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(54) Title: PD-L1 BINDING FIBRONECTIN TYPE III DOMAINS

(57) Abstract: FN3 domains that specifically bind to PD-L1, their conjugates, isolated nucleotides encoding the molecules, vectors, host cells, and methods of making and using them are useful in therapeutic and diagnostic applications.

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PD-L1 BINDING FIBRONECTIN TYPE III DOMAINS

FIELD OF THE INVENTION

The present invention relates to fibronectin type III domains that specifically bind to PD-L1 and methods of making and using the molecules.

BACKGROUND OF THE INVENTION

The immune system is tightly controlled by a network of costimulatory and co-inhibitory ligands and receptors. These molecules provide secondary signals for T cell activation and provide a balanced network of positive and negative signals to maximize immune responses against infection and tumors, while limiting immunity to self (Wang *et al.,* (Epub Mar. 7, 2011) *J Exp Med* 208(3):577-92; Lepenies *et al.*, (2008) *Endocr Metab Immune Disord Drug Targets* 8:279-288).

Programmed Death-1 (PD-1) is a key immune checkpoint receptor expressed by activated T and B cells and mediates immunosuppression. The ligand for PD-1, PD-L1, is expressed by antigen-presenting cells and many cancers such as lung, ovarian and colon carcinoma and various myelomas. Binding of PD-L1 to PD-1 on T cells downregulates T cell proliferation and activation and drives T cell anergy and exhaustion in the tumor microenvironment, facilitating tumor cell escape from T-cell mediated immune surveillance.

Therapeutic efficacy of PD-1 and PD-L1 antagonists has been validated in clinical trials. However, response rates remain low. For example, Opdivo® (Nivolumab) treatment achieved a 26% objective response rate (ORR) across the 27 clinical trials analyzed (Tie *et al.,* Int J Cancer 2016 Nov 4 doi: 10.1002/ijc.30501. [Epub ahead of print])

Measuring the expression of PD-L1 protein in the tumor tissue may aid in the early detection of cancer pathologies and may help assess the efficacy and durability of PD-L1 and PD-1 antagonists. For example, PD-L1 expression in at least 50% of tumor cells correlated with improved efficacy of Keytruda® (pembrolizumab) (Garon *et al.,* N Engl J Med 2015; 372:2018-2028), and PD-L1 expression has been correlated with poor prognosis (see for example Wang *et al.,* Eur J Surg oncol 2015 Apr; 41(4):450-6).

However, the use of PD-L1 protein expression as an accurate predictor for cancer and/or the efficacy of anti-PD-1 and anti-PD-L1 directed therapies remain challenging partially due to observed variability in results depending on the detection reagent used.

For example, the evaluation of PD-L1 expression in non-small cell lung cancer samples using commercially available assays such as PD-L1 (E1L3N®) XP® Rabbit mAb (Cell Signaling) and Ventana PD-L1 (SP142) Assay yielded discordant results (McLaughlin *et al.,* JAMA Oncol 2016 Jan;2(1):46-54)

Therefore, there is a need for reagents to accurately detect PD-L1 in tumor tissues and other samples and new therapeutics that modulate the interaction between PD-L1 and PD-1.

SUMMARY OF THE INVENTION

The invention provides an isolated FN3 domain that specifically binds to PD-L1.

The invention also provides an isolated FN3 domain that specifically binds to PD-L1 comprising the sequence of SEQ ID NOs: 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123 or 124.

The invention also provides an isolated polynucleotide encoding the FN3 domain that specifically binds to PD-L1 of the invention.

The invention also provides a vector comprising the polynucleotide of the invention.

The invention also provides a host cell comprising the vector of the invention.

The invention also provides a method of producing the FN3 domain that specifically binds to PD-L1 of the invention, comprising culturing the isolated host cell of the invention under conditions that the FN3 domain that specifically binds to PD-L1 is expressed, and purifying the FN3 domain that specifically binds PD-L1.

The invention also provides a pharmaceutical composition comprising the FN3 domain that specifically binds PD-L1 of the invention and a pharmaceutically acceptable carrier.

The invention also provides an anti-idiotypic antibody that specifically binds the FN3 domain that specifically binds to PD-L1 of the invention.

The invention also provides a kit comprising the FN3 domain of the invention.

The invention also provides a method of detecting PD-L1-expressing cancer cells in a tumor tissue, comprising

obtaining a sample of the tumor tissue from a subject; and

detecting whether PD-L1 is expressed in the tumor tissue by contacting the sample of the tumor tissue with the FN3 domain that specifically binds to PD-L1 comprising the sequence of any one of SEQ ID NOs: 34-124 and detecting the binding between PD-L1 and the FN3 domain.

The invention also provides a method of isolating or detecting PD-L1 expressing cells, comprising obtaining a sample from a subject;

contacting the sample with the FN3 domain that specifically binds to PD-L1 comprising the sequence of any one of SEQ ID NOs: 34-124, and

isolating or detecting the cells bound to the FN3 domains.

The invention also provides a method of detecting PD-L1-expressing cancer cells in a tumor tissue, comprising

conjugating the FN3 domain that specifically binds to PD-L1 comprising the sequence of any one of SEQ ID NOs: 34-124 to a detectable label to form a conjugate;

administering the conjugate to a subject; and

visualizing the PD-L1 expressing cancer cells to which the conjugate is bound.

DETAILED DESCRIPTION OF THE INVENTION

As used in this specification and the appended claims, the singular forms "a," "an," and "the" include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to "a cell" includes a combination of two or more cells, and the like.

"Fibronectin type III (FN3) domain" (FN3 domain) refers to a domain occurring frequently in proteins including fibronectins, tenascin, intracellular cytoskeletal proteins, cytokine receptors and prokaryotic enzymes (Bork and Doolittle, Proc Nat Acad Sci USA 89:8990-8994, 1992; Meinke *et al.*, J Bacteriol 175:1910-1918, 1993; Watanabe *et al.,* J Biol Chem 265:15659-15665, 1990). Exemplary FN3 domains are the 15 different FN3 domains present in human tenascin C, the 15 different FN3 domains present in human fibronectin (FN), and non-natural synthetic FN3 domains as described for example in U.S. Pat. No. 8,278,419. Individual FN3 domains are referred to by domain number and protein name, e.g., the 3rd FN3 domain of tenascin (TN3), or the 10th FN3 domain of fibronectin (FN10).

"Centyrin" refers to a FN3 domain that is based on the consensus sequence of the 15 different FN3 domains present in human tenascin C.

The term "capture agent" refers to substances that bind to a particular type of cells and enable the isolation of that cell from other cells. Exemplary capture agents are

magnetic beads, ferrofluids, encapsulating reagents, molecules that bind the particular cell type and the like.

"Sample" refers to a collection of similar fluids, cells, or tissues isolated from a subject, as well as fluids, cells, or tissues present within a subject. Exemplary samples are tissue biopsies, fine needle aspirations, surgically resected tissue, organ cultures, cell cultures and biological fluids such as blood, serum and serosal fluids, plasma, lymph, urine, saliva, cystic fluid, tear drops, feces, sputum, mucosal secretions of the secretory tissues and organs, vaginal secretions, ascites fluids, fluids of the pleural, pericardial, peritoneal, abdominal and other body cavities, fluids collected by bronchial lavage, synovial fluid, liquid solutions contacted with a subject or biological source, for example, cell and organ culture medium including cell or organ conditioned medium and lavage fluids and the like.

"Substituting" or "substituted" or 'mutating" or "mutated" refers to altering, deleting of inserting one or more amino acids or nucleotides in a polypeptide or polynucleotide sequence to generate a variant of that sequence.

"Variant" refers to a polypeptide or a polynucleotide that differs from a reference polypeptide or a reference polynucleotide by one or more modifications for example, substitutions, insertions or deletions.

"Specifically binds" or "specific binding" refers to the ability of the FN3 domain of the invention to bind PD-L1 with a dissociation constant (K_D) of about $1x10^{-6}$ M or less, for example about $1x10^{-7}$ M or less, about $1x10^{-8}$ M or less, about $1x10^{-9}$ M or less, about $1x10^{-10}$ M or less, about $1x10^{-11}$ M or less, about $1x10^{-12}$ M or less, or about $1x10^{-13}$ M or less. Alternatively, "specific binding" refers to the ability of the FN3 domain of the invention to bind PD-L1 at least 5-fold above the negative control in standard ELISA assay. The isolated FN3 domain of the invention that specifically binds PD-L1 may, however, have cross-reactivity to other related antigens, for example to the same predetermined antigen from other species (homologs), such as *Macaca Fascicularis* (cynomolgous monkey, cyno) *or Pan troglodytes* (chimpanzee).

"Library" refers to a collection of variants. The library may be composed of polypeptide or polynucleotide variants.

"Stability" refers to the ability of a molecule to maintain a folded state under physiological conditions such that it retains at least one of its normal functional activities, for example, binding to a predetermined antigen such as PD-L1.

"PD-L1" refers to human PD-L1 protein having the amino acid sequence of **SEQ ID NO: 32**. The extracellular domain of PD-L1 spans residues 1-220, the transmembrane domain spans residues 221-241 and the cytoplasmic domain spans residues 242-272.

"PD-1" refers to human PD-1 protein having the amino acid sequence of **SEQ ID NO: 33**. The extracellular domain of PD-1 spans residues 1-150, the transmembrane domain spans residues 151-171 and the cytoplasmic domain spans residues 172-268 of **SEQ ID NO: 33**.

"Tencon" refers to the synthetic fibronectin type III (FN3) domain having the sequence shown in **SEQ ID NO: 1** and described in U.S. Pat. Publ. No. 2010/0216708.

A "cancer cell" or a "tumor cell" refers to a cancerous, pre-cancerous or transformed cell, either *in vivo*, *ex vivo*, and in tissue culture, that has spontaneous or induced phenotypic changes that do not necessarily involve the uptake of new genetic material. Although transformation can arise from infection with a transforming virus and incorporation of new genomic nucleic acid, or uptake of exogenous nucleic acid, it can also arise spontaneously or following exposure to a carcinogen, thereby mutating an endogenous gene. Transformation/cancer is exemplified by, e.g., morphological changes, immortalization of cells, aberrant growth control, foci formation, proliferation, malignancy, tumor specific markers levels, invasiveness, tumor growth or suppression in suitable animal hosts such as nude mice, and the like, *in vitro*, *in vivo*, and *ex vivo* (Freshney, Culture of Animal Cells: A Manual of Basic Technique (3rd ed. 1994)).

"Vector" refers to a polynucleotide capable of being duplicated within a biological system or that can be moved between such systems. Vector polynucleotides typically contain elements, such as origins of replication, polyadenylation signal or selection markers that function to facilitate the duplication or maintenance of these polynucleotides in a biological system. Examples of such biological systems may include a cell, virus, animal, plant, and reconstituted biological systems utilizing biological components capable of duplicating a vector. The polynucleotide comprising a vector may be DNA or RNA molecules or a hybrid of these.

"Expression vector" refers to a vector that can be utilized in a biological system or in a reconstituted biological system to direct the translation of a polypeptide encoded by a polynucleotide sequence present in the expression vector.

"Polynucleotide" refers to a synthetic molecule comprising a chain of nucleotides covalently linked by a sugar-phosphate backbone or other equivalent covalent chemistry. cDNA is a typical example of a polynucleotide.

"Polypeptide" or "protein" refers to a molecule that comprises at least two amino acid residues linked by a peptide bond to form a polypeptide. Small polypeptides of less than about 50 amino acids may be referred to as "peptides".

"Valent" refers to the presence of a specified number of binding sites specific for an antigen in a molecule. As such, the terms "monovalent", "bivalent", "tetravalent", and "hexavalent" refer to the presence of one, two, four and six binding sites, respectively, specific for an antigen in a molecule.

"Subject" includes any human or nonhuman animal. "Nonhuman animal" includes all vertebrates, *e.g.,* mammals and non-mammals, such as nonhuman primates, sheep, dogs, cats, horses, cows chickens, amphibians, reptiles, etc. Except when noted, the terms "patient" or "subject" are used interchangeably.

"Isolated" refers to a homogenous population of molecules (such as synthetic polynucleotides or a polypeptide such as FN3 domains) which have been substantially separated and/or purified away from other components of the system the molecules are produced in, such as a recombinant cell, as well as a protein that has been subjected to at least one purification or isolation step. "Isolated FN3 domain" refers to an FN3 domain that is substantially free of other cellular material and/or chemicals and encompasses FN3 domains that are isolated to a higher purity, such as to 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% purity.

Compositions of matter

The present invention provides fibronectin type III (FN3) domains that specifically bind PD-L1. These molecules can be used in therapeutic and diagnostic applications and in imaging. The present invention provides polynucleotides encoding the FN3 domains of the invention or complementary nucleic acids thereof, vectors, host cells, and methods of making and using them.

The invention provides an isolated FN3 domain that specifically binds PD-L1.

The FN3 domain of the invention may bind PD-L1 with a dissociation constant (K_D) of less than about $1x10^{-7}$ M, for example less than about $1x10^{-8}$ M, less than about $1x10^{-9}$ M, less than about $1x10^{-10}$ M, less than about $1x10^{-11}$ M, less than about $1x10^{-12}$ M, or less than about $1x10^{-13}$ M as determined by surface plasmon resonance or the Kinexa method, as practiced by those of skill in the art. The measured affinity of a particular FN3 domain-antigen interaction can vary if measured under different conditions (e.g., osmolarity, pH). Thus, measurements of affinity and other antigen-binding parameters

(e.g., K_D, K_{on}, K_{off}) are made with standardized solutions of protein scaffold and antigen, and a standardized buffer, such as the buffer described herein.

The FN3 domain of the invention may bind PD-L1 at least 5-fold above the signal obtained for a negative control in standard ELISA assay.

In some embodiments, the FN3 domain that specifically binds PD-L1comprises an initiator methionine (Met) linked to the N-terminus of the molecule.

In some embodiments, the FN3 domain that specifically binds PD-L1comprises a cysteine (Cys) linked to a C-terminus of the FN3 domain.

The addition of the N-terminal Met and/or the C-terminal Cys may facilitate expression and/or conjugation of half-life extending molecules.

In some embodiments, the FN3 domain that specifically binds PD-L1 is internalized into a cell.

Internalization of the FN3 domain may facilitate delivery of a cytotoxic agent into tumor cells.

In some embodiments, the FN3 domain that specifically binds PD-L1 inhibits binding of PD-L1 to PD-1.

Inhibition of binding of PD-L1 to PD-1 by the FN3 domains of the invention may be assessed using competition ELISA. In an exemplary assay, 1 μg/ml recombinant human PD-L1 extracellular domain is bound on wells of microtiter plates, the wells are washed and blocked, and 10 μg/ml of the test FN3 domain is added. Without washing, 7.5 μg/ml PD-1 extracellular domain is added into the wells and incubated for 30 min, after which 0.5 μg/ml anti-PD-1 antibody is added and incubated for 30 min. The plates are washed and 0.5 μg/mL neutravidin-HRP conjugate polyclonal antibody is added and incubated for 30 minutes. The plates are washed and POD Chemiluminescence substrate added immediately prior to reading the luminescence signal. The FN3 domains of the invention inhibit binding of PD-L1 to PD-1 when the binding of PD-1 is reduced by at least about 80%, 85%, 90%, 95% or 100%.

In some embodiments, the FN3 domain that specifically binds PD-L1 is a PD-L1 antagonist.

In some embodiments, the FN3 domain that specifically binds PD-L1 is a PD-L1 agonist.

"Antagonist" refers to a FN3 domain that specifically binds PD-L1 that suppresses at least one reaction or activity that is induced by PD-L1 binding PD-1. A molecule is an antagonist when the at least one reaction or activity is suppressed by at least about 30%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% more than

the at least one reaction or activity suppressed in the absence of the antagonist (*e.g.*, negative control), or when the suppression is statistically significant when compared to the suppression in the absence of the antagonist. A typical reaction or activity that is induced by PD-L1 binding PD-1 is reduced antigen-specific $CD4^+$ or $CD8^+$ cell proliferation or reduced interferon-γ (IFN-γ) production by T cells.

The antagonistic FN3 domains that specifically bind PD-L1 may be used in the treatment of cancer or viral infections and in general in treatment of diseases in which activation of immune responses is desirable.

"Agonist" refers to a FN3 domain that specifically binds PD-L1 that induces at least one reaction or activity that is induced by PD-L1 binding PD-1. The FN3 domain is an agonist when the at least one reaction or activity is induced by at least about 30%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% greater than the at least one reaction or activity induced in the absence of the agonist (*e.g.*, negative control), or when the induction is statistically significant when compared to the induction in the absence of the agonist. A typical reaction or activity that is induced by PD-L1 binding PD-1 is reduced antigen-specific $CD4^+$ or $CD8^+$ cell proliferation or reduced interferon-γ (IFN-γ) production by T cells.

The agonistic FN3 domains that specifically bind PD-L1 may be used in the treatment of autoimmune or inflammatory diseases and in general diseases in which suppression of immune responses is desirable.

In some embodiments, the FN3 domain that specifically binds PD-L1 does not inhibit binding of PD-L1 to PD-1.

In some embodiments, the FN3 domain that specifically binds PD-L1 does not activate signaling downstream of PD-1.

In some embodiments, the FN3 domain that specifically binds PD-L1 is based on Tencon sequence of SEQ ID NO: 1 or Tencon 27 sequence of SEQ ID NO: 4, optionally having substitutions at residues positions 11, 14, 17, 37, 46, 73, and/or 86 (residue numbering corresponding to SEQ ID NO: 4).

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NOs: 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123 and/or 124.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 34.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 35.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 36.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 37.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 38.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 39.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 40.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 41.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 42.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 43.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 44.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 45.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 46.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 47.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 48.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 49.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 50.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 51.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 52.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 53.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 54.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 55.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 56.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 57.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 58.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 59.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 60.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 61.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 62.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 63.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 64.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 65.

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The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 71.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 72.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 73.

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The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 81.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 82.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 83.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 84.

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The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 86.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 87.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 88.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 89.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 90.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 91.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 92.

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The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 99.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 100.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 101.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 102.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 103.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 104.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 105.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 106.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 107.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 108.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 109.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 110.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 111.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 112.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 113.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 114.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 115.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 116.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 117.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 118.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 119.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 120.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 121.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 122.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 123.

The invention also provides an isolated FN3 domain that specifically binds PD-L1 comprising the amino acid sequence of SEQ ID NO: 124.

In some embodiments, the isolated FN3 domain that specifically binds PD-L1 comprises an initiator methionine (Met) linked to the N-terminus of the molecule.

in some embodiments, the isolated FN3 domain that specifically binds PD-L1 comprises an amino acid sequence that is 62%, 63%, 64% , 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 74.

In some embodiments, the isolated FN3 domain that specifically binds PD-L1 comprises an amino acid sequence that is 62%, 63%, 64% , 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of any one of SEQ ID NOs: 34-124.

Conjugates of the FN3 domains that specifically bind PD-L1 of the invention

The invention also provides an isolated FN3 domain that specifically binds PD-L1 conjugated to a heterologous molecule(s).

In some embodiments, the heterologous molecule is a detectable label or a cytotoxic agent.

The invention also provides an FN3 domain that specifically binds PD-L1 conjugated to a detectable label.

The invention also provides an FN3 domain that specifically binds PD-L1 conjugated to a cytotoxic agent.

In some embodiments, the detectable label is also a cytotoxic agent.

The FN3 domains that specifically bind PD-L1 of the invention conjugated to a detectable label can be used to evaluate expression of PD-L1 on samples such as tumor tissue *in vivo* or *in vitro*.

Detectable label includes compositions that when conjugated to the FN3 domains that specifically bind PD-L1 of the invention renders the latter detectable, via spectroscopic, photochemical, biochemical, immunochemical, or chemical means.

Exemplary detectable labels include radioactive isotopes, magnetic beads, metallic beads, colloidal particles, fluorescent dyes, electron-dense reagents, enzymes (for example, as commonly used in an ELISA), biotin, digoxigenin, haptens, luminescent molecules, chemiluminescent molecules, fluorochromes, fluorophores, fluorescent quenching agents, colored molecules, radioactive isotopes, cintillants, avidin, streptavidin, protein A, protein G, antibodies or fragments thereof, polyhistidine, Ni^{2+}, Flag tags, myc tags, heavy metals, enzymes, alkaline phosphatase, peroxidase, luciferase, electron donors/acceptors, acridinium esters, and colorimetric substrates.

A detectable label may emit a signal spontaneously, such as when the detectable label is a radioactive isotope. In other cases the detectable label emits a signal as a result of being stimulated by an external field.

Exemplary radioactive isotopes may be γ-emitting, Auger-emitting, β-emitting, an alpha-emitting or positron-emitting radioactive isotope. Exemplary radioactive isotopes include ^{3}H, ^{11}C, ^{13}C, ^{15}N, ^{18}F, ^{19}F, ^{55}Co, ^{57}Co, ^{60}Co, ^{61}Cu, ^{62}Cu, ^{64}Cu, ^{67}Cu, ^{68}Ga, ^{72}As, ^{75}Br, ^{86}Y, ^{89}Zr, ^{90}Sr, ^{94m}Tc, ^{99m}Tc, ^{115}In, $^{123}1$, $^{124}1$, ^{125}I, $^{131}1$, ^{211}At, ^{212}Bi, ^{213}Bi, ^{223}Ra, ^{226}Ra, ^{225}Ac and ^{227}Ac.

Exemplary metal atoms are metals with an atomic number greater than 20, such as calcium atoms, scandium atoms, titanium atoms, vanadium atoms, chromium atoms, manganese atoms, iron atoms, cobalt atoms, nickel atoms, copper atoms, zinc atoms, gallium atoms, germanium atoms, arsenic atoms, selenium atoms, bromine atoms, krypton atoms, rubidium atoms, strontium atoms, yttrium atoms, zirconium atoms, niobium atoms, molybdenum atoms, technetium atoms, ruthenium atoms, rhodium atoms, palladium atoms, silver atoms, cadmium atoms, indium atoms, tin atoms, antimony atoms, tellurium atoms, iodine atoms, xenon atoms, cesium atoms, barium atoms, lanthanum atoms, hafnium atoms, tantalum atoms, tungsten atoms, rhenium atoms, osmium atoms, iridium atoms, platinum atoms, gold atoms, mercury atoms, thallium atoms, lead atoms, bismuth atoms, francium atoms, radium atoms, actinium atoms, cerium atoms, praseodymium atoms, neodymium atoms, promethium atoms, samarium atoms, europium atoms, gadolinium atoms, terbium atoms, dysprosium atoms, holmium atoms, erbium atoms,

thulium atoms, ytterbium atoms, lutetium atoms, thorium atoms, protactinium atoms, uranium atoms, neptunium atoms, plutonium atoms, americium atoms, curium atoms, berkelium atoms, californium atoms, einsteinium atoms, fermium atoms, mendelevium atoms, nobelium atoms, or lawrencium atoms.

In some embodiments, the metal atoms may be alkaline earth metals with an atomic number greater than twenty.

In some embodiments, the metal atoms may be lanthanides.

In some embodiments, the metal atoms may be actinides.

In some embodiments, the metal atoms may be transition metals.

In some embodiments, the metal atoms may be poor metals.

In some embodiments, the metal atoms may be gold atoms, bismuth atoms, tantalum atoms, and gadolinium atoms.

In some embodiments, the metal atoms may be metals with an atomic number of 53 (i.e. iodine) to 83 (i.e. bismuth).

In some embodiments, the metal atoms may be atoms suitable for magnetic resonance imaging.

The metal atoms may be metal ions in the form of +1 , +2, or +3 oxidation states, such as Ba^{2+}, Bi^{3+}, Cs^{+}, Ca^{2+}, Cr^{2+}, Cr^{3+}, Cr^{6+}, Co^{2+}, Co^{3+}, Cu^{+}, Cu^{2+}, Cu^{3+}, Ga^{3+}, Gd^{3+}, Au^{+}, Au^{3+}, Fe^{2+}, Fe^{3+}, F^{3+}, Pb^{2+}, Mn^{2+}, Mn^{3+}, Mn^{4+}, Mn^{7+}, Hg^{2+}, Ni^{2+}, Ni^{3+}, Ag^{+}, Sr^{2+}, Sn^{2+}, Sn^{4+}, and Zn^{2+}. The metal atoms may comprise a metal oxide, such as iron oxide, manganese oxide, or gadolinium oxide.

Suitable dyes include any commercially available dyes such as, for example, 5(6)-carboxyfluorescein, IRDye 680RD maleimide or IRDye 800CW, ruthenium polypyridyl dyes, and the like.

Suitable fluorophores are fluorescein isothiocyante (FITC), fluorescein thiosemicarbazide, rhodamine, Texas Red, CyDyes (e.g., Cy3, Cy5, Cy5.5), Alexa Fluors (e.g., Alexa488, Alexa555, Alexa594; Alexa647), near infrared (NIR) (700-900 nm) fluorescent dyes, and carbocyanine and aminostyryl dyes.

The FN3 domains that specifically bind PD-L1 conjugated to a detectable label may be used as an imaging agent to evaluate tumor distribution, diagnosis for the presence of tumor cells and/or, recurrence of tumor.

In some embodiments, the FN3 domains that specifically bind PD-L1 of the invention are conjugated to a cytotoxic agent.

In some embodiments, the cytotoxic agent is a chemotherapeutic agent, a drug, a growth inhibitory agent, a toxin (e.g., an enzymatically active toxin of bacterial, fungal,

plant, or animal origin, or fragments thereof), or a radioactive isotope (i.e., a radioconjugate).

The FN3 domains that specifically bind PD-L1 conjugated to a cytotoxic agent of the invention may be used in the targeted delivery of the cytotoxic agent to PD-L1 expressing tumor cell, and intracellular accumulation therein, wherein systemic administration of these unconjugated cytotoxic agents may result in unacceptable levels of toxicity to normal cells.

In some embodiments, the cytotoxic agent is daunomycin, doxorubicin, methotrexate, vindesine, bacterial toxins such as diphtheria toxin, ricin, geldanamycin, maytansinoids or calicheamicin. The cytotoxic agent may elict their cytotoxic and cytostatic effects by mechanisms including tubulin binding, DNA binding, or topoisomerase inhibition.

In some embodiments, the cytotoxic agent is an enzymatically active toxins such as diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, *Aleurites fordii* proteins, dianthin proteins, *Phytolaca americana* proteins (PAPI, PAPII, and PAP-S), *momordica charantia* inhibitor, curcin, crotin, *sapaonaria officinalis* inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes.

In some embodiments, the cytotoxic agent is a radionuclide, such as ^{212}Bi, ^{131}I, ^{131}In, ^{90}Y, and ^{186}Re.

In some embodiments, the cytotoxic agent is dolastatins or dolostatin peptidic analogs and derivatives, auristatin or monomethyl auristatin phenylalanine. Exemplary molecules are disclosed in U.S. Pat No. 5,635,483 and 5,780,588. Dolastatins and auristatins have been shown to interfere with microtubule dynamics, GTP hydrolysis, and nuclear and cellular division (Woyke et al (2001) Antimicrob Agents and Chemother. 45(12):3580-3584) and have anticancerand antifungal activity. The dolastatin or auristatin drug moiety may be attached to the FN3 domain of the invention through the N (amino) terminus or the C (carboxyl) terminus of the peptidic drug moiety (WO 02/088172), or via any cysteine engineered into the FN3 domain.

The FN3 domains that specifically bind PD-L1 of the invention may be conjugated to a detectable label using known methods.

In some embodiments, the detectable label is complexed with a chelating agent.

In some embodiments, the detectable label is conjugated to the FN3 domain that specifically binds PD-L1 of the invention via a linker.

The detectable label or the cytotoxic moiety may be linked directly, or indirectly, to the FN3 domain that specifically binds PD-L1 of the invention using known methods. Suitable linkers are known in the art and include, for example, prosthetic groups, non-phenolic linkers (derivatives of N-succimidyl-benzoates; dodecaborate), chelating moieties of both macrocyclics and acyclic chelators, such as derivatives of 1,4,7,10-tetraazacyclododecane-1,4,7,10,tetraacetic acid (DOTA), derivatives of diethylenetriaminepentaacetic avid (DTPA), derivatives of S-2-(4-Isothiocyanatobenzyl)-1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA) and derivatives of 1,4,8,11-tetraazacyclodocedan-1,4,8,11-tetraacetic acid (TETA), N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis(p-azidobenzoyl)hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene) and other chelating moieties. Suitable peptide linkers are well known.

In some embodiment, the FN3 domain that specifically binds PD-L1 is removed from the blood via renal clearance.

Isolation of PD-L1 binding FN3 domains from a library based on Tencon sequence

Tencon (SEQ ID NO: 1) is a non-naturally occurring fibronectin type III (FN3) domain designed from a consensus sequence of fifteen FN3 domains from human tenascin-C (Jacobs *et al.,* Protein Engineering, Design, and Selection, 25:107-117, 2012; U.S. Pat. Publ. No. 2010/0216708). The crystal structure of Tencon shows six surface-exposed loops that connect seven beta-strands as is characteristic to the FN3 domains, the beta-strands referred to as A, B, C, D, E, F, and G, and the loops referred to as AB, BC, CD, DE, EF, and FG loops (Bork and Doolittle, Proc Natl Acad Sci USA 89:8990-8992, 1992; U.S. Pat. No. 6,673,901). These loops, or selected residues within each loop, may be randomized in order to construct libraries of fibronectin type III (FN3) domains that may be used to select novel molecules that bind Pd-L1. **Table 1** shows positions and sequences of each loop and beta-strand in Tencon (**SEQ ID NO: 1**).

Library designed based on Tencon sequence may thus have randomized FG loop, or randomized BC and FG loops, such as libraries TCL1 or TCL2 as described below. The Tencon BC loop is 7 amino acids long, thus 1, 2, 3, 4, 5, 6 or 7 amino acids may be randomized in the library diversified at the BC loop and designed based on Tencon

sequence. The Tencon FG loop is 7 amino acids long, thus 1, 2, 3, 4, 5, 6 or 7 amino acids may be randomized in the library diversified at the FG loop and designed based on Tencon sequence. Further diversity at loops in the Tencon libraries may be achieved by insertion and/or deletions of residues at loops. For example, the FG and/or BC loops may be extended by 1-22 amino acids, or decreased by 1-3 amino acids. The FG loop in Tencon is 7 amino acids long, whereas the corresponding loop in antibody heavy chains ranges from 4-28 residues. To provide maximum diversity, the FG loop may be diversified in sequence as well as in length to correspond to the antibody CDR3 length range of 4-28 residues. For example, the FG loop can further be diversified in length by extending the loop by additional 1, 2, 3, 4 or 5 amino acids.

Library designed based on Tencon sequence may also have randomized alternative surfaces that form on a side of the FN3 domain and comprise two or more beta strands, and at least one loop. One such alternative surface is formed by amino acids in the C and the F beta-strands and the CD and the FG loops (a C-CD-F-FG surface). A library design based on Tencon alternative C-CD-F-FG surface is described in U.S. Pat. Publ. No. US2013/0226834. Library designed based on Tencon sequence also includes libraries designed based on Tencon variants, such as Tencon variants having substitutions at residues positions 11, 14, 17, 37, 46, 73, or 86 (residue numbering corresponding to **SEQ ID NO: 1**), and which variants display improve thermal stability. Exemplary Tencon variants are described in US Pat. Publ. No. 2011/0274623, and include Tencon27 (**SEQ ID NO: 4**) having substitutions E11R, L17A, N46V and E86I when compared to Tencon of SEQ ID NO: 1.

Table 1.

FN3 domain	Tencon (SEQ ID NO: 1)
A strand	1-12
AB loop	13-16
B strand	17-21
BC loop	22-28
C strand	29-37
CD loop	38-43
D strand	44-50

DE loop	51-54
E strand	55-59
EF loop	60-64
F strand	65-74
FG loop	75-81
G strand	82-89

Tencon and other FN3 sequence based libraries may be randomized at chosen residue positions using a random or defined set of amino acids. For example, variants in the library having random substitutions may be generated using NNK codons, which encode all 20 naturally occurring amino acids. In other diversification schemes, DVK codons may be used to encode amino acids Ala, Trp, Tyr, Lys, Thr, Asn, Lys, Ser, Arg, Asp, Glu, Gly, and Cys. Alternatively, NNS codons may be used to give rise to all 20 amino acid residues and simultaneously reducing the frequency of stop codons. Libraries of FN3 domains with biased amino acid distribution at positions to be diversified may be synthesized for example using Slonomics® technology (http:_//www_sloning_com). This technology uses a library of pre-made double stranded triplets that act as universal building blocks sufficient for thousands of gene synthesis processes. The triplet library represents all possible sequence combinations necessary to build any desired DNA molecule. The codon designations are according to the well-known IUB code.

The FN3 domains that specifically bind PD-L1 of the invention may be isolated by producing the FN3 library such as the Tencon library using *cis* display to ligate DNA fragments encoding the scaffold proteins to a DNA fragment encoding RepA to generate a pool of protein-DNA complexes formed after *in vitro* translation wherein each protein is stably associated with the DNA that encodes it (U.S. Pat. No. 7,842,476; Odegrip *et al.,* Proc Natl Acad Sci U S A 101, 2806-2810, 2004), and assaying the library for specific binding to PSMA by any method known in the art and described in the Example. Exemplary well known methods which can be used are ELISA, sandwich immunoassays, and competitive and non-competitive assays (see, e.g., Ausubel *et al.,* eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York). The identified FN3 domains that specifically bind PD-L1 are further characterized for their binding to PD-L1, modulation of PD-L1 activity, internalization, stability, and other desired characteristics.

The FN3 domains that specifically bind PD-L1 of the invention may be generated using any FN3 domain as a template to generate a library and screening the library for molecules specifically binding PD-L1 using methods provided within. Exemplar FN3 domains that may be used are the 3rd FN3 domain of tenascin C (TN3) (SEQ ID NO: **125**), Fibcon (SEQ ID NO: **126**), and the 10th FN3 domain of fibronectin (FN10) (SEQ ID NO: **127**). Standard cloning and expression techniques are used to clone the libraries into a vector or synthesize double stranded cDNA cassettes of the library, to express, or to translate the libraries *in vitro*. For example ribosome display (Hanes and Pluckthun, Proc Natl Acad Sci USA, 94, 4937-4942, 1997), mRNA display (Roberts and Szostak, Proc Natl Acad Sci USA, 94, 12297-12302, 1997), or other cell-free systems (U.S. Pat. No. 5,643,768) can be used. The libraries of the FN3 domain variants may be expressed as fusion proteins displayed on the surface for example of any suitable bacteriophage. Methods for displaying fusion polypeptides on the surface of a bacteriophage are well known (U.S. Pat. Publ. No. 2011/0118144; Int. Pat. Publ. No. WO2009/085462; U.S. Pat. No. 6,969,108; U.S. Pat. No. 6,172,197; U.S. Pat. No. 5,223,409; U.S. Pat. No. 6,582,915; U.S. Pat. No. 6,472,147).

In some embodiments, the FN3 domain that specifically binds PD-L1 is based on Tencon sequence of SEQ ID NO: 1 or Tencon27 sequence of SEQ ID NO: 4, the SEQ ID NO: 1 or the SEQ ID NO: 4, optionally having substitutions at residues positions 11, 14, 17, 37, 46, 73, and/or 86.

The FN3 domains that specifically bind PD-L1 of the invention may be modified to improve their properties such as improve thermal stability and reversibility of thermal folding and unfolding. Several methods have been applied to increase the apparent thermal stability of proteins and enzymes, including rational design based on comparison to highly similar thermostable sequences, design of stabilizing disulfide bridges, mutations to increase alpha-helix propensity, engineering of salt bridges, alteration of the surface charge of the protein, directed evolution, and composition of consensus sequences (Lehmann and Wyss, Curr Opin Biotechnol, 12, 371-375, 2001). High thermal stability may increase the yield of the expressed protein, improve solubility or activity, decrease immunogenicity, and minimize the need of a cold chain in manufacturing. Residues that may be substituted to improve thermal stability of Tencon (**SEQ ID NO: 1**) are residue positions 11, 14, 17, 37, 46, 73, or 86, and are described in US Pat. Publ. No. 2011/0274623. Substitutions corresponding to these residues may be incorporated to the FN3 domain containing molecules of the invention.

Measurement of protein stability and protein lability can be viewed as the same or different aspects of protein integrity. Proteins are sensitive or "labile" to denaturation

caused by heat, by ultraviolet or ionizing radiation, changes in the ambient osmolarity and pH if in liquid solution, mechanical shear force imposed by small pore-size filtration, ultraviolet radiation, ionizing radiation, such as by gamma irradiation, chemical or heat dehydration, or any other action or force that may cause protein structure disruption. The stability of the molecule can be determined using standard methods. For example, the stability of a molecule can be determined by measuring the thermal melting ("T_m") temperature, the temperature in ° Celsius (°C) at which half of the molecules become unfolded, using standard methods. Typically, the higher the T_m, the more stable the molecule. In addition to heat, the chemical environment also changes the ability of the protein to maintain a particular three dimensional structure.

In one embodiment, the FN3 domain that specifically binds PD-L1 of the invention may exhibit increased stability by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% or more compared to the same domain prior to engineering measured by the increase in the T_m.

Chemical denaturation can likewise be measured by a variety of methods. Chemical denaturants include guanidinium hydrochloride, guanidinium thiocyanate, urea, acetone, organic solvents (DMF, benzene, acetonitrile), salts (ammonium sulfate, lithium bromide, lithium chloride, sodium bromide, calcium chloride, sodium chloride); reducing agents (e.g. dithiothreitol, beta-mercaptoethanol, dinitrothiobenzene, and hydrides, such as sodium borohydride), non-ionic and ionic detergents, acids (e.g. hydrochloric acid (HCl), acetic acid (CH_3COOH), halogenated acetic acids), hydrophobic molecules (e.g. phosopholipids), and targeted denaturants. Quantitation of the extent of denaturation can rely on loss of a functional property, such as ability to bind a target molecule, or by physiochemical properties, such as tendency to aggregation, exposure of formerly solvent inaccessible residues, or disruption or formation of disulfide bonds.

The FN3 domain that specifically binds PD-L1 of the invention may be generated as monomers, dimers, or multimers, for example, as a means to increase the valency and thus the avidity of target molecule binding, or to generate bi- or multispecific scaffolds simultaneously binding two or more different target molecules. The dimers and multimers may be generated by linking monospecific, bi- or multispecific protein scaffolds, for example, by the inclusion of an amino acid linker, for example a linker containing poly-glycine, glycine and serine, or alanine and proline. Exemplary linker include $(GS)_2$, (SEQ ID NO: **128**), $(GGGS)_2$ (SEQ ID NO: **129**), $(GGGGS)_5$ (SEQ ID NO: **130**), $(AP)_2$ (SEQ ID NO: **131**), $(AP)_5$ (SEQ ID NO: **132**), $(AP)_{10}$ (SEQ ID NO: **133**), $(AP)_{20}$ (SEQ ID NO: **134**) and $A(EAAAK)_5AAA$ (SEQ ID NO: **135**). The dimers and multimers may be linked

to each other in a N-to C-direction. The use of naturally occurring as well as artificial peptide linkers to connect polypeptides into novel linked fusion polypeptides is well known in the literature (Hallewell *et al., J Biol Chem* 264, 5260-5268, 1989; Alfthan *et al., Protein Eng.* 8, 725-731, 1995; Robinson & Sauer, *Biochemistry* 35, 109-116, 1996; U.S. Pat. No. 5,856,456).

Half-life extending moieties

The FN3 domains that specifically bind PD-L1 of the invention may incorporate other subunits for example via covalent interaction. In one aspect of the invention, the FN3 domains that specifically bind PD-L1 of the invention further comprise a half-life extending moiety. Exemplary half-life extending moieties are albumin, albumin variants, albumin-binding proteins and/or domains, transferrin and fragments and analogues thereof, and Fc regions. An exemplary albumin variant is shown in SEQ ID NO: **136**. Amino acid sequences of the human Fc regions are well known, and include IgG1, IgG2, IgG3, IgG4, IgM, IgA and IgE Fc regions.

All or a portion of an antibody constant region may be attached to the FN3 domain that specifically binds PD-L1 of the invention to impart antibody-like properties, especially those properties associated with the Fc region, such as Fc effector functions such as C1q binding, complement dependent cytotoxicity (CDC), Fc receptor binding, antibody-dependent cell-mediated cytotoxicity (ADCC), phagocytosis, down regulation of cell surface receptors (e.g., B cell receptor; BCR), and may be further modified by modifying residues in the Fc responsible for these activities (for review; see Strohl, *Curr Opin Biotechnol.* 20, 685-691, 2009).

Additional moieties may be incorporated into the FN3 domains that specifically bind PD-L1 of the invention such as polyethylene glycol (PEG) molecules, such as PEG5000 or PEG20,000, fatty acids and fatty acid esters of different chain lengths, for example laurate, myristate, stearate, arachidate, behenate, oleate, arachidonate, octanedioic acid, tetradecanedioic acid, octadecanedioic acid, docosanedioic acid, and the like, polylysine, octane, carbohydrates (dextran, cellulose, oligo- or polysaccharides) for desired properties. These moieties may be direct fusions with the protein scaffold coding sequences and may be generated by standard cloning and expression techniques. Alternatively, well known chemical coupling methods may be used to attach the moieties to recombinantly produced molecules of the invention.

A pegyl moiety may for example be added to the FN3 domain that specifically binds PD-L1 of the invention by incorporating a cysteine residue to the C-terminus of the

molecule, or engineering cysteines into residue positions that face away from the PD-L1 binding face of the molecule, and attaching a pegyl group to the cysteine using well known methods.

FN3 domains that specifically bind PD-L1 of the invention incorporating additional moieties may be compared for functionality by several well-known assays. For example, altered properties due to incorporation of Fc domains and/or Fc domain variants may be assayed in Fc receptor binding assays using soluble forms of the receptors, such as the FcγRI, FcγRII, FcγRIII or FcRn receptors, or using well known cell-based assays measuring for example ADCC or CDC, or evaluating pharmacokinetic properties of the molecules of the invention in *in vivo* models.

Polynucleotides, vectors, host cells

The invention also provides nucleic acids encoding the FN3 domains specifically binding PD-L1 of the invention as isolated polynucleotides or as portions of expression vectors or as portions of linear DNA sequences, including linear DNA sequences used for *in vitro* transcription/translation, vectors compatible with prokaryotic, eukaryotic or filamentous phage expression, secretion and/or display of the compositions or directed mutagens thereof. Certain exemplary polynucleotides are disclosed herein, however, other polynucleotides which, given the degeneracy of the genetic code or codon preferences in a given expression system, encode the FN3 domains of the invention are also within the scope of the invention.

The invention also provides an isolated polynucleotide encoding the FN3 domain specifically binding PD-L1 comprising the amino acid sequence of SEQ ID NOs: 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123 or 124.

The polynucleotides of the invention may be produced by chemical synthesis such as solid phase polynucleotide synthesis on an automated polynucleotide synthesizer and assembled into complete single or double stranded molecules. Alternatively, the polynucleotides of the invention may be produced by other techniques such as PCR followed by routine cloning. Techniques for producing or obtaining polynucleotides of a given known sequence are well known in the art.

The polynucleotides of the invention may comprise at least one non-coding sequence, such as a promoter or enhancer sequence, intron, polyadenylation signal, a *cis* sequence facilitating RepA binding, and the like. The polynucleotide sequences may also comprise additional sequences encoding additional amino acids that encode for example a marker or a tag sequence such as a histidine tag or an HA tag to facilitate purification or detection of the protein, a signal sequence, a fusion protein partner such as RepA, Fc or bacteriophage coat protein such as pIX or pIII.

The invention also provides a vector comprising at least one polynucleotide of the invention. Such vectors may be plasmid vectors, viral vectors, vectors for baculovirus expression, transposon based vectors or any other vector suitable for introduction of the polynucleotides of the invention into a given organism or genetic background by any means. Such vectors may be expression vectors comprising nucleic acid sequence elements that can control, regulate, cause or permit expression of a polypeptide encoded by such a vector. Such elements may comprise transcriptional enhancer binding sites, RNA polymerase initiation sites, ribosome binding sites, and other sites that facilitate the expression of encoded polypeptides in a given expression system. Such expression systems may be cell-based, or cell-free systems well known in the art.

The invention also provides a host cell comprising the vector of the invention. The FN3 domain that specifically bind PD-L1 of the invention may be optionally produced by a cell line, a mixed cell line, an immortalized cell or clonal population of immortalized cells, as well known in the art. See, e.g., Ausubel, *et al.,* ed., Current Protocols in Molecular Biology, John Wiley & Sons, Inc., NY, NY (1987-2001); Sambrook, *et al.,* Molecular Cloning: A Laboratory Manual, 2nd Edition, Cold Spring Harbor, NY (1989); Harlow and Lane, Antibodies, a Laboratory Manual, Cold Spring Harbor, NY (1989); Colligan, *et al.,* eds., Current Protocols in Immunology, John Wiley & Sons, Inc., NY (1994-2001); Colligan *et al.,* Current Protocols in Protein Science, John Wiley & Sons, NY, NY, (1997-2001).

The host cell chosen for expression may be of mammalian origin or may be selected from COS-1, COS-7, HEK293, BHK21, CHO, BSC-1, He G2, SP2/0, HeLa, myeloma, lymphoma, yeast, insect or plant cells, or any derivative, immortalized or transformed cell thereof. Alternatively, the host cell may be selected from a species or organism incapable of glycosylating polypeptides, e.g. a prokaryotic cell or organism, such as BL21, BL21(DE3), BL21-GOLD(DE3), XL1-Blue, JM109, HMS174, HMS174(DE3), and any of the natural or engineered *E. coli spp, Klebsiella spp.,* or *Pseudomonas spp* strains.

The invention also provides a method of producing the isolated FN3 domain that specifically binds PD-L1 of the invention, comprising culturing the isolated host cell of the invention under conditions such that the isolated FN3 domain that specifically binds PD-L1 is expressed, and purifying the FN3 domain.

The FN3 domains that specifically bind PD-L1 may be purified from recombinant cell cultures by well-known methods, for example by protein A purification, ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography, or high performance liquid chromatography (HPLC).

Anti-idiotypic antibodies

The present invention also provides an anti-idiotypic antibody binding to the FN3 domain of the invention.

The invention also provides an anti-idiotypic antibody that specifically binds the FN3 domain comprising any one of SEQ ID NOs: 34-124.

Kits

The invention also provides a kit comprising the FN3 domain that specifically binds PD-L1 of the invention.

The kit may be used for therapeutic uses and as a diagnostic kit.

In some embodiments, the kit comprises the FN3 domain that specifically binds PD-L1 of the invention and reagents for detecting the FN3 domain. The kit can include one or more other elements including: instructions for use; other reagents, e.g., a label, an agent useful for chelating, or otherwise coupling, a radioprotective composition; devices or other materials for preparing the FN3 domain that specifically binds PD-L1 of the invention for administration for imaging, diagnostic or therapeutic purpose; pharmaceutically acceptable carriers; and devices or other materials for administration to a subject.

In some embodiments, the kit comprises the FN3 domain that specifically binds PD-L1 comprising any one of SEQ ID NOs: 34-124.

Uses of PD-L1 binding FN3 domains of the invention

The FN3 domains that specifically bind PD-L1 of the invention may be used to diagnose, monitor, modulate, treat, alleviate, help prevent the incidence of, or reduce the

symptoms of human disease or specific pathologies in cells, tissues, organs, fluid, or, generally, a host. The FN3 domains that specifically bind PD-L1 of the invention may also be used in imaging PD-L1 positive tumor tissue in a subject. The methods of the invention may be used with an animal patient belonging to any classification. Examples of such animals include mammals such as humans, rodents, dogs, cats and farm animals.

The invention provides a method of diagnosing a subject having, or who is likely to develop cancer of a tissue based on the expression of PD-L1 by cells of the cancer tissue, methods of predicting success of immunotherapy, methods of prognosis, and methods of treatment.

The invention also provides a method of detecting PD-L1-expressing cancer cells in a tumor tissue, comprising

obtaining a sample of the tumor tissue from a subject;

detecting whether PD-L1 is expressed in the tumor tissue by contacting toe sample of the tumor tissues with the FN3 domain that specifically binds PD-L1 comprising the sequence of any one of SEQ ID NOs: 34-124 and detecting the binding between PD-L1 and the FN3 domain.

The tissue can be tissue of any organ or anatomical system, for example lung, epithelial, connective, vascular, muscle, neural, skeletal, lymphatic, prostate, cervical, breast, spleen, gastric, intestinal, oral, esophageal, uterine, ovarian, renal or testicular tissue.

PD-L1 expression may be evaluated using known methods such as immunohistochemistry or ELISA.

The invention also provides a method of isolating PD-L1 expressing cells, comprising

obtaining a sample from a subject;

contacting the sample with the FN3 domain that specifically binds PD-L1 comprising the sequence of any one of SEQ ID NOs: 34-124, and

isolating the cells bound to the FN3 domains.

The invention also provides a method of detecting PD-L1-expressing cancer cells in a tumor tissue, comprising

conjugating the FN3 domain that specifically binds PD-L1 comprising the sequence of any one of SEQ ID NOs: 34-124 to a detectable label to form a conjugate;

administering the conjugate to a subject; and

visualizing the PD-L1 expressing cancer cells to which the conjugate is bound.

The invention also provides a method of treating a subject having cancer, comprising administering to the subject a FN3 domain that specifically binds PD-L1 of the invention.

In some embodiments, the subject has a solid tumor.

In some embodiments, the subject has a hematological malignancy.

In some embodiments, the solid tumor is a melanoma.

In some embodiments, the solid tumor is a lung cancer.

In some embodiments, the solid tumor is a non-small cell lung cancer (NSCLC).

In some embodiments, the solid tumor is a squamous non-small cell lung cancer (NSCLC).

In some embodiments, the solid tumor is a non-squamous NSCLC.

In some embodiments, the solid tumor is a lung adenocarcinoma.

In some embodiments, the solid tumor is a renal cell carcinoma (RCC).

In some embodiments, the solid tumor is a mesothelioma.

In some embodiments, the solid tumor is a nasopharyngeal carcinoma (NPC).

In some embodiments, the solid tumor is a colorectal cancer.

In some embodiments, the solid tumor is a prostate cancer.

In some embodiments, the solid tumor is castration-resistant prostate cancer.

In some embodiments, the solid tumor is a stomach cancer.

In some embodiments, the solid tumor is an ovarian cancer.

In some embodiments, the solid tumor is a gastric cancer.

In some embodiments, the solid tumor is a liver cancer.

In some embodiments, the solid tumor is pancreatic cancer.

In some embodiments, the solid tumor is a thyroid cancer.

In some embodiments, the solid tumor is a squamous cell carcinoma of the head and neck.

In some embodiments, the solid tumor is a carcinomas of the esophagus or gastrointestinal tract.

In some embodiments, the solid tumor is a breast cancer.

In some embodiments, the solid tumor is a fallopian tube cancer.

In some embodiments, the solid tumor is a brain cancer.

In some embodiments, the solid tumor is an urethral cancer.

In some embodiments, the solid tumor is a genitourinary cancer.

In some embodiments, the solid tumor is an endometriosis.

In some embodiments, the solid tumor is a cervical cancer.

In some embodiments, the solid tumor is a metastatic lesion of the cancer.

In some embodiments, the hematological malignancy is a lymphoma, a myeloma or a leukemia.

In some embodiments, the hematological malignancy is a B cell lymphoma.

In some embodiments, the hematological malignancy is Burkitt's lymphoma.

In some embodiments, the hematological malignancy is Hodgkin's lymphoma.

In some embodiments, the hematological malignancy is a non-Hodgkin's lymphoma.

In some embodiments, the hematological malignancy is a myelodysplastic syndrome.

In some embodiments, the hematological malignancy is an acute myeloid leukemia (AML).

In some embodiments, the hematological malignancy is a chronic myeloid leukemia (CML).

In some embodiments, the hematological malignancy is a chronic myelomoncytic leukemia (CMML).

In some embodiments, the hematological malignancy is a multiple myeloma (MM).

In some embodiments, the hematological malignancy is a plasmacytoma.
In some embodiments, the cancer is kidney cancer.

"Treat" or "treatment" refers to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) an undesired physiological change or disorder, such as the development or spread of cancer. For purposes of this invention, beneficial or desired clinical results include, but are not limited to, alleviation of symptoms, diminishment of extent of disease, stabilized (i.e., not worsening) state of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. "Treatment" can also mean prolonging survival as compared to expected survival if not receiving treatment. Those in need of treatment include those already with the condition or disorder as well as those prone to have the condition or disorder or those in which the condition or disorder is to be prevented.

A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve a desired therapeutic result. A therapeutically effective amount of the FN3 domains that specifically bind PD-L1 of the invention may vary according to factors such as the disease state, age, sex, and weight of the individual.

Exemplary indicators of an effective FN3 domain that specifically binds PD-L1 is improved well-being of the patient, decrease or shrinkage of the size of a tumor, arrested or slowed growth of a tumor, and/or absence of metastasis of cancer cells to other locations in the body.

Administration/ Pharmaceutical Compositions

The invention provides for pharmaceutical compositions of the FN3 domains that specifically bind PD-L1, optionally conjugated to a detectable label or a cytotoxic drug of the invention and a pharmaceutically acceptable carrier. For therapeutic use, the FN3 domains that specifically bind PD-L1of the invention may be prepared as pharmaceutical compositions containing an effective amount of the domain or molecule as an active ingredient in a pharmaceutically acceptable carrier. "Carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the active compound is administered. Such vehicles can be liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. For example, 0.4% saline and 0.3% glycine can be used. These solutions are sterile and generally free of particulate matter. They may be sterilized by conventional, well-known sterilization techniques (*e.g.*, filtration). The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, stabilizing, thickening, lubricating and coloring agents, etc. The concentration of the molecules of the invention in such pharmaceutical formulation can vary widely, *i.e.*, from less than about 0.5%, usually at least about 1% to as much as 15 or 20% by weight and will be selected primarily based on required dose, fluid volumes, viscosities, etc., according to the particular mode of administration selected. Suitable vehicles and formulations, inclusive of other human proteins, e.g., human serum albumin, are described, for example, in e.g. Remington: The Science and Practice of Pharmacy, 21st Edition, Troy, D.B. ed., Lipincott Williams and Wilkins, Philadelphia, PA 2006, Part 5, Pharmaceutical Manufacturing pp 691-1092, See especially pp. 958-989.

The mode of administration for therapeutic use of the FN3 domains of the invention may be any suitable route that delivers the agent to the host, such as parenteral administration, e.g., intradermal, intramuscular, intraperitoneal, intravenous or subcutaneous, pulmonary; transmucosal (oral, intranasal, intravaginal, rectal), using a formulation in a tablet, capsule, solution, powder, gel, particle; and contained in a syringe, an implanted device, osmotic pump, cartridge, micropump; or other means appreciated by the skilled artisan, as well known in the art. Site specific administration may be achieved

by for example intrarticular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavitary, intracelial, intracerebellar, intracerebroventricular, intracolic, intracervical, intragastric, intrahepatic, intracardial, intraosteal, intrapelvic, intrapericardiac, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intrauterine, intravascular, intravesical, intralesional, vaginal, rectal, buccal, sublingual, intranasal, or transdermal delivery.

Pharmaceutical compositions can be supplied as a kit comprising a container that comprises the pharmaceutical composition as described herein. A pharmaceutical composition can be provided, for example, in the form of an injectable solution for single or multiple doses, or as a sterile powder that will be reconstituted before injection. Alternatively, such a kit can include a dry-powder disperser, liquid aerosol generator, or nebulizer for administration of a pharmaceutical composition. Such a kit can further comprise written information on indications and usage of the pharmaceutical composition.

While having described the invention in general terms, the embodiments of the invention will be further disclosed in the following examples that should not be construed as limiting the scope of the claims.

EXAMPLE 1. Construction of Tencon libraries with randomized loops

Tencon (SEQ ID NO: 1) is an immunoglobulin-like scaffold, fibronectin type III (FN3) domain, designed from a consensus sequence of fifteen FN3 domains from human tenascin-C (Jacobs *et al.,* Protein Engineering, Design, and Selection, 25:107-117, 2012; U.S. Pat. No. 8,278,419). The crystal structure of Tencon shows six surface-exposed loops that connect seven beta-strands. These loops, or selected residues within each loop, can be randomized in order to construct libraries of fibronectin type III (FN3) domains that can be used to select novel molecules that bind to specific targets.

Tencon:

LPAPKNLVVSEVTEDSLRLSWTAPDAAFDSFLIQYQESEKVGEAINLTVPGSERSY DLTGLKPGTEYTVSIYGVKGGHRSNPLSAEFTT (SEQ ID NO 1):

Various libraries were generated using the tencon scaffold and various design strategies. In general, libraries TCL1 and TCL2 produced good binders. Generation of TCL1 and TCL2 libraries are described in detail in Int. Pat. Publ. No. WO/2014081944A2.

Construction of TCL1 library

A library designed to randomize only the FG loop of Tencon (**SEQ ID NO: 1**), TCL1, was constructed for use with the *cis*-display system (Jacobs *et al.,* Protein Engineering, Design, and Selection, 25:107-117, 2012). In this system, a single-strand DNA incorporating sequences for a Tac promoter, Tencon library coding sequence, RepA coding sequence, cis-element, and ori element is produced. Upon expression in an *in vitro* transcription/translation system, a complex is produced of the Tencon-RepA fusion protein bound in *cis* to the DNA from which it is encoded. Complexes that bind to a target molecule are then isolated and amplified by polymerase chain reaction (PCR), as described below.

Construction of the TCL1 library for use with *cis*-display was achieved by successive rounds of PCR to produce the final linear, double-stranded DNA molecules in two halves; the 5' fragment contains the promoter and Tencon sequences, while the 3' fragment contains the *repA* gene and the cis- and ori elements. These two halves are combined by restriction digest in order to produce the entire construct. The TCL1 library was designed to incorporate random amino acids only in the FG loop of Tencon, KGGHRSN (SEQ ID NO: 55). NNS codons were used in the construction of this library, resulting in the possible incorporation of all 20 amino acids and one stop codon into the FG loop. The TCL1 library contains six separate sub-libraries, each having a different randomized FG loop length, from 7 to 12 residues, in order to further increase diversity.

TCL1 library (**SEQ ID NO: 2**)

LPAPKNLVVSEVTEDSLRLSWTAPDAAFDSFLIQYQESEKVGEAINLTVPGSERSY
DLTGLKPGTEYTVSIYGV$X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}X_{12}$ PLSAEFTT; wherein
X_1, X_2, X_3, X_4, X_5, X_6, X_7 is any amino acid; and
X_8, X_9, X_{10}, X_{11} and X_{12} are any amino acid or deleted

Construction of TCL2 Library

TCL2 library was constructed in which both the BC and the FG loops of Tencon were randomized and the distribution of amino acids at each position was strictly controlled. Table 3 shows the amino acid distribution at desired loop positions in the TCL2 library. The designed amino acid distribution had two aims. First, the library was biased toward residues that were predicted to be structurally important for Tencon folding and stability based on analysis of the Tencon crystal structure and/or from homology

modeling. For example, position 29 was fixed to be only a subset of hydrophobic amino acids, as this residue was buried in the hydrophobic core of the Tencon fold. A second layer of design included biasing the amino acid distribution toward that of residues preferentially found in the heavy chain HCDR3 of antibodies, to efficiently produce high-affinity binders (Birtalan *et al.,* J Mol Biol 377:1518-28, 2008; Olson *et al.,* Protein Sci 16:476-84, 2007). Towards this goal, the "designed distribution" in Table 2 refers to the distribution as follows: 6% alanine, 6% arginine, 3.9% asparagine, 7.5% aspartic acid, 2.5% glutamic acid, 1.5% glutamine, 15% glycine, 2.3% histidine, 2.5% isoleucine, 5% leucine, 1.5% lysine, 2.5% phenylalanine, 4% proline, 10% serine, 4.5% threonine, 4% tryptophan, 17.3% tyrosine, and 4% valine. This distribution is devoid of methionine, cysteine, and STOP codons.

TCL2 library (**SEQ ID NO: 3**)

LPAPKNLVVSEVTEDSLRLSW$X_1X_2X_3X_4X_5X_6X_7X_8$SFLIQYQESEKVGEAINLTVPGS ERSYDLTGLKPGTEYTVSIYGV$X_9X_{10}X_{11}X_{12}X_{13}SX_{14}X_{15}$LSAEFTT; wherein

X_1 is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val;

X_2 is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val;

X_3 Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val;

X_4 is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val;

X_5 is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val;

X_6 is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val;

X_7 is Phe, Ile, Leu, Val or Tyr;

X_8 is Asp, Glu or Thr;

X_9 is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val;

X_{10} is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val;

X_{11} is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val;

X_{12} is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val;

X_{13} is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val;

X_{14} is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val; and

X_{15} is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val.

Table 2.

Residue Position*	WT residues	Distribution in the TCL2 library
22	T	designed distribution
23	A	designed distribution
24	P	50% P + designed distribution
25	D	designed distribution
26	A	20% A + 20% G + designed distribution
27	A	designed distribution
28	F	20% F, 20% I, 20% L, 20% V, 20% Y
29	D	33% D, 33% E, 33% T
75	K	designed distribution
76	G	designed distribution
77	G	designed distribution
78	H	designed distribution
79	R	designed distribution
80	S	100% S

81	N	designed distribution
82	P	50% P + designed distribution

*residue numbering is based on Tencon sequence of SEQ ID NO: 1

Subsequently, these libraries were improved by various ways, including building of the libraries on a stabilized Tencon framework (U.S. Pat. No. 8,569,227) that incorporates substitutions E11R/L17A/N46V/E86I (Tencon27; SEQ ID NO: 4) when compared to the wild type tencon as well as altering of the positions randomized in the BC and FG loops. Tencon27 is described in Int. Pat. Appl. No. WO2013049275. From this, new libraries designed to randomize only the FG loop of Tencon (library TCL9), or a combination of the BC and FG loops (library TCL7) were generated. These libraries were constructed for use with the cis-display system (Odegrip et al., Proc Natl Acad Sci U S A 101: 2806-2810, 2004). The details of this design are shown below:

Stabilized Tencon (Tencon27) (SEQ ID NO: 4)

LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFLIQYQESEKVGEAIVLTVPGSERSY
DLTGLKPGTEYTVSIYGVKGGHRSNPLSAIFTT

TCL7 (randomized FG and BC loops) (SEQ ID NO: 5)

LPAPKNLVVSRVTEDSARLSW$X_1X_2X_3X_4X_5X_6X_7X_8X_9$FDSFLIQYQESEKVGEAIVLT
VPGSERSYDLTGLKPGTEYTVSIYGV$X_{10}X_{11}X_{12}X_{13}X_{14}X_{15}X_{16}X_{17}X_{18}X_{19}$SNPLSAIFTT;

wherein

X_1, X_2, X_3, X_4, X_5, X_6, X_{10}, X_{11}, X_{12}, X_{13}, X_{14}, X_{15} and X_{16} is A, D, E, F, G, H, I, K, L, N, P, Q, R, S, T, V, W or Y; and

X_7, X_8, X_9, X_{17}, X_{18} and X_{19}, is A, D, E, F, G, H, I, K, L, N, P, Q, R, S, T, V, W, Y or deleted.

TCL9 (randomized FG loop) (SEQ ID NO: 6)

LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFLIQYQESEKVGEAIVLTVPGSERSY DLTGLKPGTEYTVSIYGV $X_1X_2X_3X_4X_5X_6X_7X_8X_9$ $X_{10}X_{11}X_{12}$SNPLSAIFTT;

X_1, X_2, X_3, X_4, X_5, X_6 and X_7, is A, D, E, F, G, H, I, K, L, N, P, Q, R, S, T, V, W or Y; and

X_8, X_9, X_{10}, X_{11} and X_{12} is A, D, E, F, G, H, I, K, L, N, P, Q, R, S, T, V, W, Y or deleted.

For library construction, DNA fragments encoding randomized BC loops (lengths 6-9 positions) or FG loops (lengths 7-12 positions) were synthesized using Slonomics technology (Sloning Biotechnology GmbH) so as to control the amino acid distribution of the library and to eliminate stop codons. Two different sets of DNA molecules randomizing either the BC loop or the FG loops were synthesized independently and later combined using PCR to produce the full library product.

Construction of FG loop libraries (TCL9)

A set of synthetic DNA molecules consisting of a 5' Tac promoter followed by the complete gene sequence of Tencon with the exception of randomized codons in the FG loop was produced (**SEQ ID NOs: 26-31**). For FG loop randomization, all amino acids except cysteine and methionine were encoded at equal percentages. The lengths of the diversified portion are such that they encode for 7, 8, 9, 10, 11, or 12 amino acids in the FG loop. Sub-libraries of each length variation were synthesized individually at a scale of 2ug and then amplified by PCR using oligos Sloning-FOR (**SEQ ID NO: 9**) and Sloning-Rev (**SEQ ID NO: 10**).

The 3' fragment of the library is a constant DNA sequence containing elements for display, including a PspOMI restriction site, the coding region of the repA gene, and the cis- and ori elements. PCR reactions were performed to amplify this fragment using a plasmid (pCR4Blunt) (Invitrogen) as a template with M13 Forward and M13 Reverse primers. The resulting PCR products were digested by PspOMI overnight and gel-purified. To ligate the 5' portion of library DNA to the 3' DNA containing repA gene, 2 pmol (~540ng to 560ng) of 5' DNA was ligated to an equal molar (~1.25 µg) of 3' repA DNA in the presence of NotI and PspOMI enzyme and T4 ligase at 37°C overnight. The ligated library product was amplified by using 12 cycles of PCR with oligos POP2250 (**SEQ ID NO: 11**) and DigLigRev (**SEQ ID NO: 12**). For each sub-library, the resulting DNA from 12 PCR reactions were combined and purified by Qiagen spin column. The yield for each sub-library of TCL9 ranged from 32-34 µg.

Construction of FG/BC Loop libraries (TCL7)

The TCL7 library provides for a library with randomized Tencon BC and FG loops. In this library, BC loops of lengths 6-9 amino acids were mixed combinatorially with randomized FG loops of 7-12 amino acids in length. Synthetic Tencon fragments BC6, BC7, BC8, and BC9 (**SEQ ID NOs: 13-16,** respectively) were produced to include the Tencon gene encoding for the N-terminal portion of the protein up to and including residue VX such that the BC loop is replaced with either 6, 7, 8, or 9 randomized amino acids. These fragments were synthesized prior to the discovery of L17A, N46V and E83I mutations (CEN5243) but these mutations were introduced in the molecular biology steps described below. In order to combine this fragment with fragments encoding for randomized FG loops, the following steps were taken.

First, a DNA fragment encoding the Tac promoter and the 5' sequence of Tencon up to the nucleotide encoding for amino acid A17 (130mer-L17A, **SEQ ID NO: 17**) was produced by PCR using oligos POP2222ext (**SEQ ID NO: 18**) and LS1114 (**SEQ ID NO: 19**). This was done to include the L17A mutation in the library (CEN5243). Next, DNA fragments encoding for Tencon residues R18-V75 including randomized BC loops were amplified by PCR using BC6, BC7, BC8, or BC9 as a templates and oligos LS1115 (**SEQ ID NO: 20**) and LS1117 (**SEQ ID NO: 21**). This PCR step introduced a BsaI site at the 3' end. These DNA fragments were subsequently joined by overlapping PCR using oligos POP2222ext and LS1117 as primers. The resulting PCR product of 240bp was pooled and purified by Qiagen PCR purification kit. The purified DNA was digested with BsaI-HF and gel purified.

Fragments encoding the FG loop were amplified by PCR using FG7, FG8, FG9, FG10, FG11, and FG12 as templates with oligonucleotides SDG10 (**SEQ ID NO: 22**) and SDG24 (**SEQ ID NO: 23**) to incorporate a BsaI restriction site and N46V and E86I variations (CEN5243).

The digested BC fragments and FG fragments were ligated together in a single step using a 3-way ligation. Four ligation reactions in the 16 possible combinations were set up, with each ligation reaction combining two BC loop lengths with 2 FG loop lengths. Each ligation contained ~300 ng of total BC fragment and 300 ng of the FG fragment. These 4 ligation pools were then amplified by PCR using oligos POP2222 (**SEQ ID NO: 24**) and SDG28 **SEQ ID N: 25**). 7.5 µg of each reaction product were then digested with Not1 and cleaned up with a Qiagen PCR purification column. 5.2 µg of this DNA, was

ligated to an equal molar amount of RepA DNA fragment (~14 μg) digested with PspOMI and the product amplified by PCR using oligos POP2222.

EXAMPLE 2: Generation of Tencon libraries having alternative binding surfaces

The choice of residues to be randomized in a particular library design governs the overall shape of the interaction surface created. X-ray crystallographic analysis of an FN3 domain containing scaffold protein selected to bind maltose binding protein (MBP) from a library in which the BC, DE, and FG loops were randomized was shown to have a largely curved interface that fits into the active site of MBP (Koide et al., Proc Natl Acad Sci U S A 104: 6632-6637, 2007). In contrast, an ankyrin repeat scaffold protein that was selected to bind to MBP was found to have a much more planar interaction surface and to bind to the outer surface of MBP distant from the active (Binz et al., Nat Biotechnol 22: 575-582, 2004). These results suggest that the shape of the binding surface of a scaffold molecule (curved vs. flat) may dictate what target proteins or specific epitopes on those target proteins are able to be bound effectively by the scaffold. Published efforts around engineering protein scaffolds containing FN3 domains for protein binding has relied on engineering adjacent loops for target binding, thus producing curved binding surfaces. This approach may limit the number of targets and epitopes accessible by such scaffolds.

Tencon and other FN3 domains contain two sets of CDR-like loops lying on the opposite faces of the molecule, the first set formed by the BC, DE, and FG loops, and the second set formed by the AB, CD, and EF loops. The two sets of loops are separated by the beta-strands that form the center of the FN3 structure. If the image of the Tencon is rotated by 90 degrees, an alternative surface can be visualized. This slightly concave surface is formed by the CD and FG loops and two antiparallel beta- strands, the C and the F beta-strands, and is herein called the C-CD-F-FG surface. The C-CD-F-FG surface can be used as a template to design libraries of protein scaffold interaction surfaces by randomizing a subset of residues that form the surface. Beta-strands have a repeating structure with the side chain of every other residue exposed to the surface of the protein. Thus, a library can be made by randomizing some or all surface exposed residues in the beta strands. By choosing the appropriate residues in the beta-strands, the inherent stability of the Tencon scaffold should be minimally compromised while providing a unique scaffold surface for interaction with other proteins.

Library TCL14 (**SEQ ID NO: 7**), was designed into Tencon27 scaffold (**SEQ ID NO: 4**).

A full description of the methods used to construct this library is described in US. Pat. Publ. No. US2013/0226834.

TCL14 library (SEQ ID NO: 7):

LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFX$_1$IX$_2$YX$_3$EX$_4$X$_5$X$_6$X$_7$GEAIVLTVPGS ERSYDLTGLKPGTEYX$_8$VX$_9$IX$_{10}$GVKGGX$_{11}$X$_{12}$SX$_{13}$PLSAIFTT;

wherein

X$_1$, X$_2$, X$_3$, X$_4$, X$_5$, X$_6$, X$_7$, X$_8$, X$_9$, X$_{10}$, X$_{11}$, X$_{12}$ and X$_{13}$ are A, D, E, F, G, H, I, K, L, N, P, Q, R, S, T, V, W, Y, C or M.

The two beta strands forming the C-CD-F-FG surface in Tencon27 have a total of 9 surface exposed residues that could be randomized; C-strand: S30, L32, Q34, Q36; F-strand: E66, T68, S70, Y72, and V74, while the CD loop has 6 potential residues: S38, E39, K40, V41, G42, and E43 and the FG loop has 7 potential residues: K75, G76, G77, H78, R79, S80, and N81. Select residues were chosen for inclusion in the TCL14 design due to the larger theoretical size of the library if all 22 residues were randomized.

Thirteen positions in Tencon were chosen for randomizing: L32, Q34 and Q36 in C-strand, S38, E39, K40 and V41 in CD-loop, T68, S70 and Y72 in F-strand, H78, R79, and N81 in FG-loop. In the C and F strands S30 and E66 were not randomized as they lie just beyond the CD and FG loops and do not appear to be as apparently a part of the C-CD-F-FG surface. For the CD loop, G42 and E43 were not randomized as glycine, providing flexibility, can be valuable in loop regions, and E43 lies at the junction of the surface. The FG loop had K75, G76, G77, and S80 excluded. The glycines were excluded for the reasons above while careful inspection of the crystal structures revealed S80 making key contacts with the core to help form the stable FG loop. K75 faces away from the surface of the C-CD-F-FG surface and was a less appealing candidate for randomization. Although the above mentioned residues were not randomized in the original TCL14 design, they could be included in subsequent library designs to provide additional diversity for de novo selection or for example for an affinity maturation library on a select TCL14 target specific hit.

Subsequent to the production of TCL14, 3 additional Tencon libraries of similar design were produced. These two libraries, TCL19, TCL21 and TCL23, are randomized

at the same positions as TCL14 (see above) however the distribution of amino acids occurring at these positions is altered (**Table 3**). TCL19 and TCL21 were designed to include an equal distribution of 18 natural amino acids at every position (5.55% of each), excluding only cysteine and methionine. TCL23 was designed such that each randomized position approximates the amino acid distribution found in the HCDR3 loops of functional antibodies (Birtalan et al., J Mol Biol 377: 1518-1528, 2008) as described in **Table 3**. As with the TCL21 library, cysteine and methionine were excluded.

A third additional library was built to expand potential target binding surface of the other libraries library. In this library, TCL24, 4 additional Tencon positions were randomized as compared to libraries TCL14, TCL19, TCL21, and TCL23. These positions include N46 and T48 from the D strand and S84 and I86 from the G strand. Positions 46, 48, 84, and 86 were chosen in particular as the side chains of these residues are surface exposed from beta-strands D and G and lie structurally adjacent to the randomized portions of the C and F strand, thus increasing the surface area accessible for binding to target proteins. The amino acid distribution used at each position for TCL24 is identical to that described for TCL19 and TCL21 in **Table 3**.

TCL24 Library (**SEQ ID NO: 8**)

LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFX_1IX_2YX_3E$X_4X_5X_6X_7$GEAIX_8LX_9VPG SERSYDLTGLKPGTEYX_{10}VX_{11}IX_{12}GVKGG$X_{13}X_{14}$SX_{15}PLX_{16}AX_{17}FTT;

wherein

X_1, X_2, X_3, X_4, X_5, X_6, X_{10}, X_{11}, X_{12}, X_{13}, X_{14}, X_{15}, X_{16} and X_{17} are A, D, E, F, G, H, I, K, L, N, P, Q, R, S, T, V, Y or W.

Table 3. Amino acid frequency (%) at each randomized position for TCL21, TCL23, and TCL24.

Amino Acid	TCL19	TCL21	TCL23	TCL24
Ala	5.6	5.6	6.0	5.6
Arg	5.6	5.6	6.0	5.6

Asn	5.6	5.6	3.9	5.6
Asp	5.6	5.6	7.5	5.6
Cys	0.0	0.0	0.0	0.0
Gln	5.6	5.6	1.5	5.6
Glu	5.6	5.6	2.5	5.6
Gly	5.6	5.6	15.0	5.6
His	5.6	5.6	2.3	5.6
Ile	5.6	5.6	2.5	5.6
Leu	5.6	5.6	5.0	5.6
Lys	5.6	5.6	1.5	5.6
Met	0.0	0.0	0.0	0.0
Phe	5.6	5.6	2.5	5.6
Pro	5.6	5.6	4.0	5.6
Ser	5.6	5.6	10.0	5.6
Thr	5.6	5.6	4.5	5.6
Trp	5.6	5.6	4.0	5.6
Tyr	5.6	5.6	17.3	5.6
Val	5.6	5.6	4.0	5.6

Generation of TCL21, TCL23, and TCL24 libraries

The TCL21 library was generated using Colibra library technology (Isogenica) in order to control amino acid distributions. TCL19, TCL23, and TCL24 gene fragments were generated using Slonomics technology (Morphosys) to control amino acid distributions. PCR was used to amplify each library following initial synthesis followed by ligation to the gene for RepA in order to be used in selections using the CIS-display system (Odegrip et al., Proc Natl Acad Sci U S A 101: 2806-2810, 2004) as described above for the loop libraries.

EXAMPLE 3: Selection of fibronectin type III (FN3) domains that bind PD-L1

Panning

FN3 domains specific for human PD-L1 were selected via CIS-Display (Odegrip *et al* 2004) using recombinant biotinylated PD-L1 (rhPD-L1/Fc Chimera, R&D Systems 156-B7). For *in vitro* transcription and translation (ITT), 3 μg of DNA from libraries TCL18, TCL19, TCL21, TCL23, and TCL24 were incubated at 30°C with 0.1 mM complete amino acids, 1X S30 premix components, and 15 μL of S30 extract (Isogenica) in a total volume of 50 μL. After 1 hour, 375 μL of blocking solution (2% BSA in PBS, Invitrogen) was added and reactions were incubated on a cold block for 15 minutes. Unbound library members were removed by successive washes with TBST and TBS. After washing, DNA was eluted from the target protein by heating to 75°C for 10 minutes and amplified by PCR using KOD polymerase for further rounds of panning. High affinity binders were isolated by successively lowering the concentration of target PD-L1 during each round from 400 nM to 100 nM and increasing the washing stringency.

Outputs from the fifth round panning were subjected to four additional rounds of off-rate selection. Library transcription and translation was performed as described above after which the ITT reactions were incubated with biotinylated recombinant PD-L1 proteins and captured on neutravidin or streptavidin coated magnetic beads, before being washed in TBST extensively then subsequently washed in 5 μM cold recombinant PD-L1 protein for 1 hour. The biotinylated target antigen concentration was reduced from 25 nM in rounds 6 and 7 to 2.5 nM in rounds 8 and 9.

Following panning, genes encoding the selected FN3 domains were amplified by PCR, subcloned into a pET vector modified to include a ligase independent cloning site, and transformed into BL21 (DE3) (Stratagene) cells for soluble expression in *E. coli* using standard molecular biology techniques. A gene sequence encoding a C-terminal poly-histidine tag was added to each FN3 domain to enable purification and detection. Cultures were grown to an optical density of 0.6-0.8 in TB medium supplemented with 100 μg/mL carbenicillin in 1 mL 96-well blocks at 37°C before the addition of IPTG to 1 mM, at which point the temperature was reduced to 30°C. Cells were harvested approximately 16 hours later by centrifugation and frozen at -20°C. Cell lysis was achieved by incubating each pellet in 0.6 mL of BugBuster® HT lysis buffer (Novagen EMD Biosciences) supplemented with 0.2 mg/mL lysozyme with shaking at room temperature for 30 minutes.

Biochemical Screening for FN3 domains that bind Recombinant PD-L1

Streptavidin-coated Maxisorp plates (Nunc catalog 436110) were blocked for 1h in Starting Block T20 (Pierce) and then coated with biotinylated PD-L1 (using same antigen as in panning) or negative controls (an unrelated Fc-fused recombinant protein and human serum albumin) for 1h. Plates were rinsed with TBST and diluted lysate was applied to plates for 1h. Following additional rinses, wells were treated with HRP-conjugated anti-FN3 domain antibody (PAB25) for 1h and then assayed with POD (Roche catalog 11582950001). The DNA from FN3 domain lysates with ELISA binding signals to PD-L1 at least 5-fold above both Fc and HSA controls were sequenced resulting in 57 (**Table 4**) and 37 (**Table 5**) unique, readable FN3 domain sequences isolated from Round 5 and Round 9 screening respectively.

High-throughput Expression of anti-PD-L1 FN3 domains

40 isolated clones from unique hits identified by biochemical binding ELISA from Round 9 were combined for growth into 96-well block plate; clones grew in 1 mL cultures (LB media supplemented with kanamycin for selection) at 37°C overnight with shaking. For protein expression in 96-block plates, 1 mL TB media supplemented with kanamycin was inoculated with 50 µL of the overnight culture and grown at 37°C with continual shaking at 300rpm until OD_{600} = 0.6-1. Once the target OD was reached, protein expression was induced with addition of IPTG to 1 mM; plates were transferred to 30°C (300 rpm) for overnight growth. Overnight cultures were centrifuged to harvest the cells; bacterial pellets were stored at -80°C until ready for use. Pellets were lysed with BugBuster® HT lysis buffer (Novagen EMD Biosciences) and His-tagged Centyrins purified from the clarified lysates with His MultiTrap™ HP plates (GE Healthcare) and eluted in buffer containing 20 mM sodium phosphate, 500 mM sodium chloride, and 250 mM imidazole at pH 7.4. Purified samples were exchanged into PBS pH 7.4 for analysis using PD MultiTrap™ G-25 plates (GE Healthcare).

Size Exclusion Chromatography Analysis

Size exclusion chromatography was used to determine the aggregation state of anti-PD-L1 FN3 domains. Aliquots (10 µL) of each purified FN3 domain were injected onto a Superdex 75 5/150 column (GE Healthcare) at a flow rate of 0.3 mL/min in a

mobile phase of PBS pH 7.4. Elution from the column was monitored by absorbance at 280 nm. Tencon protein was included in each run as a control. Agilent ChemStation software was used to analyse the elution profiles. 20 anti-PD-L1 FN3 domains demonstrated a retention time between 5.2 and 6.4 minutes and only a single SEC peak indicative of monomeric protein (**Table 6**).

Table 4.

Clone	ELISA PD-L1 Fc (RSU)	ELISA Fc Control (RSU)	ELISA HSA (RSU)	SEQ ID NO:
ISOP121HR5P1G9	17760	880	1760	34
ISOP121BR5P1F7	12880	720	880	35
ISOP121BR5P1A6	10960	720	720	36
ISOP121BR5P1C5	11680	400	720	37
ISOP121BR5P1D7	12800	800	720	38
ISOP121BR5P1C6	13360	720	720	39
ISOP121AR5P1G6	16960	1200	880	40
ISOP121BR5P1B7	11360	640	480	41
ISOP121FR5P1G1	10000	640	400	42
ISOP121GR5P1B4	16160	800	560	43
ISOP121BR5P1G2	16720	800	560	44
ISOP121HR5P1H2	20960	720	560	45
ISOP121FR5P1G11	18560	880	480	46
ISOP121AR5P1E7	327200	4240	6560	47
ISOP121GR5P1F6	32080	640	640	48
ISOP121BR5P1E9	42000	960	800	49
ISOP121AR5P1F2	51040	880	960	50
ISOP121AR5P1F7	64000	720	1040	51
ISOP121BR5P1H6	74640	1440	1040	52
ISOP121GR5P1A2	61680	720	720	53
ISOP121BR5P1D3	75760	800	800	54
ISOP121AR5P1F9	136080	1120	1040	55
ISOP121AR5P1H5	170800	960	1120	56
ISOP121AR5P1G10	231920	1360	1280	57
ISOP121AR5P1F3	180160	800	960	58
ISOP121BR5P1E2	137280	800	720	59

ISOP121BR5P1D1	186240	1040	960	60
ISOP121BR5P1C9	226400	1120	1040	61
ISOP121GR5P1G11	239600	960	1040	62
ISOP121BR5P1A7	388640	800	1120	63
ISOP121BR5P1C3	177040	640	480	64
ISOP121AR5P1D11	392800	640	1040	65
ISOP121ER5P1E7	251120	480	560	66
ISOP121GR5P1G7	367760	800	800	67
ISOP121AR5P1A8	515920	560	1040	68
ISOP121BR5P1E7	411760	800	640	69
ISOP121FR5P1H8	430640	560	640	70
ISOP121GR5P1D2	513280	720	640	71
ISOP121AR5P1H2	926720	880	1120	72
ISOP121GR5P1F10	577120	640	640	73
ISOP121BR5P1A2	742800	720	800	74
ISOP121GR5P1F7	697200	640	720	75
ISOP121AR5P1B8	591600	640	560	76
ISOP121GR5P1D7	791920	720	720	77
ISOP121BR5P1G3	770800	560	640	78
ISOP121AR5P1C5	732480	640	560	79
ISOP121FR5P1H9	1195520	720	880	80
ISOP121AR5P1A10	788560	1120	560	81
ISOP121HR5P1F2	906960	480	640	82
ISOP121AR5P1H1	1475280	880	880	83
ISOP121BR5P1D10	1538800	480	880	84
ISOP121BR5P1F10	1422880	560	720	85
ISOP121BR5P1D11	2442960	800	1120	86
ISOP121AR5P1E11	1842000	720	720	87
ISOP121BR5P1D6	2435760	560	880	88
ISOP121BR5P1B5	1483520	720	400	89

Table 5.

Clone	ELISA PD-L1 Fc	ELISA Fc Control (RSU)	ELISA HSA (RSU)	SEQ ID NO:

	(RSU)			
ISOP194ER9P1G3	4288320	560	720	90
ISOP194AR9P1F2	16271040	1920	7520	91
ISOP194AR9P1H10	5212800	4400	2400	92
ISOP194BR9P1H4	4064960	3040	3840	93
ISOP194AR9P1D8	923200	12000	6560	94
ISOP194BR9P1D1	2152080	1360	1280	95
ISOP194AR9P1E8	3404480	6960	67680	96
ISOP194AR9P1E9	19719920	5520	1600	97
ISOP194AR9P1H9	2592720	21280	11360	98
ISOP194BR9P1A9	19046640	2320	3200	99
ISOP194BR9P1A5	3182000	800	1280	100
ISOP194BR9P1F7	15151120	1920	1760	101
ISOP194AR9P1G7	15914000	1280	560	102
ISOP194AR9P1E3	4566880	1120	800	103
ISOP194AR9P1C5	4371120	3440	1040	104
ISOP194AR9P1H3	17746800	9200	4880	105
ISOP194GR9P1E9	2821920	720	1200	106
ISOP194HR9P1B10	385360	560	1840	107
ISOP194ER9P1A11	4352240	800	880	108
ISOP194ER9P1A3	2360160	560	800	109
ISOP194ER9P1H9	3042800	720	880	110
ISOP194HR9P1B2	5656400	400	1840	111
ISOP194HR9P1D11	6620160	480	1680	112
ISOP194GR9P1F6	319200	400	1200	113
ISOP194GR9P1F9	105280	320	800	114
ISOP194GR9P1C11	164320	1040	1440	115
ISOP194ER9P1E6	8982160	240	720	116
ISOP194BR9P1G9	14376560	640	960	117
ISOP194BR9P1E4	9791680	640	1440	118
ISOP194AR9P1H1	21445040	15680	6800	119
ISOP194BR9P1D10	1666880	720	1120	120
ISOP194BR9P1C8	6110640	640	1280	121
ISOP194AR9P1C10	13863040	38240	14960	122
ISOP194AR9P1D11	1043280	28160	12720	123
ISOP194AR9P1C3	3548240	56400	5920	124

Table 6.

Clone	SEC Retention Time (min)	SEC Peak Height (mAU)	Monomeric?	SEQ ID NO:
ISOP194ER9P1G3	5.951	167.47	FALSE	90
ISOP194AR9P1F2	5.901	552.30	TRUE	91
ISOP194AR9P1H10	5.976	12.80	FALSE	92
ISOP194BR9P1H4	5.688	394.40	TRUE	93
ISOP194AR9P1D8	5.711	162.07	FALSE	94
ISOP194BR9P1D1	6.696	88.56	TRUE	95
ISOP194AR9P1E8	5.549	570.07	TRUE	96
ISOP194AR9P1E9	5.79	493.72	TRUE	97
ISOP194AR9P1H9	5.694	511.99	TRUE	98
ISOP194BR9P1A9	5.662	225.76	FALSE	99
ISOP194BR9P1A5	7.82	15.28	FALSE	100
ISOP194BR9P1F7	5.982	94.57	TRUE	101
ISOP194AR9P1G7	5.845	50.19	TRUE	102
ISOP194AR9P1E3	6.939	15.65	FALSE	103
ISOP194AR9P1C5	No peak		FALSE	104
ISOP194AR9P1H3	6.238	155.66	TRUE	105
ISOP194GR9P1E9	6.343	20.59	TRUE	106
ISOP194HR9P1B10	5.911	398.72	TRUE	107
ISOP194ER9P1A11	5.957	154.65	TRUE	108
ISOP194ER9P1A3	5.976	341.20	TRUE	109
ISOP194ER9P1H9	No peak		FALSE	110

ISOP194HR9P1B2	6.274	2.33	FALSE	111
ISOP194HR9P1D11	6.002	433.98	FALSE	112
ISOP194GR9P1F6	6.12	29.42	TRUE	113
ISOP194GR9P1F9	No peak		FALSE	114
ISOP194GR9P1C11	12.458	2.90	FALSE	115
ISOP194ER9P1E6	6.125	149.28	TRUE	116
ISOP194BR9P1G9	6.622	84.28	FALSE	117
ISOP194BR9P1E4	5.714	456.33	TRUE	118
ISOP194AR9P1H1	6.247	12.76	FALSE	119
ISOP194BR9P1D10	6.059	10.60	FALSE	120
ISOP194BR9P1C8	No peak		FALSE	121
ISOP194AR9P1C10	5.715	98.64	TRUE	122
ISOP194AR9P1D11	No peak		FALSE	123
ISOP194AR9P1C3	5.588	700.26	TRUE	124

Sequences

SEQ ID No. 1= Original Tencon Sequence
LPAPKNLVVSEVTEDSLRLSWTAPDAAFDSFLIQYQESEKVGEAINLTVPGSERSY
DLTGLKPGTEYTVSIYGVKGGHRSNPLSAEFTT

SEQ ID No. 2= TCL1 library
LPAPKNLVVSEVTEDSLRLSWTAPDAAFDSFLIQYQESEKVGEAINLTVPGSERSY
DLTGLKPGTEYTVSIYGV$X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}X_{12}$ PLSAEFTT;
wherein
X_1, X_2, X_3, X_4, X_5, X_6, X_7 is any amino acid; and
X_8, X_9, X_{10}, X_{11} and X_{12} are any amino acid or deleted

SEQ ID No. 3=TCL2 library
LPAPKNLVVSEVTEDSLRLSW$X_1X_2X_3X_4X_5X_6X_7X_8$SFLIQYQESEKVGEAINLTVPGS
ERSYDLTGLKPGTEYTVSIYGV$X_9X_{10}X_{11}X_{12}X_{13}SX_{14}X_{15}$LSAEFTT;
wherein
X_1 is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val;

X_2 is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val;
X_3 Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val;
X_4 is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val;
X_5 is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val;
X_6 is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val;
X_7 is Phe, Ile, Leu, Val or Tyr;
X_8 is Asp, Glu or Thr;
X_9 is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val;
X_{10} is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val;
X_{11} is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val;
X_{12} is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val;
X_{13} is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val;
X_{14} is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val; and
X_{15} is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val.

SEQ ID No. 4= Stabilized Tencon
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFLIQYQESEKVGEAIVLTVPGSERSY
DLTGLKPGTEYTVSIYGVKGGHRSNPLSAIFTT

SEQ ID No. 5= TCL7 (FG and BC loops)
LPAPKNLVVSRVTEDSARLSW$X_1X_2X_3X_4X_5X_6X_7X_8X_9$FDSFLIQYQESEKVGEAIVLT
VPGSERSYDLTGLKPGTEYTVSIYGV$X_{10}X_{11}X_{12}X_{13}X_{14}X_{15}X_{16}X_{17}X_{18}X_{19}$SNPLSAIFTT;
wherein
X_1, X_2, X_3, X_4, X_5, X_6, X_{10}, X_{11}, X_{12}, X_{13}, X_{14}, X_{15} and X_{16} are A, D, E, F, G, H, I, K, L, N, P, Q, R, S, T, V, W or Y; and
X_7, X_8, X_9, X_{17}, X_{18} and X_{19}, are A, D, E, F, G, H, I, K, L, N, P, Q, R, S, T, V, W, Y or deleted

SEQ ID No. 6= TCL9 (FG loop)
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFLIQYQESEKVGEAIVLTVPGSERSY
DLTGLKPGTEYTVSIYGV$X_1X_2X_3X_4X_5X_6X_7X_8X_9$ $X_{10}X_{11}X_{12}$SNPLSAIFTT;
wherein
X_1, X_2, X_3, X_4, X_5, X_6 and X_7, is A, D, E, F, G, H, I, K, L, N, P, Q, R, S, T, V, W or Y; and
X_8, X_9, X_{10}, X_{11} and X_{12} is A, D, E, F, G, H, I, K, L, N, P, Q, R, S, T, V, W, Y or deleted.

TCL14 library (SEQ ID NO: 7):
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFX_1IX_2YX_3E$X_4X_5X_6X_7$GEAIVLTVPGS
ERSYDLTGLKPGTEYX_8VX_9IX_{10}GVKGG$X_{11}X_{12}$SX_{13}PLSAIFTT;
wherein

X_1, X_2, X_3, X_4, X_5, X_6, X_7, X_8, X_9, X_{10}, X_{11}, X_{12} and X_{13} are A, D, E, F, G, H, I, K, L, N, P, Q, R, S, T, V, W, Y, C or M.

TCL24 Library (SEQ ID NO: 8)
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFX$_1$IX$_2$YX$_3$EX$_4$X$_5$X$_6$X$_7$GEAIX$_8$LX$_9$VPG SERSYDLTGLKPGTEYX$_{10}$VX$_{11}$IX$_{12}$GVKGGX$_{13}$X$_{14}$SX$_{15}$PLX$_{16}$AX$_{17}$FTT;
wherein
X_1, X_2, X_3, X_4, X_5, X_6, X_{10}, X_{11}, X_{12}, X_{13}, X_{14}, X_{15}, X_{16} and X_{17} are A, D, E, F, G, H, I, K, L, N, P, Q, R, S, T, V, Y or W.

SEQ ID No. 9 = Sloning-FOR
GTGACACGGCGGTTAGAAC

SEQ ID No. 10 = Sloning-REV
GCCTTTGGGAAGCTTCTAAG

SEQ ID No. 11 = POP2250
CGGCGGTTAGAACGCGGCTACAATTAATAC

SEQ ID No. 12 = DigLigRev
CATGATTACGCCAAGCTCAGAA

SEQ ID No. 13 = BC9
GTGACACGGCGGTTAGAACGCGGCTACAATTAATACATAACCCCATCCCCCTG
TTGACAATTAATCATCGGCTCGTATAATGTGTGGAATTGTGAGCGGATAACAA
TTTCACACAGGAAACAGGATCTACCATGCTGCCGGCGCCGAAAAACCTGGTTG
TTTCTGAAGTTACCGAAGACTCTCTGCGTCTGTCTTGGNNNNNNNNNNNNNNN
NNNNNNNNNNNNTTYGACTCTTTCCTGATCCAGTACCAGGAATCTGAAAAAGT
TGGTGAAGCGATCAACCTGACCGTTCCGGGTTCTGAACGTTCTTACGACCTGA
CCGGTCTGAAACCGGGTACCGAATACACCGTTTCTATCTACGGTGTTCTTAGA
AGCTTCCCAAAGGC

SEQ ID No. 14 = BC8
GTGACACGGCGGTTAGAACGCGGCTACAATTAATACATAACCCCATCCCCCTG
TTGACAATTAATCATCGGCTCGTATAATGTGTGGAATTGTGAGCGGATAACAA
TTTCACACAGGAAACAGGATCTACCATGCTGCCGGCGCCGAAAAACCTGGTTG
TTTCTGAAGTTACCGAAGACTCTCTGCGTCTGTCTTGGNNNNNNNNNNNNNNN
NNNNNNNNNTTYGACTCTTTCCTGATCCAGTACCAGGAATCTGAAAAAGTTGG
TGAAGCGATCAACCTGACCGTTCCGGGTTCTGAACGTTCTTACGACCTGACCG
GTCTGAAACCGGGTACCGAATACACCGTTTCTATCTACGGTGTTCTTAGAAGC
TTCCCAAAGGC

SEQ ID No. 15 = BC7
GTGACACGGCGGTTAGAACGCGGCTACAATTAATACATAACCCCATCCCCCTG
TTGACAATTAATCATCGGCTCGTATAATGTGTGGAATTGTGAGCGGATAACAA
TTTCACACAGGAAACAGGATCTACCATGCTGCCGGCGCCGAAAAACCTGGTTG
TTTCTGAAGTTACCGAAGACTCTCTGCGTCTGTCTTGGNNNNNNNNNNNNNNN
NNNNNNTTYGACTCTTTCCTGATCCAGTACCAGGAATCTGAAAAAGTTGGTGA
AGCGATCAACCTGACCGTTCCGGGTTCTGAACGTTCTTACGACCTGACCGGTC
TGAAACCGGGTACCGAATACACCGTTTCTATCTACGGTGTTCTTAGAAGCTTCC
CAAAGGC

SEQ ID No. 16 = BC6
GTGACACGGCGGTTAGAACGCGGCTACAATTAATACATAACCCCATCCCCCTG
TTGACAATTAATCATCGGCTCGTATAATGTGTGGAATTGTGAGCGGATAACAA
TTTCACACAGGAAACAGGATCTACCATGCTGCCGGCGCCGAAAAACCTGGTTG
TTTCTGAAGTTACCGAAGACTCTCTGCGTCTGTCTTGGNNNNNNNNNNNNNNN
NNNTTYGACTCTTTCCTGATCCAGTACCAGGAATCTGAAAAAGTTGGTGAAGC
GATCAACCTGACCGTTCCGGGTTCTGAACGTTCTTACGACCTGACCGGTCTGA
AACCGGGTACCGAATACACCGTTTCTATCTACGGTGTTCTTAGAAGCTTCCCA
AAGGC

SEQ ID No. 17 = 130mer-L17A
CGGCGGTTAGAACGCGGCTACAATTAATACATAACCCCATCCCCCTGTTGACA
ATTAATCATCGGCTCGTATAATGTGTGGAATTGTGAGCGGATAACAATTTCAC
ACAGGAAACAGGATCTACCATGCTG

SEQ ID No. 18 = POP222ext
CGG CGG TTA GAA CGC GGC TAC AAT TAA TAC

SEQ ID No. 19 = LS1114
CCA AGA CAG ACG GGC AGA GTC TTC GGT AAC GCG AGA AAC AAC CAG
GTT TTT CGG CGC CGG CAG CAT GGT AGA TCC TGT TTC

SEQ ID No. 20 = LS1115
CCG AAG ACT CTG CCC GTC TGT CTT GG

SEQ ID No. 21 = LS1117
CAG TGG TCT CAC GGA TTC CTG GTA CTG GAT CAG GAA AGA GTC GAA

SEQ ID No. 22 = SDG10
CATGCGGTCTCTTCCGAAAAAGTTGGTGAAGCGATCGTCCTGACCGTTCCGGG
T

SEQ ID No. 23 = SDG24
GGTGGTGAAGATCGCAGACAGCGGGTTAG

SEQ ID No. 24 = POP2222
CGGCGGTTAGAACGCGGCTAC

SEQ ID No. 25 = SDG28
AAGATCAGTTGCGGCCGCTAGACTAGAACCGCTGCCACCGCCGGTGGTGAAG
ATCGCAGAC

SEQ ID No. 26 = FG12
GTGACACGGCGGTTAGAACGCGGCTACAATTAATACATAACCCCATCCCCCTG
TTGACAATTAATCATCGGCTCGTATAATGTGTGGAATTGTGAGCGGATAACAA
TTTCACACAGGAAACAGGATCTACCATGCTGCCGGCGCCGAAAAACCTGGTTG
TTTCTCGCGTTACCGAAGACTCTGCGCGTCTGTCTTGGACCGCGCCGGACGCG
GCGTTCGACTCTTTCCTGATCCAGTACCAGGAATCTGAAAAAGTTGGTGAAGC
GATCGTGCTGACCGTTCCGGGTTCTGAACGTTCTTACGACCTGACCGGTCTGA
AACCGGGTACCGAATACACCGTTTCTATCTACGGTGTTNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNTCTAACCCGCTGTCTGCGATCTTCACCACC

GGCGGTCACCATCACCATCACCATGGCAGCGGTTCTAGTCTAGCGGCCGCAAC
TGATCTTGGC

SEQ ID No. 27 = FG11
GTGACACGGCGGTTAGAACGCGGCTACAATTAATACATAACCCCATCCCCCTG
TTGACAATTAATCATCGGCTCGTATAATGTGTGGAATTGTGAGCGGATAACAA
TTTCACACAGGAAACAGGATCTACCATGCTGCCGGCGCCGAAAAACCTGGTTG
TTTCTCGCGTTACCGAAGACTCTGCGCGTCTGTCTTGGACCGCGCCGGACGCG
GCGTTCGACTCTTTCCTGATCCAGTACCAGGAATCTGAAAAAGTTGGTGAAGC
GATCGTGCTGACCGTTCCGGGTTCTGAACGTTCTTACGACCTGACCGGTCTGA
AACCGGGTACCGAATACACCGTTTCTATCTACGGTGTTNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNTCTAACCCGCTGTCTGCGATCTTCACCACCGGC
GGTCACCATCACCATCACCATGGCAGCGGTTCTAGTCTAGCGGCCGCAACTGA
TCTTGGC

SEQ ID No. 28 = FG10
GTGACACGGCGGTTAGAACGCGGCTACAATTAATACATAACCCCATCCCCCTG
TTGACAATTAATCATCGGCTCGTATAATGTGTGGAATTGTGAGCGGATAACAA
TTTCACACAGGAAACAGGATCTACCATGCTGCCGGCGCCGAAAAACCTGGTTG
TTTCTCGCGTTACCGAAGACTCTGCGCGTCTGTCTTGGACCGCGCCGGACGCG
GCGTTCGACTCTTTCCTGATCCAGTACCAGGAATCTGAAAAAGTTGGTGAAGC
GATCGTGCTGACCGTTCCGGGTTCTGAACGTTCTTACGACCTGACCGGTCTGA
AACCGGGTACCGAATACACCGTTTCTATCTACGGTGTTNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNTCTAACCCGCTGTCTGCGATCTTCACCACCGGCGGTC
ACCATCACCATCACCATGGCAGCGGTTCTAGTCTAGCGGCCGCAACTGATCTT
GGC

SEQ ID No. 29 = FG9
GTGACACGGCGGTTAGAACGCGGCTACAATTAATACATAACCCCATCCCCCTG
TTGACAATTAATCATCGGCTCGTATAATGTGTGGAATTGTGAGCGGATAACAA
TTTCACACAGGAAACAGGATCTACCATGCTGCCGGCGCCGAAAAACCTGGTTG
TTTCTCGCGTTACCGAAGACTCTGCGCGTCTGTCTTGGACCGCGCCGGACGCG
GCGTTCGACTCTTTCCTGATCCAGTACCAGGAATCTGAAAAAGTTGGTGAAGC
GATCGTGCTGACCGTTCCGGGTTCTGAACGTTCTTACGACCTGACCGGTCTGA
AACCGGGTACCGAATACACCGTTTCTATCTACGGTGTTNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNTCTAACCCGCTGTCTGCGATCTTCACCACCGGCGGTCACC
ATCACCATCACCATGGCAGCGGTTCTAGTCTAGCGGCCGCAACTGATCTTGGC

SEQ ID No. 30 = FG8
GTGACACGGCGGTTAGAACGCGGCTACAATTAATACATAACCCCATCCCCCTG
TTGACAATTAATCATCGGCTCGTATAATGTGTGGAATTGTGAGCGGATAACAA
TTTCACACAGGAAACAGGATCTACCATGCTGCCGGCGCCGAAAAACCTGGTTG
TTTCTCGCGTTACCGAAGACTCTGCGCGTCTGTCTTGGACCGCGCCGGACGCG
GCGTTCGACTCTTTCCTGATCCAGTACCAGGAATCTGAAAAAGTTGGTGAAGC
GATCGTGCTGACCGTTCCGGGTTCTGAACGTTCTTACGACCTGACCGGTCTGA
AACCGGGTACCGAATACACCGTTTCTATCTACGGTGTTNNNNNNNNNNNNNN
NNNNNNNNNNTCTAACCCGCTGTCTGCGATCTTCACCACCGGCGGTCACCATC
ACCATCACCATGGCAGCGGTTCTAGTCTAGCGGCCGCAACTGATCTTGGC

SEQ ID No. 31 = FG7
GTGACACGGCGGTTAGAACGCGGCTACAATTAATACATAACCCCATCCCCCTG
TTGACAATTAATCATCGGCTCGTATAATGTGTGGAATTGTGAGCGGATAACAA

TTTCACACAGGAAACAGGATCTACCATGCTGCCGGCGCCGAAAAACCTGGTTG
TTTCTCGCGTTACCGAAGACTCTGCGCGTCTGTCTTGGACCGCGCCGGACGCG
GCGTTCGACTCTTTCCTGATCCAGTACCAGGAATCTGAAAAAGTTGGTGAAGC
GATCGTGCTGACCGTTCCGGGTTCTGAACGTTCTTACGACCTGACCGGTCTGA
AACCGGGTACCGAATACACCGTTTCTATCTACGGTGTTNNNNNNNNNNNNNN
NNNNNNNTCTAACCCGCTGTCTGCGATCTTCACCACCGGCGGTCACCATCACC
ATCACCATGGCAGCGGTTCTAGTCTAGCGGCCGCAACTGATCTTGGC

SEQ ID NO: 32 = human mature PD-L1

FTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYWEMEDKNIIQFVHGEED
LKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRIT
VKVNAPYNKINQRILVVDPVTSEHELTCQAEGYPKAEVIWTSSDHQVLSGKTTTT
NSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPPNER

SEQ ID NO: 33 = human mature PD-1

PGWFLDSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQT
DKLAAFPEDRSQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSGTYLCGAISLAPK
AQIKESLRAELRVTERRAEVPTAHPSPSPRPAGQFQTLVVGVVGGLLGSLVLLVW
VLAVICSRAARGTIGARRTGQPLKEDPSAVPVFSVDYGELDFQWREKTPEPPVPCV
PEQTEYATIVFPSGMGTSSPARRGSADGPRSAQPLRPE DGHCSWPL

Clone	SEQ ID NO:	AA Sequence
ISOP121HR5P1G9	34	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFPINYGERATKGE AINLYVPGSERSYDLTGLKPGTEYWVLIGGVKGGLKSSPLWAW FTT
ISOP121BR5P1F7	35	LPAPKNLVVSRVTEDSARLSWHDATWQYFDSFLIQYQESEKVG EAIVLTVPGSERSYDLTGLKPGTEYTVSIYGVFHRKHIDFVSNPLS AIFTT
ISOP121BR5P1A6	36	LPAPKNLVVSRVTEDSARLSWASWLVAFFDSFLIQYQESEKVG EAIVLTVPGSERSYDLTGLKPGTEYTVSIYGVYQRHASAFVSNPL SAIFTT
ISOP121BR5P1C5	37	LPAPKNLVVSRVTEDSARLSWFRLRIVQTFDSFLIQYQESEKVG EAIVLTVPGSERSYDLTGLKPGTEYTVSIYGVITVVELLQQSNPLS AIFTT
ISOP121BR5P1D7	38	LPAPKNLGCFSRYRRLSRLSWETPYPSLSNFDSFLIQYQESEKVG EAIVLTVPGSERSYDLTGLKPGTEYTVSIYGVLKLSAAWWPSNP LSAIFTT
ISOP121BR5P1C6	39	LPAPKNLVVSRVTEDSARLSWRKQEQYFDSFLIQYQESEKVGE AIVLTVPGSERSYDLTGLKPGTEYTVSIYGVYSRPKAEFTSNPLSA IFTT

ISOP121AR5P1G6	40	LPAPKNLVVSRVTEDSARLSWHATFGDPFDSFLIQYQESEKVG EAIVLTVPGSERSYDLTGLKPGTEYTVSIYGVGRHYTVYDSNPLS AIFTT
ISOP121BR5P1B7	41	LPAPKNLVVSRITEDSARLSWKWEEGFFDSFLIQYQESEKVGEA IVLTVPGSERSYDLTGLKPGTEYTVSIYGVYQRHASAFVSNPLSA IFTT
ISOP121FR5P1G1	42	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFWIHYTEAPVHGE AIVLTVPGSERSYDLTGLKPGTEYTVVIWGVKGGTWSSPLSAIF TT
ISOP121GR5P1B4	43	LPAPKNLIVSRVTEDSARLSWTAPDAAFDSFPINYGERATKGEA INLYVPGSERSYDLTGLKPGTEYWVLIGGVKGGLKSSPLWAWF TT
ISOP121BR5P1G2	44	LPAPKNLVVSRVTEDSARLSWADELHHANHFDSFLIQYQESEK VGEAIVLTVPGSERSYDLTGLKPGTEYTVSIYGVYDRHYEIHFYS NPLSAIFTT
ISOP121HR5P1H2	45	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFDIYYLEYDYSGEA IVLTVPGSERSYDLTGLKPGTEYDVLIIGVKGGSLSTPLSAIFTT
ISOP121FR5P1G11	46	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFSIWYLEIVAHGE AIVLTVPGSERSYDLTGLKPGTEYEVIIHGVKGCGPSGPLSAIFTT
ISOP121AR5P1E7	47	LPAPKNLVVSRVTEDSARLSWHVYHEIDYFDSFLIQYQESEKVG EAIVLTVPGSERSYDLTGLKPGTEYTVSIYGVYQRKVEFYSNPLS AIFTT
ISOP121GR5P1F6	48	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFDIRYHEYTWPGE AIVLLVPGSERSYDLTGLKPGTEYGVYINGVKGGFRSKPLFAWF TTGG
ISOP121BR5P1E9	49	LPAPKNLVVSRVTEDSARLSWDSYRDYFDSFLIQYQESEKVGEA IVLTVPGSERSYDLTGLKPGTEYTVSIYGVYSRKHVVFVQSNPLS AIFTT
ISOP121AR5P1F2	50	LPAPKNLVISRVTEDSARLSWGWSELIATHFDSFLIQYQESEKV GEAIVLTVPGSERSYDLTGLKPGTEYTVSIYGVYNRKVNFYSNPL SAIFTT
ISOP121AR5P1F7	51	LPAPKNLVVSRVTEDSARLSWQEHWDTSSNFDSFLIQYQESEK VGEAIVLTVPGSERSYDLTGLKPGTEYTISIYGVYNRKVLFYSNPL SAIFTT
ISOP121BR5P1H6	52	LPAPKNLVVSRVTEDSARLSWGYIDVSYFDSFLIQYQESEKVGE AIVLTVPGSERSYDLTGLKPGTEYTVSIYGVYSRPKAEFTSNPLSA IFTT
ISOP121GR5P1A2	53	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFKIQYIERYIPGEAI QLNVPGSERSYDLTGLKPGTEYSVIIPGVKGGRNSFPLWAWFT T
ISOP121BR5P1D3	54	LPAPKNLVVSRVTEDSARLSWYEDNTERFDSFLIQYQESEKVGE AIVLTVPGSERSYDLTGLKPGTEYTVSIYGVYIRVQVLWFSNPLS AIFTT
ISOP121AR5P1F9	55	LPAPKNLVVSRVTEDSARLSWGWSELIATHFDSFLIQYQESEKV GEAIVLTVPGSERSYDLTGLKPGTEYTVSIYGVYNRKVNFYSNPL SAIFTT

ISOP121AR5P1H5	56	LPAPKNLVVSRVTEDSARLSWEDAVKHIWFDSFLIQYQESEKV GEAIVLTVPGSERSYDLTGLKPGTEYTVSIYGVWIASVWRSNPL SAIFTT
ISOP121AR5P1G10	57	LPAPKNLVVSRVTEDSARLSWEWLEHFDSFLIQYQESEKVGEAI VLTVPGSERSYDLTGLKPGTEYTVSIYGVYQRKVEFHSNPLSAIF TTT
ISOP121AR5P1F3	58	LPAPKNLVVSRVTEDSARLSWPFNNYSEHFDSFLIQYQESEKVG EAIVLTVPGSERSYDLTGLKPGTEYTVSIYGVYERKTAFYSNPLSA IFTT
ISOP121BR5P1E2	59	LPAPKNLVVSRVTEDSARLSWWFPLEWFDSFLIQYQESEKVGE AIVLTVPGSERSYDLTGLKPGTEYTVSIYGVYTREHKSVWASNP LSAIFTT
ISOP121BR5P1D1	60	LPAPKNLVVSRVTEDSARLSWKWGGEFFDSFLIQYQESEKVGE AIVLTVPGSERSYDLTGLKPGTEYTVSIYGVYQRNWHHWYSNP LSAIFTT
ISOP121BR5P1C9	61	LPAPKNLVVSRVTEDSARLSWIWPDKHEFFDSFLIQYQESEKVG EAIVLTVPGSERSYDLTGLKPGTEYTVSIYGVYDRKYANWSSNP LSAIFTT
ISOP121GR5P1G11	62	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFQINYHEYGQNG EAIQLIVPGSERSYDLTGLKPGTEYGVWIWGVKGGIRSKPLWA FFTT
ISOP121BR5P1A7	63	LPAPKNLVVSRVTEDSARLSWTTAFHNEYFDSFLIQYQESEKVG EAIVLTVPGSERSYDLTGLKPGTEYTVSIYGVYSRPKAEFTSNPLS AIFTT
ISOP121BR5P1C3	64	LPAPKNLVVSRVTEDSARLSWASARDYFDSFLIQYQESEKVGEA IVLTVPGSERSYDLTGLKPGTEYTVSIYGVLAIAQITHWFSNPLS AIFTT
ISOP121AR5P1D11	65	LPAPKNLVVSRVTEDSARLSWEWLEHFDSFLIQYQESEKVGEAI VLTVPGSERSYDLTGLKPGTEYTVSIYGVYQRKVEFHSNPLSAIF TT
ISOP121ER5P1E7	66	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFTIGYTETPPRGEA IVLTVPGSERSYDLTGLKPGTKYYVSILGVKGGLGSWPLSAIFTT
ISOP121GR5P1G7	67	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFHIRYHEYDKNGE AIQLYVPGSERSYDLTGLKPGTEYGVYIHGVKGGGRSKPLWAH FTT
ISOP121AR5P1A8	68	LPAPKNLVVSRVTEDSARLSWGLEWAYQFFDSFLIQYQESEKV GEAIVLTVPGSERSYDLTGLKPGTEYTVSIYGVYLRAIEFYSNPLS AIFTT
ISOP121BR5P1E7	69	LPAPKNLVVSRVTEDSARLSWRKQEQYFDSFLIQYQESEKVGE AIVLTVPGSERSYDLTGLKPGTEYTVSIYGVKKWPSTTTTSNPLS AIFTT
ISOP121FR5P1H8	70	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFVIYYSEQHFYGE AIVLTVPGSERSYDLTGLKPGTEYVVKIYGVKGGETSKPLSAIFTT
ISOP121GR5P1D2	71	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFHILYQERAQSGE AIGLVVPGSERSYDLTGLKPATEYSVQIFGVKGGKLSNPLWAW FTT

ISOP121AR5P1H2	72	LPAPKNLVVSRVTEDSARLSWVIDEFIPLFDSFLIQYQESEKVGE AIVLTVPGSERSYDLTGLKPGTEYTVSIYGVLAKNIGISNPLSAIFT T
ISOP121GR5P1F10	73	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFFIDYVERATVGE AIALNVPGSKRSYALTGLKPGTEYFVKIRGVKGGLKSKPLWAW FTT
ISOP121BR5P1A2	74	LPAPKNLVVSRVTEDSARLSWRFSQEWFDSFLIQYQESEKVGE AIVLTVPGSERSYDLTGLKPGTEYTVSIYGVYARGIHKWLSNPLS AIFTT
ISOP121GR5P1F7	75	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFGINYVERASEGE AIDLGVPGSERSYDLTGLKPGTEYFVKIFGVKGGIPSVPLWAWF TT
ISOP121AR5P1B8	76	LPAPKNLVISRVTEDSARLSWDKRTQFAFDSFLIQYQESEKVGE AIVLTVPGSERSYDLTGLKPGTEYTVSIYGVPTWSGRTQSNPLS AIFTT
ISOP121GR5P1D7	77	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFKIWYQERSIVGE AIFLLVPGSERSYDLTGLKPGTEYIVQIFGVKGGPYSNPLWAPFT T
ISOP121BR5P1G3	78	LPAPKNLVVSRVTEDSARLSWKQRTSFHFDSFLIQYQESEKVGE AIVLTVPGSERSYDLTGLKPGTEYTVSIYGVPFWQQWQPESNP LSAIFTT
ISOP121AR5P1C5	79	LPAPKNLVVSRVTEDSARLSWKRSDDEWFDSFLIQYQESEKVG EAIILTVPGSERSYDLTGLKPGTEYTVSIYGVYQRAALWFSNPLS AIFTT
ISOP121FR5P1H9	80	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFSILYGETAPIGEAI VLTVPGSERSYDLTGLKPGTEYVVYIQGVKGGNYSQPLSAIFTT
ISOP121AR5P1A10	81	LPAPKNLVVSRVTEDSARLSWPDWSNSEYFDSFLIQYQESEKV GEAIVLTVPGSERSYDLTGLKPGTEYTVSIYGVYARHRLFVSNPL SAIFTT
ISOP121HR5P1F2	82	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFTILYGETYSGGEA IVLTVPGSERSYDLTGLKPGTEYVVYIFGVKGGKWSRPLSAIFTT
ISOP121AR5P1H1	83	LPAPKNLVVSRVTEDSARLSWKQATKFVFDSFLIQYQESEKVGE AIVLTVPGSERSYDLTGLKPGTEYTVSIYGVPDWFVLESNPLSAI FTT
ISOP121BR5P1D10	84	LPAPKNLVVSRVTEDSARLSWGKKSHFFDSFLIQYQESEKVGEA IVLTVPGSERSYDLTGLKPGTEYTVSIYGVYTRGQCEWESNQLS AIFFTT
ISOP121BR5P1F10	85	LPAPKNLVVSRVTEDSARLSWPLNLEYFDSFLIQYQESEKVGEAI VLTVPGSERSYDLTGLKPGTEYTVSIYGVYGRYGGPFVSNPLSAI FTT
ISOP121BR5P1D11	86	LPAPKNLVVSRVTEDSARLSWFNADEEYFDSFLIQYQESEKVGE AIVLTVPGSERSYDLTGLKPGTEYTVSIYGVYVRAVRFVSSNPLS AIFTT
ISOP121AR5P1E11	87	LPAPKNLVVSRVTEDSARLSWSVQTSFVFDSFLIQYQESEKVGE AIVLTVPGSERSYDLTGLKPGTEYTVSIYGVPLWHGFDSNPLSAI FTT

ISOP121BR5P1D6	88	LPAPKNLVVSRVTEDSARLSWKQGTSFHFDSFLIQYQESEKVGE AIVLTVPGSERSYDLTGLKPGTEYTVSIYGVQLLANDIISSNPLSAI FTT
ISOP121BR5P1B5	89	LPAPKNLVVSRVTEDSARLSWRKQEQYFDSFLIQYQESEKVGE AIVLTVPGSERSYDLTGLKPGTEYTVSIYGVYQRGYHNWFSNPL SAIFTT
ISOP194ER9P1G3	90	LPAPKNLIVSRVTEDSARLSWTAPDAAFDSFRIAYYETMVSGEA IVLTVPGSERSYDLTGLKPGTEYAVIIKGVKGGKPSWPLSAIFTT
ISOP194AR9P1F2	91	LPAPKNLVISRVTEDSARLSWEWLEHFDSFLIQYQESEKVGEAI VLTVPGSERSYDLTGLKPGTEYTVSIYGVYNRKVNFYSNPLSAIF TT
ISOP194AR9P1H10	92	LPAPKNLVISRVTEDSARLSWPAHYHSAFFDSFLIQYQESEKVG EAIVLTVPGSERSYDLTGLKPGTEYTVSIYGVYQRKVEFHSNPLS AIFTT
ISOP194BR9P1H4	93	LPAPKNLVVSRVTEDSACLSWTTAFHNEYFDSFLIQYQESEKVG EAIVLTVPGSERSYDLTGLKPGTEYTVSIYGVYSRPKAEFTSNPLS AIFTT
ISOP194AR9P1D8	94	LPAPKNLVVSRVTEDSARLSWDTWNDFFDSFLIQYQESEKVGE AIVLTVPGSERSYDLTGLKPGTEYTVSIYGVYQRKVIWLSNPLSA IFTT
ISOP194BR9P1D1	95	LPAPKNLVVSRVTEDSARLSWEHSLLNDQWFDSFLIQYQESEK VGEAIVLTVPGSERSYDLTGLKPGTEYTVSIYGVYQRGRALWYS NPLSAIFTT
ISOP194AR9P1E8	96	LPAPKNLVVSRVTEDSARLSWEWLEHFDSFLIQYQESEKVGEAI VLTIPGSERSYDLTGLKPGTEYTVSIYGVYQRKVEFHSNPLSAIFT T
ISOP194AR9P1E9	97	LPAPKNLVVSRVTEDSARLSWEWLEHFDSFLIQYQESEKVGEAI VLTVPGSERSYDLTGLKPGTEYTVSIYGVYQRKVNFYSNPLSAIF TT
ISOP194AR9P1H9	98	LPAPKNLVVSRVTEDSARLSWEWLEHFDSFQIQYQESEKVGEA IVLTVPGSERSYDLTGLKPGTEYTVSIYGVYQRKVEFHSNPLSAIF TT
ISOP194BR9P1A9	99	LPAPKNLVVSRVTEDSARLSWFNADEEYFDSFLIQYQESEKVGE AIVLTVPGSERSYDLTGLKPGTEYTVSIYGVYDRKVKFVQSNPLS AIFTT
ISOP194BR9P1A5	100	LPAPKNLVVSRVTEDSARLSWFNADEEYFDSFLIQYQESEKVGE AIVLTVPGSERSYDLTGLKPGTEYTVSIYGVYQRGYHNWFSNPL SAIFTT
ISOP194BR9P1F7	101	LPAPKNLVVSRVTEDSARLSWFNADEEYFDSFLIQYQESEKVGE AIVLTVPGSERSYDLTGLKPGTEYTVSIYGVYTRGRYEWRESNPL SAIFTT
ISOP194AR9P1G7	102	LPAPKNLVVSRVTEDSARLSWGDDFNSEYFDSFLIQYQESEKV GEAIVLTVPGSERSYDLTGLKPGTEYTVSIYGVYTRAVVFTSNPL SAIFTT
ISOP194AR9P1E3	103	LPAPKNLVVSRVTEDSARLSWKRSDDEWFDSFLIQYQESEKVG EAIVLTVPGSERSYDLTGLKPGTEYTVSIYGVYQRAALWFSNPLS AIFTT

ISOP194AR9P1C5	104	LPAPKNLVVSRVTEDSARLSWLRDFNGRAFFDSFLIQYQESEKV GEAIVLTVPGSERSYDPTGLKPGTEYTVSIYGVFITWIHVRSNPL SAIFTT
ISOP194AR9P1H3	105	LPAPKNLVVSRVTEDSARLSWNASWISHNFFDSFLIQYQESEK VGEAIVLTVPGSERSYDLTGLKPGTEYTVSIYGVYERKTAFYSNP LSAIFTT
ISOP194GR9P1E9	106	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFHIRYHEYDKNGE AIQLYVPGSERSYDLTGLKPGTEYGVFIWGVKGGLKSKPLWAW FTT
ISOP194HR9P1B10	107	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFPIRYYERANGEAI VLTVPGSERSYDLTGLKPGTEYIVWIYGVKGGGRSGPLSAIFTT
ISOP194ER9P1A11	108	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFRIAYYETMVSGE AIVLTVPGSERSYDLTGLKPGTEYAVIIKGVKGGKPSWPLSAIFT T
ISOP194ER9P1A3	109	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFRIAYYETMVSGE AIVLTVPGSERSYDLTGLKPGTEYAVIIKGVKGGMVSWPLSAIFT T
ISOP194ER9P1H9	110	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFRIAYYETMVSGE AIVLTVPGSERSYDLTGPKPGTEYAVIIKGVKGGKPSWPLSAIFT T
ISOP194HR9P1B2	111	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFSILYGELIGDGEAI VLTVPGSERSYDLTGLKPGSEYTVYIFGVKGGRYSRPLSAIFTT
ISOP194HR9P1D11	112	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFSILYGELIGDGEAI VLTVPGSERSYDLTGLKPGTEYTVYIFGVKGGRYSRPLSAIFTT
ISOP194GR9P1F6	113	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFWIDYWERLSEGE AIALRVPGSERSYDLTGLKPGTEYYVWIVGVKGGKFSQPLRAW FTT
ISOP194GR9P1F9	114	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFWIFYNERWQNG EAIRLIVPGSERSYDLTGLKPGTEYSVIIPGVKGGRNSFPLWAWF TT
ISOP194GR9P1C11	115	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFWIFYNERWQNG EAIRLTVPGSERSYDLTGLKPGTEYWVLIGGVKGGLKSSPLWA WFTT
ISOP194ER9P1E6	116	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFWIKYYEKRNPGE AIVLTVPGSERSYDLTGLKPGTEYLVIISGVKGGSRSVPLSAIFTT
ISOP194BR9P1G9	117	LPAPKNLVVSRVTEDSARLSWTTAFHNEYFDSFLIQYQESEKVG EAIVLTVPGSERSYDLTGLKPGTEYTVSIYGVYIRVQVLWFSNPL SAIFTT
ISOP194BR9P1E4	118	LPAPKNLVVSRVTEDSARLSWTTAFHNEYFDSFLIQYQESEKVG EAIVLTVPGSERSYDLTGLKPGTEYTVSIYGVYQRGYHNWFSNP LSAIFTT
ISOP194AR9P1H1	119	LPAPKNLVVSRVTEDSARLSWWRVLGHSHFFDSFLIQYQESEK VGEAIVLTVPGSERSYDLTGLKPGTEYTVSIYGVYNRKVNFYSNP LSAIFTT

ISOP194BR9P1D10	120	LPAPKNLVVSRVTEDSARLSWYEDNTERFDSFLIQYQESEKVVE AIVLTVPGSERSYDLTGLKPGTEYTVSIYGVYIRVQVLWFSNPLS AIFTT
ISOP194BR9P1C8	121	LPAPKNLVVSRVTEDSARLSWYFAGELWFDSFLIQYQESEKVG EAIVLTVPGSERSYDLTGLKPGTEYTVSIYGVYQRGYHNWFSNP LSAIFTT
ISOP194AR9P1C10	122	LPAPKNLVVSRVTEDSARPSWEWLEHFDSFLIQYQESEKVGEAI VLTVPGSERSYDLTGLKPGTEYTVSIYGVYNRKVNFYSNPLSAIF TT
ISOP194AR9P1D11	123	LPAPKNLVVSRVTEDSGRLSWQHHISFFDSFLIQYQESEKVGEA IVLTVPGSERSYDLTGLKPGTEYTVSIYGVYNRKVNFYSNPLSAIF TT
ISOP194AR9P1C3	124	LPAPKNLVVSRVTQNSARLSWEWLEHFDSFLIHYQESEKVGEA IVLTVPGSERSYDLTGLKPGTEYTVSIYGVYQRKVEFHSNPLSAIF TT
3rd FN3 domain of tenascin C (TN3	125	DAPSQIEVKDVTDTTALITWFKPLAEIDGIELTYGIKDVP GDRTTIDLTEDENQYSIGNLKPDTEYEVSLISRR GDMSSNPAKETFTT
Fibcon	126	LDAPTDLQVTNVTDTSITVSWTPPSATITGYRITYTPSNG PGEPKELTVPPSSTSVTITGLTPGVEYVVSLYAL KDNQESPPLVGTQTT
10th FN3 domain of fibronectin	127	VSDVPRDLEVVAATPTSLLISWDAPAVTVRYYRITYGET GGNSPVQEFTVPGSKSTATISGLKPGVDYTITVY AVTGRGDSPASSKPISINYRT
Linker	128	GSGS
Linker	129	GGGSGGGS
Linker	130	GGGGSGGGGSGGGGSGGGGSGGGGS
Linker	131	APAP
Linker	132	APAPAPAPAP
Linker	133	APAPAPAPAPAPAPAPAPAP
Linker	134	APAPAPAPAPAPAPAPAPAPAPAPAPAPAPAPAPAPAP
Linker	135	EAAAKEAAAKEAAAKEAAAKEAAAKAAA

Albumin variant	136	DAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQSPFED HVKLVNEVTEFAKTCVADESAENCDKSLHTLFG DKLCTVATLRETYGEMADCCAKQEPERNECFL QHKDDNPNLPRLVRPEVDVMCTAFHDNEETFL KKYLYEIARRHPYFYAPELLFFAKRYKAAFTEC CQAADKAACLLPKLDELRDEGKASSAKQRLKC ASLQKFGERAFKAWAVARLSQRFPKAEFAEVSK LVTDLTKVHTECCHGDLLECADDRADLAKYICE NQDSISSKLKECCEKPLLEKSHCIAEVENDEMPA DLPSLAADFVESKDVCKNYAEAKDVFLGMFLY EYARRHPDYSVVLLLRLAKTYETTLEKCCAAAD PHECYAKVFDEFKPLVEEPQNLIKQNCELFEQLG EYKFQNALLVRYTKKVPQVSTPTLVEVSRNLGK VGSKCCKHPEAKRMPCAEDYLSVVLNQLCVLH EKTPVSDRVTKCCTESLVNRRPCFSALEVDETY VPKEFNAETFTFHADICTLSEKERQIKKQTALVE LVKHKPKATKEQLKAVMDDFAAFVEKCCKAD DKETCFAEEGKKLVAASQAALGL

WHAT IS CLAIMED

1) An isolated FN3 domain that specifically binds to PD-L1.
2) The FN3 domain of claim 1, wherein the FN3 domain is based on Tencon 27 (SEQ ID NO: 4) or Tencon 1 (SEQ ID NO: 1), or on the amino acid sequence of SEQ ID NO: 4, optionally having substitutions at residue positions 11, 14, 17, 37, 46, 73 and/or 86 corresponding to SEQ ID NO: 4.
3) The FN3 domain of claim 1 or 2, wherein the FN3 domain is conjugated to a heterologous molecule.
4) The FN3 domain of claim 3, wherein the heterologous molecule is a detectable label or a cytotoxic agent, or both.
5) The FN3 domain of any of claims 1-4, wherein the detectable label is a radioactive isotope, magnetic beads, metallic beads, colloidal particles, a fluorescent dye, an electron-dense reagent, an enzyme, biotin, digoxigenin, or hapten.
6) The FN3 domain of any of claims 1-5, wherein the detectable label is auristatin, monomethyl auristatin phenylalanine, dolostatin, chemotherapeutic agent, a drug, a growth inhibitory agent, a toxin, or a radioactive isotope.
7) The FN3 domain of any of claims 1-6, comprising the amino acid sequence of SEQ ID NOs: 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123 or 124.
8) The FN3 domain of any of claims 1-7, further comprising a methionine at the N-terminus of the FN3 domain.
9) The FN3 domain of any of claims 1-8, wherein the FN3 domain is coupled to a half-life extending moiety.
10) The FN3 domain of claim 9, wherein the half-life extending moiety is an albumin binding molecule, a polyethylene glygol (PEG), albumin, albumin variant, or at least a portion of an Fc region of an immunoglobulin.
11) An isolated polynucleotide encoding the FN3 domain of claim 7.
12) A vector comprising the polynucleotide of claim 11.
13) A host cell comprising the vector of claim 12.

14) A method of producing an FN3 domain that specifically binds PD-L1, comprising culturing the isolated host cell of claim 13 under conditions that the FN3 domain is expressed, and purifying the FN3 domain.

15) A pharmaceutical composition comprising the FN3 domain of any of claims 1-10 and a pharmaceutically acceptable carrier.

16) An anti-idiotypic antibody that specifically binds to the FN3 domain of claim 7.

17) A kit comprising the FN3 domain of claim 7.

18) A method of detecting PD-L1-expressing cancer cells in a tumor tissue, comprising

a) obtaining a sample of the tumor tissue from a subject; and

b) detecting whether PD-L1 is expressed in the tumor tissue by contacting the sample of the tumor tissue with the FN3 domain that specifically binds to PD-L1 comprising the sequence of any one of the amino acid sequences of SEQ ID NOs: 34-124 and detecting the binding between PD-L1 and the FN3 domain.

19) A method of isolating or detecting PD-L1 expressing cells, comprising

a) obtaining a sample from a subject;

b) contacting the sample with the FN3 domain that specifically binds to PD-L1 comprising the sequence of any one of the amino acid sequences of SEQ ID NOs: 34-124, and

c) isolating or detecting the cells bound to the FN3 domains.

20) A method of detecting PD-L1-expressing cancer cells in a tumor tissue, comprising

a) conjugating the FN3 domain that specifically binds to PD-L1 comprising the sequence of any one of the amino acid sequences of SEQ ID NOs: 34-124 to a detectable label to form a conjugate;

b) administering the conjugate to a subject; and

c) visualizing the PD-L1 expressing cancer cells to which the conjugate is bound.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/65980

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 39/00, C07K 16/28 (2018.01)
CPC - A61K 39/0011, A61K 2039/5156

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ------ Y	WO 2016/086021 A1 (BRISTOL-MYERS SQUIBB COMPANY) 02 June 2016 (02.06.2016) abstract; para [0005]; [0006]; [0026]-[0028]; [0084]; [0087]; [0111]; [0165]; [0221]-[0224]; [0342].	1, 3/1, 4/1 ------ 2, 3/2, 4/2, 18-20
Y	US 2016/0041182 A1 (JANSSEN BIOTECH, INC.) 11 February 2016 (11.02.2016) claims 1-9; para [0028]; SEQ ID NO: 27.	2, 3/2, 4/2, 18-20

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 April 2018	07 MAY 2018
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/65980

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 5-17
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claims 1-4, directed to an isolated FN3 domain that specifically binds to PD-L1.

Group II, claims 18-20, directed to a method of isolating or detecting PD-L1 expressing cells.

The inventions listed as Groups I-II do not relate to a single special technical feature under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

--continued on first extra sheet--

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☒ No protest accompanied the payment of additional search fees.

--continued from Box No: III Observations where unity of invention is lacking--

Special technical features:

Group I has the special technical feature of an isolated FN3 domain that specifically binds to PD-L1, that is not required by Group II.

Group II has the special technical feature of detecting whether PD-LI is expressed in a tumor tissue by contacting the sample of the tumor tissue with the FN3 domain that specifically binds to PD-L1, that is not required by Group I.

Common technical features:

Groups I-II share the common technical feature of a FN3 domain that specifically binds to PD-L1. However, this shared technical feature does not represent a contribution over prior art, because this shared technical feature is anticipated by WO 2016/086021 A1 to BRISTOL-MYERS SQUIBB COMPANY (hereinafter BMS).

BMS teaches a FN3 domain that specifically binds to PD-L1 (abstract "Provided herein are novel 10th Fn3 domains which specifically bind to PD-L1, as well as imaging agents based on the same for diagnostics").

As the technical features were known in the art at the time of the invention, they cannot be considered special technical features that would otherwise unify the groups.

Therefore, Group I-II inventions lack unity under PCT Rule 13 because they do not share the same or corresponding special technical feature.

NOTE, claims 5-17 are held unsearchable because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).