 315 (AS25222 sdAb amino acid sequence; CDRs are underlined)
QIQLVESGGGSVQAGGSLRLSCAVTGISISPDCMGWFRQAPGKKREAVATIFTNTASTRYGDSVKGRF
TSSQGNGKNTLYLQMDSLNVDDTATYYCAARYTGGNCFNLEPLAWHFWGQGTQVTVSS
SEQ ID NO: 316 (AS25396 sdAb amino acid sequence; CDRs are underlined)
QVHLMESGGGSVQAGGSLRLSCVVSGISISPDCMGWFRQAPGKKREAVATIFTNTRRTRYGDSVKGR
VTSSQGNAKNTLYLKMDNLRHDDTATYYCAARYTGGDCFNLDPLSWRFWGQGTQVTVSS
SEQ ID NO: 317 (AS25457 sdAb amino acid sequence; CDRs are underlined)
QVQLVESGGGSVQAGGSLRLSCAVSGISISPDCMGWFRQAPGKKREAVATIFTNTRSTRYGDSVKGRF
TSSQGNAKNTLYLQMDSLKLDDTATYYCAARYTGGDCFNLEPVAWRFWGQGTQVTVSS
SEQ ID NO: 318 (AS25487 sdAb amino acid sequence; CDRs are underlined)

EVQLVESGGGLVQPGGSLRLSCAASGFTFSVWSMSWVRQAPGEGLEWVSTITGSGAQTYYASSVRG
RFTTSRDNAKNTVYLQMNSLKSDDTAVYYCERGNGQTAMEALINPPERPGTQVTVSS
SEQ ID NO: 319 (AS25095 sdAb amino acid sequence; CDRs are underlined)
QVQLVESGGGLMQPGGSLRLSCAASGFTFSSYWMYWVRQAPGKGLEWVSVINRAGDSAWYADSVT
GRFTISRDNAKNTVYLQMDSLKPEDTAMYYCAADSRGYGGDWYKLLSDFNYCGQGTQVTVSS
SEQ ID NO: 320 (AS25435 sdAb amino acid sequence; CDRs are underlined)
QVQLVESGGGSVQAGGSLRLSCAATAYTASFYCMGWFRQAPGKEREAVASINDDGVTMYADSVKG
QFTISQDSATKTLYLQMNRLKPEDTAMYYCAATPEGYCYAERLSTWRYTFWGQGTQVTVSS
SEQ ID NO: 321 (AS15193VH8 sdAb amino acid sequence; CDRs are underlined)
EVQLVESGGGLVQPGGSLRLSCAVSGNIYNRNFMGWFRQAPGKGLEGVSAIYTGTSRTYYADSVKG
RFTISRDNSKNTVYLQMNSLRAEDTAVYYCAADLRDGFWDTGVWNTWGQGTLVTVSS
SEQ ID NO: 322 (AS15193VH8M1 sdAb amino acid sequence; CDRs are underlined)
EVQLVESGGGLVQPGGSLRLSCAVSGNIYNRNFMGWFRQAPGKGLEGVSAIYTGTSRTYYADSVKG
RFTISRDNSKNTVYLQMNSLRAEDTAVYYCAADLREGFWDTGVWNTWGQGTLVTVSS
SEQ ID NO: 323 (AS15193VH18 sdAb amino acid sequence; CDRs are underlined)
EVQLVESGGGLVQPGGSLRLSCAVSGNIYNRNFMGWFRQAPGKGREGVSAIYTGTSRTYYADSVKG
RFTISRDNAKNTVYLQMNSLRPEDTAVYYCAADLRDGFWDTGVWNTWGQGTLVTVSS
SEQ ID NO: 324 (AS15193VH18M1 sdAb amino acid sequence; CDRs are underlined)
EVQLVESGGGLVQPGGSLRLSCAVSGNIYNRNFMGWFRQAPGKGREGVSAIYTGTSRTYYADSVKG
RFTISRDNAKNTVYLQMNSLRPEDTAVYYCAADLREGFWDTGVWNTWGQGTLVTVSS
SEQ ID NO: 325 (A31543 HCAb amino acid sequence; CDRs are underlined, linker is bolded)
QVQLVESGGGKVQPGGSLRLSCAASGGTLDYYAIGWFRQAPGKEREAVSCISSSDGSTYYADSVKGR
FTISRDNAKNTVYLQMNSLKPGDTAVYHCATDRACGSSWLGAESWAQGTQVTVSS**ESKYGPPCPPC**
PAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQF
NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQV
SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHE
ALHNHYTQKSLSLSLGK
SEQ ID NO: 326 (AS06962 HCAb amino acid sequence; CDRs are underlined, linker is bolded)
QVHLVDSGGGLVQPGGSLRLSCAASGSITSRNTMGWYRQVPGKQRELVALIATFVTHYADSVKGRFT
ISRDNARKMVFLEMNSLQPEDTGAYYCYVDVSPYWGRGTQVTVSS**ESKYGPPCPPCP**APEFLGGPS
VFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVL
TVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFY
PSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKS
LSLSLGK
SEQ ID NO: 327 (AS15090 HCAb amino acid sequence; CDRs are underlined, linker is bolded)
QVQLVESGGGSVQAGGSLRLSCAASGYTYIPNCMAWFRQAPGKEREGVTLIFTGDGTSTYVDSVKGR
FTISQDNAKNTVYLQMNSLKPEDTALYYCAAAERCSGSNDRISFWGISYWGQGTQVTVSS**ESKYGPP**
CPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPR
EEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTK
NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSV
MHEALHNHYTQKSLSLSLGK
SEQ ID NO: 328 (AS15140 HCAb amino acid sequence; CDRs are underlined, linker is bolded)
QVQLVESGGGSVQAGGSLRLSCTASAYTYSNICLGWLRQAPGGGLEAVATIYIADQTSYYADSVKGR
FRISKDAAKNAVYLQMSSLRPEDTAMYYCASRYGSTCGEYLADYTSRAQGTQVTVSS**ESKYGPPCP**
PCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREE
QFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVM
HEALHNHYTQKSLSLSLGK
SEQ ID NO: 329 (AS15152 HCAb amino acid sequence; CDRs are underlined, linker is bolded)
QMQLVESGGGSVQAGGSLRLSCAVSGYIYNRNFMGWFRQAPGKEREGVAAIYTGGPYTYYTDSVQG
RFTISQDNTKNTVYLQMNSLKPEDTAMYYCVSDLSDGTWDQGRWNYWGQGTQVTVSS**ESKYGPPC**
PPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREE
QFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKN

QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVM
HEALHNHYTQKSLSLSLGK

SEQ ID NO: 330 (AS15156 HCAb amino acid sequence; CDRs are underlined, linker is bolded)
QVQLVESGGGSAQAGGSLRLSCAVSGYIYNRNFMGWFRQVPGKVREGVAAIYTGTERTYYADSVKG
RFTISQDNAKNTVYLQMNSLKPEDTAMYYCVADLRDGTWDTGVWNTWGQGTQVTVSS**ESKYGPP**
CPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPR
EEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTK
NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSV
MHEALHNHYTQKSLSLSLGK

SEQ ID NO: 331 (AS15193 HCAb amino acid sequence; CDRs are underlined, linker is bolded)
QIQLVESGGGSAQAGGSLRLSCVVSGNIYNRNFMGWFRQAPGKVREGVAAIYTGTSRTYYADSVKG
RFTISQDNAKNTVYLQMNSLKPEDTAMYYCAADLRDGFWDTGVWNTWGQGTQVTVSS**ESKYGPP**
CPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPR
EEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTK
NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSV
MHEALHNHYTQKSLSLSLGK

SEQ ID NO: 332 (AS15872 HCAb amino acid sequence; CDRs are underlined, linker is bolded)
QVQLVESGGGLVQPGGSLTLSCAASGFTFSTAAMSWVRQVPEEGLEWVASIDSSGSRTYYAGSVKGR
FTISRDNAKNTLYLQLNSLKAEDTAMYYCAKDHMSWLPRGQGTQVTVSS**ESKYGPPCPPCP**APEFL
GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRV
VSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLV
KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHY
TQKSLSLSLGK

SEQ ID NO: 333 (AS15881 HCAb amino acid sequence; CDRs are underlined, linker is bolded)
QVQLVESGGGSVQAGGSLRLSCAASGFTDSSYCGAWFRQVPGKEREGVAIIDRYGGTMYKDSVKGR
FTISKDTAKNILYLQMNSLKLEDTAMYYCAAAEYRGSSCDAESGYWGQGTQVTVSS**ESKYGPPCPP**
CPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQ
FNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQ
VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMH
EALHNHYTQKSLSLSLGK

SEQ ID NO: 334 (AS15883 HCAb amino acid sequence; CDRs are underlined, linker is bolded)
QVHLMESGGGSVQAGGSLTLSCAASVFTDSNYCMAWFRQVPGKEREGVAIIDRYGGTMYKDSVKG
RFTISKDTAKNILYLQMNSLKLEDTAMYYCAAAGYRGSSCDADSGYWGQGTQVTVSS**ESKYGPPCP**
PCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREE
QFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVM
HEALHNHYTQKSLSLSLGK

SEQ ID NO: 335 (AS15892 HCAb amino acid sequence; CDRs are underlined, linker is bolded)
QIQLVESGGGSVQAGGSLRLSCAASGFTDSSYCGAWFRQVPGKEREGVAIIDRYGGTMYKDSVKGRF
TISKDTAKNILYLQMNSLKLEDTAMYYCAAAEYRGSSCDAESGYWGQGTQVTVSS**ESKYGPPCPPC**
PAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQF
NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQV
SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHE
ALHNHYTQKSLSLSLGK

SEQ ID NO: 336 (AS15899 HCAb amino acid sequence; CDRs are underlined, linker is bolded)
QVQLVESGGGSVQAGGSLRLSCAASGYTAGSLCMGWFRQAPGKEREGVAAIYTGGGSTYYADSVK
GRFTISQDNAKNTVYLQMNSLKPEDTAQYYCGAGSREDYCDRGYIYDHWGQGTQVTVSS**ESKYGPP**
CPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPR
EEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTK
NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSV
MHEALHNHYTQKSLSLSLGK

SEQ ID NO: 337 (AS17049 HCAb amino acid sequence; CDRs are underlined, linker is bolded)

QVKLVESGGGSVQAGGYLRLSCAASGDTNNLNFRGWFRQAPGKEREGVAVITHSGSTYYAESVKGR
FTISQDLAKNTMYLQMNSLKPEDTAMYYCAAADVWRISWSFVPELFSYWGQGTQVTVSS**ESKYGPP**
CPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPR
EEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTK
NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSV
MHEALHNHYTQKSLSLSLGK

SEQ ID NO: 338 (AS17118 HCAb amino acid sequence; CDRs are underlined, linker is bolded)

QVQLVESGGGSVQAGGSLRLSCAGSGFTFNNYAMGWFRQAPGKEREGIAGIWTGGGSTYYADSVKG
RFTISEDVAKNTVYLQMDSLKPEDTAMYYCAAERWDYSDWRRLKRGDYNYWGQGTQVTVSS**ESK**
YGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAK
TKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQE
EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVF
SCSVMHEALHNHYTQKSLSLSLGK

SEQ ID NO: 339 (AS24984 HCAb amino acid sequence; CDRs are underlined, linker is bolded)

QVHLMESGGGSVPSGGSLRLSCAVSGSGYSYSRGCFAWFQQRPGKEREGVAIINMDGHTRYSDSVQG
RFIISQDKAKNTLHLQMNTLRPDDTAMYYCAYDRSQCYVLSDRLRLPGTFSDWGQGTQVTVSS**ESK**
YGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAK
TKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQE
EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVF
SCSVMHEALHNHYTQKSLSLSLGK

SEQ ID NO: 340 (AS25037 HCAb amino acid sequence; CDRs are underlined, linker is bolded)

EVQLAESGGGSVQAGGSLKLSCLASQWISSDCGMAWYRQAPGKERELVSRISSDDTTTYADSVKGRF
TISQDSAKNTLYLQMNKLKTEDTGVYYCAAEAKSTITSLCYPLNYWGQGTQVTVSS**ESKYGPPCPPC**
PAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQF
NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQV
SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHE
ALHNHYTQKSLSLSLGK

SEQ ID NO: 341 (AS25064 HCAb amino acid sequence; CDRs are underlined, linker is bolded)

QVQLVESGGGSVQAGGSLRLTCAATGYSWRPDCMGWYRQAAEKEREGVAVIDADGITSYADAAKG
RFTISRDNNKITLYLQMLKPDDTGMYVCVVGWRVSSGGNCQFNDYWGQGTQVTVSS**ESKYGPPCP**
PCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREE
QFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVM
HEALHNHYTQKSLSLSLGK

SEQ ID NO: 342 (AS25067 HCAb amino acid sequence; CDRs are underlined, linker is bolded)

QVHLMESGGGAVQTGGSLRLSCAVSGISISPDCMGWFRQAPGKKREAVTTIFANTGSARYGDSVKGR
FTSSQGNAKNTLYLQMDSVKLDDTGTYYCAARFTGGDCFDHQPLAWRFWGQGTQVTVSS**ESKYGP**
PCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKP
REEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMT
KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCS
VMHEALHNHYTQKSLSLSLGK

SEQ ID NO: 343 (AS25071 HCAb amino acid sequence; CDRs are underlined, linker is bolded)

EVQLAESGGGSVQSGGSLRLSCAVSGSGYSYSRGCFAWFQQRPGKEREGVAIINSDGHTAYSDSVQG
RFIISQDKAKNTLYLQMNSLKPDDTAMYYCAYDRSQCYVLRDRLRLPDTFTDWGQGTQVTVSS**ESK**
YGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAK
TKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQE
EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVF
SCSVMHEALHNHYTQKSLSLSLGK

SEQ ID NO: 344 (AS25115 HCAb amino acid sequence; CDRs are underlined, linker is bolded)

QVHLVESGGASVQAGGSLRLSCAATAYTASNYCMGWFRQSPGKEREAVASINDDGVTSYADSVKGR
FTISQDSAKKTLYLQMNRLKPEDTAMYYCAATPDGYCYAERLSRWRYEFWGQGTQVTVSS**ESKYGP**
PCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKP
REEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMT

KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCS
VMHEALHNHYTQKSLSLSLGK

SEQ ID NO: 345 (AS25117 HCAb amino acid sequence; CDRs are underlined, linker is bolded)
EVQLAESGGGSVQAGGSLRLSCVISGTSISPDCMGWFRQAPGKKREAVMSIFTNTGSTRYGDSVKGRF
TSSQGNAKNTLYLQMDSLKLDDTATYYCAARYTGGDCFNLEPLAWRFWGQGTQVTVSS**ESKYGPP**
CPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPR
EEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTK
NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSV
MHEALHNHYTQKSLSLSLGK

SEQ ID NO: 346 (AS25119 HCAb amino acid sequence; CDRs are underlined, linker is bolded)
QVQLVESGGGSVQAGGPLRLTCAATGYSWRPDCMGWYRQAAEKEREGVAVIDADGITSYADAAKG
RFTISRDNNNITLYLQMLKPDDTGMYVCVIGWRVSSGGNCQFNDYWGQGTQVTVSS**ESKYGPPCPP**
CPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQ
FNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQ
VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMH
EALHNHYTQKSLSLSLGK

SEQ ID NO: 347 (AS25149 HCAb amino acid sequence; CDRs are underlined, linker is bolded)
QVQLVESGGGSVQSGGSLKLSCAVSGSGYSYSRGCFAWFQQRPGKEREGVAIINSDGHTRYSDSVQG
RFIISQDKAKNTLYLQMNSLKPDDAAMYYCAYDRNQCYVLLDRLRLPGTFSDWGQGTQVTVSS**ESK**
YGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAK
TKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQE
EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVF
SCSVMHEALHNHYTQKSLSLSLGK

SEQ ID NO: 348 (AS25156 HCAb amino acid sequence; CDRs are underlined, linker is bolded)
EVQLVESGGGSVQSGGSLRLSCAVSGSGYSYSRGCFAWFQQRPGKEREGVAIINSDGHTRYSDSVQG
RFIISQDKAKNTLYLQMNSLKPDDTAMYYCAYDCSQCYVLRDRLRLPDTFTDWGQGTQVTVSS**ESK**
YGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAK
TKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQE
EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVF
SCSVMHEALHNHYTQKSLSLSLGK

SEQ ID NO: 349 (AS25164 HCAb amino acid sequence; CDRs are underlined, linker is bolded)
EVQLVESGGGSVQSGGSLRLSCAVSGSGYSYNRGCFAWFQQRPGKEREGVAIINSDGHTTYGDSVQG
RFIISQDKAKNTLDLQMNSLKPDDTAMYYCAYDRNQCYVLRDRLRLPDTFTDWGQGTQVTVSS**ESK**
YGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAK
TKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQE
EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVF
SCSVMHEALHNHYTQKSLSLSLGK

SEQ ID NO: 350 (AS25170 HCAb amino acid sequence; CDRs are underlined, linker is bolded)
QVKLVESGGGLVQSGGSLRLSCAVSGSGYSYSRGCFAWFQQRPGKEREGVAIINMDGHTMYSDLAQ
GRFIISQDKAKNTLYLQMNSLKPDDTAMYYCAYDRDQCYVLRDRLRLPDTFNDWGQGTQVTVSS**ES**
KYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNA
KTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQ
EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNV
FSCSVMHEALHNHYTQKSLSLSLGK

SEQ ID NO: 351 (AS25222 HCAb amino acid sequence; CDRs are underlined, linker is bolded)
QIQLVESGGGSVQAGGSLRLSCAVTGISISPDCMGWFRQAPGKKREAVATIFTNTASTRYGDSVKGRF
TSSQGNGKNTLYLQMDSLNVDDTATYYCAARYTGGNCFNLEPLAWHFWGQGTQVTVSS**ESKYGPP**
CPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPR
EEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTK
NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSV
MHEALHNHYTQKSLSLSLGK

SEQ ID NO: 352 (AS25396 HCAb amino acid sequence; CDRs are underlined, linker is bolded)

QVHLMESGGGSVQAGGSLRLSCVVSGISISPDCMGWFRQAPGKKREAVATIFTNTRRTRYGDSVKGR
VTSSQGNAKNTLYLKMDNLRHDDTATYYCAARYTGGDCFNLDPLSWRFWGQGTQVTVSS**ESKYGP**
PCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKP
REEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMT
KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCS
VMHEALHNHYTQKSLSLSLGK

SEQ ID NO: 353 (AS25457 HCAb amino acid sequence; CDRs are underlined, linker is bolded)
QVQLVESGGGSVQAGGSLRLSCAVSGISISPDCMGWFRQAPGKKREAVATIFTNTRSTRYGDSVKGRF
TSSQGNAKNTLYLQMDSLKLDDTATYYCAARYTGGDCFNLEPVAWRFWGQGTQVTVSS**ESKYGPP**
CPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPR
EEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTK
NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSV
MHEALHNHYTQKSLSLSLGK

SEQ ID NO: 354 (AS25487 HCAb amino acid sequence; CDRs are underlined, linker is bolded)
EVQLVESGGGLVQPGGSLRLSCAASGFTFSVWSMSWVRQAPGEGLEWVSTITGSGAQTYYASSVRG
RFTTSRDNAKNTVYLQMNSLKSDDTAVYYCERGNGQTAMEALINPPERPGTQVTVSS**ESKYGPPCPP**
CPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQ
FNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQ
VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMH
EALHNHYTQKSLSLSLGK

SEQ ID NO: 355 (AS25095 HCAb amino acid sequence; CDRs are underlined, linker is bolded)
QVQLVESGGGLMQPGGSLRLSCAASGFTFSSYWMYWVRQAPGKGLEWVSVINRAGDSAWYADSVT
GRFTISRDNAKNTVYLQMDSLKPEDTAMYYCAADSRGYGGDWYKLLSDFNYCGQGTQVTVSS**ESK**
YGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAK
TKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQE
EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVF
SCSVMHEALHNHYTQKSLSLSLGK

SEQ ID NO: 356 (AS25435 HCAb amino acid sequence; CDRs are underlined, linker is bolded)
QVQLVESGGGSVQAGGSLRLSCAATAYTASFYCMGWFRQAPGKEREAVASINDDGVTMYADSVKG
QFTISQDSATKTLYLQMNRLKPEDTAMYYCAATPEGYCYAERLSTWRYTFWGQGTQVTVSS**ESKYG**
PPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTK
PREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEM
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCS
VMHEALHNHYTQKSLSLSLGK

SEQ ID NO: 357 (AS15193VH8 HCAb amino acid sequence; CDRs are underlined, linker is bolded)
EVQLVESGGGLVQPGGSLRLSCAVSGNIYNRNFMGWFRQAPGKGLEGVSAIYTGTSRTYYADSVKG
RFTISRDNSKNTVYLQMNSLRAEDTAVYYCAADLRDGFWDTGVWNTWGQGTLVTVSS**ESKYGPPC**
PPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREE
QFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVM
HEALHNHYTQKSLSLSLGK

SEQ ID NO: 358 (AS15193VH8M1 HCAb amino acid sequence; CDRs are underlined, linker is bolded)
EVQLVESGGGLVQPGGSLRLSCAVSGNIYNRNFMGWFRQAPGKGLEGVSAIYTGTSRTYYADSVKG
RFTISRDNSKNTVYLQMNSLRAEDTAVYYCAADLREGFWDTGVWNTWGQGTLVTVSS**ESKYGPPC**
PPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREE
QFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVM
HEALHNHYTQKSLSLSLGK

SEQ ID NO: 359 (AS15193VH18 HCAb amino acid sequence; CDRs are underlined, linker is bolded)
EVQLVESGGGLVQPGGSLRLSCAVSGNIYNRNFMGWFRQAPGKGREGVSAIYTGTSRTYYADSVKG
RFTISRDNAKNTVYLQMNSLRPEDTAVYYCAADLRDGFWDTGVWNTWGQGTLVTVSS**ESKYGPPC**
PPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREE
QFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKN

QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVM
HEALHNHYTQKSLSLSLGK
SEQ ID NO: 360 (AS15193VH18M1 HCAb amino acid sequence; CDRs are underlined, linker is bolded)
EVQLVESGGGLVQPGGSLRLSCAVSGNIYNRNFMGWFRQAPGKGREGVSAIYTGTSRTYYADSVKG
RFTISRDNAKNTVYLQMNSLRPEDTAVYYCAADLREGFWDTGVWNTWGQGTLVTVSS**ESKYGPPC**
PPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREE
QFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVM
HEALHNHYTQKSLSLSLGK
SEQ ID NO: 361 (human PD-1 amino acid sequence, excluding the leader peptide)
PGWFLDSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKLAAFPEDRSQ
PGQDCRFRVTQLPNGRDFHMSVVRARRNDSGTYLCGAISLAPKAQIKESLRAELRVTERRAEVPTAHP
SPSPRPAGQFQTLVVGVVGGLLGSLVLLVWVLAVICSRAARGTIGARRTGQPLKEDPSAVPVFSVDYG
ELDFQWREKTPEPPVPCVPEQTEYATIVFPSGMGTSSPARRGSADGPRSAQPLRPEDGHCSWPL
SEQ ID NO: 362 (extracellular domain of human PD-1 amino acid sequence)
PGWFLDSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKLAAFPEDRSQ
PGQDCRFRVTQLPNGRDFHMSVVRARRNDSGTYLCGAISLAPKAQIKESLRAELRVTERRAEVPTAHP
SPSPRPAGQFQTLV
SEQ ID NO: 363 (IgG4 Fc amino acid sequence)
APEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNS
TYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSL
TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEAL
HNHYTQKSLSLSLGK
SEQ ID NO: 364 (IgG1 inert Fc amino acid sequence)
APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYA
STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS
LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA
LHNHYTQKSLSLSPGK
SEQ ID NO: 365 (IgG1 Fc amino acid sequence)
APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN
STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSL
TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL
HNHYTQKSLSLSPGK
SEQ ID NO: 366 (human acceptor amino acid sequence)
QVQLVESGGGVVQPGGSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVSVIYSGGSSTYYADSVKG
RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK
SEQ ID NO: 367 (human IgG4 (hIgG4) hinge amino acid sequence)
ESKYGPPCPPCP
SEQ ID NO: 368 (mutated human IgG1 (hIgG1) hinge amino acid sequence)
EPKSSDKTHTSPPSP
SEQ ID NO: 369 (human IgG1 (hIgG1) hinge amino acid sequence)
EPKSCDKTHTCPPCP
SEQ ID NO: 370 (linker peptide (9GS) amino acid sequence)
GGGGSGGGS
SEQ ID NO: 371 (linker peptide amino acid sequence)
GGGGSGGGGSGGGGS
SEQ ID NO: 372 (linker peptide amino acid sequence, n is an integer of at least one)
$(G)_n$
SEQ ID NO: 373 (linker peptide amino acid sequence, n is an integer of at least one)
$(GS)_n$
SEQ ID NO: 374 (linker peptide amino acid sequence, n is an integer of at least one)
$(GSGGS)_n$
SEQ ID NO: 375 (linker peptide amino acid sequence, n is an integer of at least one)

$(GGGS)_n$
SEQ ID NO: 376 (Linker peptide amino acid sequence, n is an integer of at least one)
$(GGGGS)_n$
SEQ ID NO: 377 (anti-TIGIT mAb tiragolumab heavy chain amino acid sequence)
EVQLQQSGPGLVKPSQTLSLTCAISGDSVSSNSAAWNWIRQSPSRGLEWLGKTYYRFKWYSDYAVSV
KGRITINPDTSKNQFSLQLNSVTPEDTAVFYCTRESTTYDLLAGPFDYWGQGTLVTVSSASTKGPSVFP
LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ
TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVD
VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPA
PIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD
SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
SEQ ID NO: 378 (anti-TIGIT mAb tiragolumab light chain amino acid sequence)
DIVMTQSPDSLAVSLGERATINCKSSQTVLYSSNNKKYLAWYQQKPGQPPNLLIYWASTRESGVPDRE
SGSGSGTDFTLTISSLQAEDVAVYYCQQYYSTPFTFGPGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV
VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTH
QGLSSPVTKSFNRGEC
SEQ ID NO: 379 (anti-LAG-3 mAb relatlimab heavy chain amino acid sequence)
QVQLQQWGAGLLKPSETLSLTCAVYGGSFSDYYWNWIRQPPGKGLEWIGEINHRGSTNSNPSLKSRV
TLSLDTSKNQFSLKLRSVTAADTAVYYCAFGYSDYEYNWFDPWGQGTLVTVSSASTKGPSVFPLAPC
SRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTC
NVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPE
VQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISK
AKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL
YSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLG
SEQ ID NO: 380 (anti-LAG-3 mAb relatlimab light chain amino acid sequence)
EIVLTQSPATLSLSPGERATLSCRASQSISSYLAWYQQKPGQAPRLLIYDASNRATGIPARFSGSGSGTD
FTLTISSLEPEDFAVYYCQQRSNWPLTFGQGTNLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFY
PREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTK
SFNRGEC
SEQ ID NO: 381 (anti-TIM-3 mAb MBG453 heavy chain amino acid sequence)
QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYNMHWVRQAPGQGLEWIGDIYPGQGDTSYNQKFK
GRATMTADKSTSTVYMELSSLRSEDTAVYYCARVGGAFPMDYWGQGTLVTVSSASTKGPSVFPLAP
CSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYT
CNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDP
EVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTIS
KAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF
LYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK
SEQ ID NO: 382 (anti-TIM-3 mAb MBG453 light chain amino acid sequence)
DIVLTQSPDSLAVSLGERATINCRASESVEYYGTSLMQWYQQKPGQPPKLLIYAASNVESGVPDRFSG
SGSGTDFTLTISSLQAEDVAVYYCQQSRKDPSTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVV
CLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQ
GLSSPVTKSFNRGEC
SEQ ID NO: 383 (anti-CTLA-4 mAb Ipilimumab heavy chain amino acid sequence)
QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYTMHWVRQAPGKGLEWVTFISYDGNNKYYADSVKG
RFTISRDNSKNTLYLQMNSLRAEDTAIYYCARTGWLGPFDYWGQGTLVTVSSASTKGPSVFPLAPSSK
STSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICN
VNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE
DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK
TISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG
SFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
SEQ ID NO: 384 (anti-CTLA-4 mAb Ipilimumab light chain amino acid sequence)
EIVLTQSPGTLSLSPGERATLSCRASQSVGSSYLAWYQQKPGQAPRLLIYGAFSRATGIPDRFSGSGSGT
DFTLTISRLEPEDFAVYYCQQYGSSPWTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNN

FYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPV
TKSFNRGEC

SEQ ID NO: 385 (anti-PD-1 mAb Pembrolizumab (IgG4 S228P) heavy chain amino acid sequence)

QVQLVQSGVEVKKPGASVKVSCKASGYTFTNYYMYWVRQAPGQGLEWMGGINPSNGGTNFNEKFK
NRVTLTTDSSTTTAYMELKSLQFDDTAVYYCARRDYRFDMGFDYWGQGTTVTVSSASTKGPSVFPL
APCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKT
YTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQ
EDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEK
TISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG
SFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

SEQ ID NO: 386 (anti-PD-1 mAb Pembrolizumab (IgG4 S228P) light chain amino acid sequence)

EIVLTQSPATLSLSPGERATLSCRASKGVSTSGYSYLHWYQQKPGQAPRLLIYLASYLESGVPARFSGS
GSGTDFTLTISSLEPEDFAVYYCQHSRDLPLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCL
LNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGL
SSPVTKSFNRGEC