

[0006a] In one aspect, the present invention refers to a target-binding polypeptide comprising an amino acid sequence of

MGSWX₅X₆FKX₉X₁₀LAX₁₃IKX₁₆X₁₇LEALGGSEAELAX₃₀FEX₃₃X₃₄IAX₃₇FEX₄₀X₄₁LQ
X₄₄YKGKGNPEVEALRKEAAAIRDELQAYRHN (SEQ ID NO:2),
MGSWAEFKQRLAAIKTRLEALGGSEAEAAFX₃₂X₃₃EIX₃₆AFX₃₉X₄₀ELX₄₃AYKGKGNPEVE
ALX₅₇X₅₈EAX₆₁AIX₆₄X₆₅ELX₆₈AYRHN (SEQ ID NO:3),
MGSWX₅EFX₈X₉RLX₁₂AIX₁₅X₁₆RLX₁₉ALGGSEAEAAFEKEIAAFESSELQAYKGKGNPEVEX₅
₅LRX₅₈X₅₉AAX₆₂IRX₆₅X₆₆LQAYRHN (SEQ ID NO:4),
MGSWX₅X₆FKX₉X₁₀LAX₁₃IKX₁₆X₁₇LEALGGSEAEAAFX₃₂X₃₃EIX₃₆AFX₃₉X₄₀ELX₄₃AYKGK
GNPEVEX₅₅LRX₅₈X₅₉AAX₆₂IRX₆₅X₆₆LQAYRHN (SEQ ID NO:5),
MGSWX₅EFX₈X₉RLX₁₂AIX₁₅X₁₆RLX₁₉ALGGSEAEALAX₃₀FEX₃₃X₃₄IAX₃₇FEX₄₀X₄₁LQ
X₄₄YKGKGNPEVEALX₅₇X₅₈EAX₆₁AIX₆₄X₆₅ELX₆₈AYRHN (SEQ ID NO:6),
MGSWX₅X₆FKX₉X₁₀LAX₁₃IKX₁₆X₁₇LEALZ₁EAELAX₂₈FEX₃₁X₃₂IAX₃₅FEX₃₈X₃₉LQX₄₂YZ₂NPE
VEALRKEAAAIRDELQAYRHN (SEQ ID NO:7),
MGSWAEFKQRLAAIKTRLEALZ₁EAELAAFX₃₀X₃₁EIX₃₄AFX₃₇X₃₈ELX₄₁AYZ₂NPEVEALX₅₂
X₅₃EAX₅₆AIX₅₉X₆₀ELX₆₃AYRHN (SEQ ID NO:8),
MGSWX₅EFX₈X₉RLX₁₂AIX₁₅X₁₆RLX₁₉ALZ₁EAELAAFEKEIAAFESSELQAYZ₂NPEVE
X₅₀LRX₅₃X₅₄AAX₅₇IRX₆₀X₆₁LQAYRHN (SEQ ID NO:9),
MGSWX₅X₆FKX₉X₁₀LAX₁₃IKX₁₆X₁₇LEALZ₁EAELAAFX₃₀X₃₁EIX₃₄AFX₃₇X₃₈ELX₄₁AYZ₂NPEV
EX₅₀LRX₅₃X₅₄AAX₅₇IRX₆₀X₆₁LQAYRHN (SEQ ID NO:10) or
MGSWX₅EFX₈X₉RLX₁₂AIX₁₅X₁₆RLX₁₉ALZ₁EAELAX₂₈FEX₃₁X₃₂IAX₃₅FEX₃₈X₃₉LQX₄₂Y
Z₂NPEVEALX₅₂X₅₃EAX₅₆AIX₅₉X₆₀ELX₆₃AYRHN (SEQ ID NO:11), wherein

- (a) X_n is a natural or non-natural amino acid;
- (b) Z₁ and Z₂ comprise between about 2 to about 30 natural or non-natural amino acids;
- (c) the target-binding polypeptide specifically binds a target of interest, wherein the specific binding of the target-binding polypeptide to the target of interest is greater than the binding of a reference polypeptide comprising the amino acid sequence of SEQ ID NO:1 to the target of interest; and
- (d) the target-binding polypeptide does not comprise the amino acid sequence of SEQ ID NO:50.

[0006b] In another aspect, the present invention refers to a de novo binding domain polypeptide (DBDpp) comprising three anti-parallel alpha helices joined by linker peptides, wherein

- (a) the DBDpp is a synthetic peptide derived from modifications to the amino acid sequence of SEQ ID NO:1, wherein the modifications comprise one or more conservative or non-conservative amino acid substitutions of solvent accessible or solvent inaccessible residues;
- (b) the DBDpp specifically binds a target of interest, wherein the specific binding of the DBDpp to the target of interest is greater than the binding of a reference polypeptide comprising the amino acid sequence of SEQ ID NO:1 to the target of interest; and
- (c) the DBDpp does not comprise the amino acid sequence of SEQ ID NO:50.

[0006c] In yet another aspect, the present invention refers to a fusion protein comprising a first and second DBDpp as disclosed herein wherein the first and second DBDpp exhibits binding specificity for a tumor target, optionally wherein the first and second DBDpp exhibits binding specificity for different tumor targets.

[0006d] In yet another aspect, the present invention refers to a pharmaceutical composition comprising the target-binding polypeptide as disclosed herein, the DBDpp as disclosed herein, or the fusion protein as disclosed herein and further comprising a pharmaceutically acceptable carrier.

[0006e] In yet another aspect, the present invention refers to a kit comprising the target-binding polypeptide as disclosed herein, the DBDpp as disclosed herein, or the fusion protein as disclosed herein.

[0006f] In yet another aspect, the present invention refers to an isolated nucleic acid molecule encoding the target-binding polypeptide as disclosed herein, the DBDpp as disclosed herein, or the fusion protein as disclosed herein.

[0006g] In a further aspect, the present invention refers to a vector comprising the isolated nucleic acid molecule as disclosed herein, and optionally further comprising a nucleotide sequence which regulates the expression of the target-binding polypeptide, DBDpp, or fusion protein encoded by the nucleic acid molecule.

[0006h] In yet another aspect, the present invention refers to a host cell comprising the nucleic acid molecule as disclosed herein or the vector as disclosed herein.

[0006i] In yet another aspect, the present invention refers to a cell line engineered to express the target-binding polypeptide as disclosed herein, the DBDpp as disclosed herein, or the fusion protein as disclosed herein.

[0006j] In yet another aspect, the present invention refers to a chimeric antigen receptor (CAR), wherein the CAR comprises

- (a) a targeting domain,
- (b) a transmembrane domain, and
- (c) an intracellular signaling domain,

wherein the targeting domain comprises the target-binding polypeptide as disclosed herein, the DBDpp as disclosed herein, or the fusion protein as disclosed herein.

[0006k] In yet another aspect, the present invention refers to an isolated nucleic acid molecule comprising a sequence encoding the CAR as disclosed herein.

[0006l] In yet another aspect, the present invention refers to a cell comprising a nucleic acid molecule comprising a sequence encoding the CAR as disclosed herein, optionally wherein the cell is a T cell or a natural killer (NK) cell, and optionally wherein the cell exhibits an anti-tumor immunity when the target-binding polypeptide, DBDpp, or fusion protein binds to its corresponding tumor antigen.

[0006m] In yet another aspect, the present invention refers to the use of an immune cell comprising the CAR as disclosed herein for the manufacture of a medicament for the treatment of cancer.

[0006n] In yet another aspect, the present invention refers to the use of an immune cell comprising a chimeric antigen receptor (CAR) for the manufacture of a medicament for the treatment of cancer, wherein the CAR comprises:

- (a) a target binding domain comprising a target-binding polypeptide having an amino acid sequence selected from the group consisting of

MSWX₅X₆FKX₉X₁₀LAX₁₃IKX₁₆X₁₇LEALGGSEAELAX₃₀FEX₃₃X₃₄IAX₃₇FEX₄₀X₄₁LQX₄₄YKGKGNPEVEALRKEAAAIRDELQAYRHN (SEQ ID NO:2),

MGSWAEFKQRLAAIKTRLEALGGSEAEAAFX₃₂X₃₃EIX₃₆AFX₃₉X₄₀ELX₄₃AYKGKGNPEVEALX₅₇X₅₈EAX₆₁AIX₆₄X₆₅ELX₆₈AYRHN (SEQ ID NO:3),

MSWX₅EFX₈X₉RLX₁₂AIX₁₅X₁₆RLX₁₉ALGGSEAEAAFEKEIAAFESELQAYKGKGNPEVEX₅

₅LRX₅₈X₅₉AAX₆₂IRX₆₅X₆₆LQAYRHN (SEQ ID NO:4),
 MGSWX₅X₆FKX₉X₁₀LAX₁₃IKX₁₆X₁₇LEALGGSEAELAAFX₃₂X₃₃EIX₃₆AFX₃₉X₄₀ELX₄₃AYK GK
 GNPEVEX₅₅LRX₅₈X₅₉AAX₆₂IRX₆₅X₆₆LQAYRHN (SEQ ID NO:5), and
 MGSWX₅EFX₈X₉RLX₁₂AIX₁₅X₁₆RLX₁₉ALGGSEAELAX₃₀FEX₃₃X₃₄IAX₃₇FEX₄₀X₄₁LQX₄₄YK G
 KGNPEVEALX₅₇X₅₈EAX₆₁AIX₆₄X₆₅ELX₆₈AYRHN (SEQ ID NO:6), wherein X_n is a natural or
 non-natural amino acid, wherein X_n is not a cysteine or a proline residue, wherein the target-binding
 polypeptide specifically binds a target of interest expressed by a cancer cell, and wherein the specific
 binding of the target-binding polypeptide to the target of interest is greater than binding of a
 polypeptide according to SEQ ID NO:1 to the target of interest,

(b) a transmembrane domain selected from 41BB and CD28, and

(c) an intracellular domain, wherein the intracellular domain comprises a signaling domain
 selected from an alpha, beta, or zeta chain of the T cell receptor.

[0006o] In yet another aspect, the present invention refers to a method for
 transforming a reference polypeptide into a target-binding polypeptide capable of specifically
 binding to a target of interest, the method comprising:

- (a) modifying a plurality of amino acid residues from the reference polypeptide to generate a
 plurality of candidate target-binding polypeptides; wherein the candidate target-binding
 polypeptides comprise a variant of the amino acid sequence of SEQ ID NO: 1, wherein the
 candidate target-binding polypeptides comprise three anti-parallel alpha helices joined by
 linker peptides, wherein the amino acid residues modified are solvent accessible or solvent
 inaccessible, and wherein the modification comprises one or more conservative or non-
 conservative amino acid substitutions and does not include a substitution with a cysteine or a
 proline;
- (b) packaging the plurality of candidate target-binding polypeptides in a plurality of vectors to
 generate a candidate library; and
- (c) screening the candidate library for candidate target-binding polypeptides that exhibit specific
 binding to the target of interest,

wherein the candidate target-binding polypeptides comprise an amino acid sequence selected
 from the group consisting of

MGSWX₅X₆FKX₉X₁₀LAX₁₃IKX₁₆X₁₇LEALGGSEAELAX₃₀FEX₃₃X₃₄IAX₃₇FEX₄₀X₄₁LQ

X_{44} YKGKGNPEVEALRKEAAAIRDELQAYRHN (SEQ ID NO:2),
 MGSWAEFKQRLAAIKTRLEALGGSEAELAAFX₃₂X₃₃EIX₃₆AFX₃₉X₄₀ELX₄₃AYKGKGNPEVE
 ALX₅₇X₅₈EAX₆₁AIX₆₄X₆₅ELX₆₈AYRHN (SEQ ID NO:3),
 MGSWX₅EFX₈X₉RLX₁₂AIX₁₅X₁₆RLX₁₉ALGGSEAELAAFEKEIAAFESSELQAYKGKGNPEVEX₅
₅LRX₅₈X₅₉AAX₆₂IRX₆₅X₆₆LQAYRHN (SEQ ID NO:4),
 MGSWX₅X₆FKX₉X₁₀LAX₁₃IKX₁₆X₁₇LEALGGSEAELAAFX₃₂X₃₃EIX₃₆AFX₃₉X₄₀ELX₄₃AYKGK
 GNPEVEX₅₅LRX₅₈X₅₉AAX₆₂IRX₆₅X₆₆LQAYRHN (SEQ ID NO:5),
 MGSWX₅EFX₈X₉RLX₁₂AIX₁₅X₁₆RLX₁₉ALGGSEAELAX₃₀FEX₃₃X₃₄IAX₃₇FEX₄₀X₄₁LQ
 X_{44} YKGKGNPEVEALX₅₇X₅₈EAX₆₁AIX₆₄X₆₅ELX₆₈AYRHN (SEQ ID NO:6),
 MGSWX₅X₆FKX₉X₁₀LAX₁₃IKX₁₆X₁₇LEALZ₁EAELAX₂₈FEX₃₁X₃₂IAX₃₅FEX₃₈X₃₉LQX₄₂YZ₂NPE
 VEALRKEAAAIRDELQAYRHN (SEQ ID NO:7),
 MGSWAEFKQRLAAIKTRLEALZ₁EAELAAFX₃₀X₃₁EIX₃₄AFX₃₇X₃₈ELX₄₁AYZ₂NPEVEALX₅₂
 X₅₃EAX₅₆AIX₅₉X₆₀ELX₆₃AYRHN (SEQ ID NO:8),
 MGSWX₅EFX₈X₉RLX₁₂AIX₁₅X₁₆RLX₁₉ALZ₁EAELAAFEKEIAAFESSELQAYZ₂NPEVE
 X_{50} LRX₅₃X₅₄AAX₅₇IRX₆₀X₆₁LQAYRHN (SEQ ID NO:9),
 MGSWX₅X₆FKX₉X₁₀LAX₁₃IKX₁₆X₁₇LEALZ₁EAELAAFX₃₀X₃₁EIX₃₄AFX₃₇X₃₈ELX₄₁AYZ₂NPEV
 EX₅₀LRX₅₃X₅₄AAX₅₇IRX₆₀X₆₁LQAYRHN (SEQ ID NO:10) and
 MGSWX₅EFX₈X₉RLX₁₂AIX₁₅X₁₆RLX₁₉ALZ₁EAELAX₂₈FEX₃₁X₃₂IAX₃₅FEX₃₈X₃₉LQX₄₂Y
 Z₂NPEVEALX₅₂X₅₃EAX₅₆AIX₅₉X₆₀ELX₆₃AYRHN (SEQ ID NO:11), wherein X_n is a natural or
 non-natural amino acid and Z₁ and Z₂ comprise between about 2 to about 30 natural or non-natural
 amino acids.

[0006p] In yet another aspect, the present invention refers to virus-like particles (VLPs) comprising a fusion polypeptide comprising the DBDpp as disclosed herein, wherein the VLPs are suitable for use as immunogens for antibody generation, wherein said antibodies are directed against the DBDpp.

[0007] Several examples of the disclosure relate to agents that specifically bind targets of interest, such as the de novo binding domain (DBD) containing polypeptides (DBDpp) disclosed herein. Nucleic acids encoding the DBDpp and vectors and host cells containing the nucleic acids are also provided, as are DBDpp libraries and methods for producing and screening such libraries and the DBDpp identified from such libraries and/or screens. DBDpp including DBDpp fusion proteins are also provided, as are methods of making and using the DBDpp. Non-limiting examples of such uses include, but are not limited to, affinity purification, target analysis, diagnostic and/or therapeutic applications.

[0008] In several examples, there is provided a binding agent that binds with a high degree of specificity to a target of interest. In several examples the binding agent is a non-antibody agent. In several examples, the binding agent is a polypeptide. In several examples, there are provided polypeptides for binding a target of interest that have a sequence that differs, at least at one position, from the sequence of SEQ ID NO:1. In several such examples, the agent (e.g., a polypeptide) exhibits specific binding to the target of interest, that binding being greater than the binding of a polypeptide according to SEQ ID NO:1 to the target of interest. In several examples, there is provided a polypeptide for binding a target of interest, the polypeptide comprising an amino acid sequence comprising MGSWX₅EFX₈RLX₁₂AIX₁₅X₁₆RLX₁₉ALGGSEAELAAFEKEIAAFESELQAYKKGKNPEVEX₅₅LRX₅₈X₅₉AAX₆₂IRX₆₅X₆₆LQAYRHN (SEQ ID NO:4), wherein the sequence differs in sequence from the sequence of SEQ ID NO:1 (e.g., by modifications to the amino acid sequence of SEQ ID NO:1). In several examples, the polypeptide specifically binds a target of interest

used. In some examples, the substitutions do not include substitution with a cysteine (e.g., no cysteines are added to the sequence). In some examples, wherein the substitutions do not include substitution with a proline (e.g., no prolines are added to the sequence). In some examples, neither cysteine nor proline is substituted into the sequence of the polypeptide.

[0010] Various targets of interest can be bound by the agents disclosed herein. For example, in several examples, the target of interest specifically bound by the polypeptide is a cancer antigen. In some examples, the cancer antigen specifically bound by polypeptide is PD-L1. In several such examples, target-binding polypeptide comprises or consists essentially of an amino acid sequence selected from the group consisting of SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO: 43, SEQ ID and NO:44. In some examples, the cancer antigen specifically bound by polypeptide is CD137. In some such example, the polypeptide comprises or consists essentially of an amino acid sequence selected from the group consisting of SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO: 17, SEQ ID NO:18, and SEQ ID NO:19. In some examples, the cancer antigen specifically bound by polypeptide is CD123. In some such examples, the polypeptide comprises or consists essentially of an amino acid sequence selected from SEQ ID NOS: 92-127. In some examples, a combination of cancer antigens is targeted, for example by coupling or otherwise combining various target-binding polypeptides. In some examples, two, three, four or more different cancer antigens are targeted. In some examples, multiple target-binding polypeptides are used to enhance the ability and/or capacity to bind a single target (e.g., dimers, trimers, etc.)

[0011] Additionally provided for in several examples is a method for transforming a reference polypeptide into a polypeptide having specific binding for a target of interest.

In some examples, the method comprising modifying a plurality of amino acid residues from a reference polypeptide to generate a plurality of candidate binding polypeptides, packaging the plurality of candidate binding polypeptides in a plurality of vectors to generate a candidate library, and screening the candidate library for candidate binding polypeptides that exhibit specific binding to the target of interest. In several examples, the reference polypeptide comprises a variant of a non-naturally occurring polypeptide and comprises three anti-parallel alpha helices joined by linker peptides. In several examples the amino acid residues to be modified are solvent accessible or solvent inaccessible

amino acids. In several examples, a greater degree of solvent accessible amino acids are modified, while in some examples a greater degree of solvent inaccessible amino acids are modified. In some examples, the modification comprises amino acid substitutions. As discussed above, the substitutions can comprise conservative amino acid substitutions, non-conservative amino acid substitutions, and/or combinations thereof. Optionally, in several examples, the substitution does not comprise substitution in of a cysteine, does not comprise substitution in of a proline, and in some cases does not comprise substitution in of a cysteine or a proline.

[0012] In several examples, the method further comprises identifying potentially immunogenic amino acid residues in the candidate binding polypeptides and modifying at least one of the potentially immunogenic amino acid residues (e.g., to reduce the potential immunogenicity of the resultant polypeptides that bind a target of interest). In several examples, the modification to reduce immunogenicity comprises an amino acid substitution (e.g., conservative and/or non-conservative substitutions).

[0013] In several examples, there is provided a de novo binding domain polypeptide (DBDpp) that comprises or consists essentially of three anti-parallel alpha helices, the DBDpp being a variant of a synthetic polypeptide, wherein the DBDpp immunospecifically binds to a protein that is at least 95% identical to CD123. In several examples, the DBDpp has a dissociation constant (KD) between about 10^{-4} M and about 10^{-12} M. In some examples, the target to which the

bioluminescent label. In some examples, the label is a biotin moiety. In several examples, a streptavidin moiety can be used. In some examples, a His-tag, FLAG-tag or other tag is used. In some examples, the label is luciferase, green fluorescent protein, red fluorescent protein, or other similar agent.

[0015] In several examples, the target-binding agent (e.g., a polypeptide) is conjugated to a therapeutic or cytotoxic agent (e.g., chemotherapeutic agent, radiotherapeutic agent, etc.). Depending on the example, the target-binding agent may optionally comprise a pharmaceutically acceptable carrier.

[0016] In several examples, there are provided kits comprising any of the target-binding agents disclosed herein (e.g., a therapeutic kit, a diagnostic kit, a kit for research use, etc.).

[0017] Several examples also provide for isolated nucleic acid molecules encoding the any of the target-binding polypeptides disclosed herein. Still additional examples provide for a vector (e.g., a plasmid, viral vector, or non-viral vector) containing the isolated nucleic acid molecule. Several such examples may also include standard components for expression of protein encoded by the nucleic acid (e.g., promoters, packaging components, etc.). For example, in several examples, the vector further comprises an additional nucleotide sequence which regulates the expression of the polypeptide encoded by the nucleic acid molecule. In several examples, the additional nucleic acid sequence is an inducible promoter.

full or partial antagonists, and the like. Those agents that compete for binding to a target of interest (either to the same epitope, an overlapping epitope, or a non-overlapping epitope that leads to steric or other hindrances to the agent binding a target of interest) can be identified by competitive binding assays.

[0020] Also provided for herein are polypeptides (either alone or expressed by a cell) that bind to a tumor. In several examples, the binding is based on the polypeptide having been generated and identified as having specific binding for one or more markers expressed by the tumor. The tumor, depending on the example, may be a suspension tumor or a solid tumor.

[0021] Several examples, also provide for a chimeric antigen receptor (CAR), wherein the CAR includes a targeting domain, a transmembrane domain, and an intracellular signaling domain. In several examples, the targeting domain is made up of, at least in part, a target-binding polypeptide as disclosed herein. In several examples, the intracellular signaling domain is selected from the group consisting of a human CD3 zeta domain, 41BB domain, a CD28 domain and any combination thereof. Depending on the example, the costimulatory signaling region comprises the intracellular domain of a costimulatory molecule selected from the group consisting of CD27, CD28, 4-1BB, OX40, CD30, CD40, PD-1, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, a ligand that specifically binds with CD83, and any combination thereof. In several examples, the CAR comprises a fusion protein that includes an additional target-binding polypeptide. Also provided for are isolated nucleic acid sequences encoding CARs that include the target-binding polypeptides as part (or all) of the targeting region.

[0022] Further provided for herein are cells comprising a nucleic acid sequence encoding a CAR, wherein the CAR comprises an antigen binding domain made up of, at least in part, a polypeptide that binds a target of interest, a transmembrane domain, and a signaling domain. In several examples, the polypeptide binds specifically to a tumor antigen (and thus functions to deliver the cell expressing the CAR to the tumor. In several examples, the tumor antigen is associated with a hematologic malignancy. In additional examples, tumor antigen is associated with a solid tumor. Both solid and hematologic tumors can be simultaneously targeted in some examples. In several examples, the tumor antigen is selected from the group consisting of CD137, PD-L1, CD123, CTLA4, CD47, KIR, DR5, TIM3, PD1, EGFR, TCR, CD19, CD20, CD22,

ROR 1, mesothelin, CD33/IL3Ra, cMet, PSMA, Glycolipid F77, EGFRvIII, GD2, NY-ESO- 1, MAGE A3, and combinations thereof. Depending on the example, the cell expressing the CAR can be a T cell or a natural killer (NK) cell. In several examples, the cell (whether T cell, NK cell or other cell type) exhibits an anti-tumor immunity when the polypeptide binds to its corresponding tumor antigen.

[0023] Still additional examples provide for amino acids having the sequence of SEQ ID 4, wherein X_n is not cysteine or proline.

[0024] Also provided for in one aspect is mammalian cells that generate membrane-bound virus-like particles (VLPs), wherein the mammalian cell is engineered to express a fusion protein comprising a de novo binding domain polypeptide (DBDpp) fused to a chimeric antigen receptor (CAR), the fusion protein being expressed on the generated VLPs (e.g., as transmembrane proteins). Depending on the examples, the VLPs produced by the mammalian cells are suitable for use as immunogens for antibody generation. In some such examples, the antibodies are directed against the de novo binding domain polypeptide (DBDpp) (e.g., the antibodies bind to the DBDpp and can be used to detect the DBDpp, isolate the DBDpp, etc.

[0025] The target-binding polypeptides disclosed herein are also useful in a therapeutic context, e.g., for treatment and/or diagnosis of a disease, such as a cancer (e.g., a solid or hematologic malignancy).

There are also provided, in several examples methods of treating a subject having cancer, comprising administering to the subject an immune cell comprising a chimeric antigen receptor (CAR), wherein the CAR comprises a target binding domain, wherein the target binding domain comprises a polypeptide having an amino acid sequence comprising: MGSWX₅EFX₈X₉RLX₁₂AIX₁₅X₁₆RLX₁₉ALGGSEAELAAFEKEIAAFESELQAYKGKGNPEVEX₅₅LRX₅₈X₅₉AAX₆₂IRX₆₅X₆₆LQAYRHN (SEQ ID NO:4), a transmembrane domain, and an intracellular domain (comprising a signaling domain). Upon administration to a subject having cancer, the target binding domain specifically binds to a target of interest expressed by a cancer cell, and the binding of the target of interest induces the immune cell to generate cytotoxic signals that result in cytotoxic effects on the cancer cell, thereby treating the cancer. In several examples, the polypeptide has a sequence that differs from SEQ ID NO:1 (e.g., the polypeptide is generated by modifying the amino acid sequence of SEQ ID NO:1). As a result of the differing sequence, the

[0031] In several examples, the administration of the immune cells with a CAR is intravenous, though other routes, such as intra-arterial, intramuscular, local, or other acceptable route can be used for a given treatment scenario.

[0032] There are also provided, in several examples, methods of treating a subject having cancer, comprising, administering to the subject an immune cell comprising a chimeric antigen receptor (CAR), wherein the CAR comprises a target binding domain, wherein the target binding domain comprises a polypeptide having an amino acid sequence selected from of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6, wherein no cysteine or proline residues are substituted into any of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6, wherein the polypeptide specifically binds a target of interest expressed by a cancer cell, and wherein the polypeptide's specific binding to the target of interest is greater than binding of a polypeptide according to SEQ ID NO:1 to the target of interest, a transmembrane domain, and an intracellular domain, wherein the intracellular domain comprises a signaling domain, wherein, upon administration to a subject having cancer, the target binding domain specifically binds to the target of interest expressed by a cancer cell, and wherein the binding of the target of interest induces the

WHAT IS CLAIMED IS:

1. A target-binding polypeptide comprising an amino acid sequence of

MGSWX₅X₆FKX₉X₁₀LAX₁₃IKX₁₆X₁₇LEALGGSEAELAX₃₀FEX₃₃X₃₄IAX₃₇FEX₄₀X₄₁LQ
 X₄₄YKGKGNPEVEALRKEAAAIRDELQAYRHN (SEQ ID NO:2),
 MGSWAEFKQRLAAIKTRLEALGGSEAELAAFX₃₂X₃₃EIX₃₆AFX₃₉X₄₀ELX₄₃AYKGKGNPE
 VEALX₅₇X₅₈EAX₆₁AIX₆₄X₆₅ELX₆₈AYRHN (SEQ ID NO:3),
 MGSWX₅EFX₈X₉RLX₁₂AIX₁₅X₁₆RLX₁₉ALGGSEAELAAFEKEIAAFESSELQAYKGKGNPEV
 EX₅₅LRX₅₈X₅₉AAX₆₂IRX₆₅X₆₆LQAYRHN (SEQ ID NO:4),
 MGSWX₅X₆FKX₉X₁₀LAX₁₃IKX₁₆X₁₇LEALGGSEAELAAFX₃₂X₃₃EIX₃₆AFX₃₉X₄₀ELX₄₃AYK
 GKGNPEVEX₅₅LRX₅₈X₅₉AAX₆₂IRX₆₅X₆₆LQAYRHN (SEQ ID NO:5),
 MGSWX₅EFX₈X₉RLX₁₂AIX₁₅X₁₆RLX₁₉ALGGSEAELAX₃₀FEX₃₃X₃₄IAX₃₇FEX₄₀X₄₁LQ
 X₄₄YKGKGNPEVEALX₅₇X₅₈EAX₆₁AIX₆₄X₆₅ELX₆₈AYRHN (SEQ ID NO:6),
 MGSWX₅X₆FKX₉X₁₀LAX₁₃IKX₁₆X₁₇LEALZ₁EAELAX₂₈FEX₃₁X₃₂IAX₃₅FEX₃₈X₃₉LQX₄₂YZ₂
 NPEVEALRKEAAAIRDELQAYRHN (SEQ ID NO:7),
 MGSWAEFKQRLAAIKTRLEALZ₁EAELAAFX₃₀X₃₁EIX₃₄AFX₃₇X₃₈ELX₄₁AYZ₂NPEVEALX
₅₂ X₅₃EAX₅₆AIX₅₉X₆₀ELX₆₃AYRHN (SEQ ID NO:8),
 MGSWX₅EFX₈X₉RLX₁₂AIX₁₅X₁₆RLX₁₉ALZ₁EAELAAFEKEIAAFESSELQAYZ₂NPEVE
 X₅₀LRX₅₃X₅₄AAX₅₇IRX₆₀X₆₁LQAYRHN (SEQ ID NO:9),
 MGSWX₅X₆FKX₉X₁₀LAX₁₃IKX₁₆X₁₇LEALZ₁EAELAAFX₃₀X₃₁EIX₃₄AFX₃₇X₃₈ELX₄₁AYZ₂NP
 EVEX₅₀LRX₅₃X₅₄AAX₅₇IRX₆₀X₆₁LQAYRHN (SEQ ID NO:10) or
 MGSWX₅EFX₈X₉RLX₁₂AIX₁₅X₁₆RLX₁₉ALZ₁EAELAX₂₈FEX₃₁X₃₂IAX₃₅FEX₃₈X₃₉LQX₄₂YZ₂N
 PEVEALX₅₂X₅₃EAX₅₆AIX₅₉X₆₀ELX₆₃AYRHN (SEQ ID NO:11), wherein

- (a) X_n is a natural or non-natural amino acid;
- (b) Z₁ and Z₂ comprise between about 2 to about 30 natural or non-natural amino acids;
- (c) the target-binding polypeptide specifically binds a target of interest, wherein the specific binding of the target-binding polypeptide to the target of interest is greater than the binding of a reference polypeptide comprising the amino acid sequence of SEQ ID NO:1 to the target of interest; and
- (d) the target-binding polypeptide does not comprise the amino acid sequence of SEQ ID NO:50.

2. The target-binding polypeptide of claim 1 comprising an amino acid sequence of SEQ ID NO: 4.
3. The target-binding polypeptide of claim 1 or claim 2, wherein X_n is not a cysteine or a proline.
4. The target-binding polypeptide of any one of claim 1 to 3, wherein the target of interest is a cancer antigen, optionally wherein the cancer antigen is PD-L1, CD137, or CD123, and optionally wherein
 - (a) the target of interest is PD-L1 and the target-binding polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO: 43, and SEQ ID NO:44;
 - (b) the target of interest is CD137 and the target-binding polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, and SEQ ID NO:19;
 - (c) the target of interest is CD123 and the target-binding polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 92-126 and SEQ ID NO:127;
or
 - (d) the target of interest is CD123 and the target-binding polypeptide competes with the target-binding polypeptide of (c) for binding to CD123.
5. A de novo binding domain polypeptide (DBDpp) comprising three anti-parallel alpha helices joined by linker peptides, wherein
 - (a) the DBDpp is a synthetic peptide derived from modifications to the amino acid sequence of SEQ ID NO:1, wherein the modifications comprise one or more conservative or non-conservative amino acid substitutions of solvent accessible or solvent inaccessible residues;
 - (b) the DBDpp specifically binds a target of interest, wherein the specific binding of the DBDpp to the target of interest is greater than the binding of a reference polypeptide comprising the amino acid sequence of SEQ ID NO:1 to the target of interest; and
 - (c) the DBDpp does not comprise the amino acid sequence of SEQ ID NO:50.

6. The DBDpp of claim 5 comprising an amino acid sequence of

MGSWX₅X₆FKX₉X₁₀LAX₁₃IKX₁₆X₁₇LEALGGSEAELAX₃₀FEX₃₃X₃₄IAX₃₇FEX₄₀X₄₁LQ
X₄₄YKGKGNPEVEALRKEAAAIRDELQAYRHN (SEQ ID NO:2),
MGSWAEFKQRLAAIKTRLEALGGSEAELAAFX₃₂X₃₃EIX₃₆AFX₃₉X₄₀ELX₄₃AYKGKGNPE
VEALX₅₇X₅₈EAX₆₁AIX₆₄X₆₅ELX₆₈AYRHN (SEQ ID NO:3),
MGSWX₅EFX₈X₉RLX₁₂AIX₁₅X₁₆RLX₁₉ALGGSEAELAAFEKEIAAFESELQAYKGKGNPEV
EX₅₅LRX₅₈X₅₉AAX₆₂IRX₆₅X₆₆LQAYRHN (SEQ ID NO:4),
MGSWX₅X₆FKX₉X₁₀LAX₁₃IKX₁₆X₁₇LEALGGSEAELAAFX₃₂X₃₃EIX₃₆AFX₃₉X₄₀ELX₄₃AYK
GKGNPEVEX₅₅LRX₅₈X₅₉AAX₆₂IRX₆₅X₆₆LQAYRHN (SEQ ID NO:5),
MGSWX₅EFX₈X₉RLX₁₂AIX₁₅X₁₆RLX₁₉ALGGSEAELAX₃₀FEX₃₃X₃₄IAX₃₇FEX₄₀X₄₁LQ
X₄₄YKGKGNPEVEALX₅₇X₅₈EAX₆₁AIX₆₄X₆₅ELX₆₈AYRHN (SEQ ID NO:6),
MGSWX₅X₆FKX₉X₁₀LAX₁₃IKX₁₆X₁₇LEALZ₁EAELAX₂₈FEX₃₁X₃₂IAX₃₅FEX₃₈X₃₉LQX₄₂YZ₂
NPEVEALRKEAAAIRDELQAYRHN (SEQ ID NO:7),
MGSWAEFKQRLAAIKTRLEALZ₁EAELAAFX₃₀X₃₁EIX₃₄AFX₃₇X₃₈ELX₄₁AYZ₂NPEVEALX
₅₂ X₅₃EAX₅₆AIX₅₉X₆₀ELX₆₃AYRHN (SEQ ID NO:8),
MGSWX₅EFX₈X₉RLX₁₂AIX₁₅X₁₆RLX₁₉ALZ₁EAELAAFEKEIAAFESELQAYZ₂NPEVE
X₅₀LRX₅₃X₅₄AAX₅₇IRX₆₀X₆₁LQAYRHN (SEQ ID NO:9),
MGSWX₅X₆FKX₉X₁₀LAX₁₃IKX₁₆X₁₇LEALZ₁EAELAAFX₃₀X₃₁EIX₃₄AFX₃₇X₃₈ELX₄₁AYZ₂NP
EVEX₅₀LRX₅₃X₅₄AAX₅₇IRX₆₀X₆₁LQAYRHN (SEQ ID NO:10) or
MGSWX₅EFX₈X₉RLX₁₂AIX₁₅X₁₆RLX₁₉ALZ₁EAELAX₂₈FEX₃₁X₃₂IAX₃₅FEX₃₈X₃₉LQX₄₂YZ₂N
PEVEALX₅₂X₅₃EAX₅₆AIX₅₉X₆₀ELX₆₃AYRHN (SEQ ID NO:11), wherein

(a) X_n is a natural or non-natural amino acid; and

(b) Z₁ and Z₂ comprise between about 2 to about 30 natural or non-natural amino acids.

7. The DBDpp of claim 5 or claim 6 that specifically binds to a protein comprising amino acids 19-305 of CD123 (SEQ ID NO: 187) or a protein that is at least 95% identical to CD123, optionally wherein the DBDpp is characterized by one or more of

(a) the DBDpp binds to the protein comprising amino acids 19-305 of CD123 (SEQ ID NO: 187) or the protein that is at least 95% identical to CD123 with a dissociation constant (K_D) between about 10⁻⁴M and about 10⁻¹²M,

- (b) the DBDpp comprises an amino acid sequence of
 MGSWX₅EFX₈X₉RLX₁₂AIX₁₅X₁₆RLX₁₉ALGGSEAELAAFEKEIAAFESELQAYKGKGNP
 EVEX₅₅LRX₅₈X₅₉AAX₆₂IRX₆₅X₆₆LQAYRHN (SEQ ID NO:4), wherein X_n is a natural or
 non-natural amino acid,
- (c) the DBDpp comprises an amino acid sequence of
 MGSWX₅EFX₈X₉RLX₁₂AIX₁₅X₁₆RLX₁₉ALGGSEAELAAFEKEIAAFESELQAYKGKGNP
 EVEX₅₅LRX₅₈X₅₉AAX₆₂IRX₆₅X₆₆LQAYRHN (SEQ ID NO:4), wherein X_n is a natural or
 non-natural amino acid, and wherein X_n is not cysteine or proline, and
- (d) the DBDpp comprises an amino acid sequence at least 85% identical to the amino acid
 sequence of any one of SEQ ID NO:60 – SEQ ID NO: 136, and
- (e) the DBDpp is capable of binding to a tumor.
8. A fusion protein comprising a first and second DBDpp according to any one of claims 5 to 7
 wherein the first and second DBDpp exhibits binding specificity for a tumor target, optionally
 wherein the first and second DBDpp exhibits binding specificity for different tumor targets.
9. The target-binding polypeptide of any one of claim 1 to 4, the DBDpp of any one of claims 5 to
 7, or the fusion protein of claim 8, wherein the target-binding polypeptide, DBDpp, or fusion
 protein is labeled and optionally, wherein the label is a biotin moiety or is selected from the
 group consisting of an enzymatic label, a fluorescent label, a luminescent label, and a
 bioluminescent label.
10. The target-binding polypeptide of any one of claim 1 to 4 and 9, the DBDpp of any one of claims
 5 to 7 and 9, or the fusion protein of claim 8 or claim 9, wherein the target-binding polypeptide,
 DBDpp, or fusion protein is conjugated to a therapeutic or cytotoxic agent.
11. A pharmaceutical composition comprising the target-binding polypeptide of any one of claim 1
 to 4, 9 and 10, the DBDpp of any one of claims 5 to 7, 9 and 10, or the fusion protein of any one
 of claims 8 to 10 and further comprising a pharmaceutically acceptable carrier.

12. A kit comprising the target-binding polypeptide of any one of claim 1 to 4, 9 and 10, the DBDpp of any one of claims 5 to 7, 9 and 10, or the fusion protein of any one of claims 8 to 10.
13. An isolated nucleic acid molecule encoding the target-binding polypeptide of any one of claim 1 to 4, the DBDpp of any one of claims 5 to 7, or the fusion protein of claim 8.
14. A vector comprising the isolated nucleic acid molecule of claim 13, and optionally further comprising a nucleotide sequence which regulates the expression of the target-binding polypeptide, DBDpp, or fusion protein encoded by the nucleic acid molecule.
15. A host cell comprising the nucleic acid molecule of claim 13 or the vector of claim 14.
16. A cell line engineered to express the target-binding polypeptide of any one of claim 1 to 4, the DBDpp of any one of claims 5 to 7, or the fusion protein of claim 8.
17. A chimeric antigen receptor (CAR), wherein the CAR comprises
 - (a) a targeting domain,
 - (b) a transmembrane domain, and
 - (c) an intracellular signaling domain,wherein the targeting domain comprises the target-binding polypeptide of any one of claim 1 to 4, the DBDpp of any one of claims 5 to 7, or the fusion protein of claim 8.
18. The CAR of claim 17, which is characterized by one or more of
 - (a) the intracellular signaling domain is selected from the group consisting of a human CD3 zeta domain, 41BB domain, a CD28 domain and any combination thereof,
 - (b) the target-binding polypeptide, DBDpp, or fusion protein binds to a tumor antigen associated with a hematologic malignancy or a solid tumor,
 - (c) the target-binding polypeptide, DBDpp, or fusion protein binds to a tumor antigen selected from the group consisting of CD137, PD-L1, CD123, CTLA4, CD47, KIR, DR5, TIM3, PD1, EGFR, TCR, CD19, CD20, CD22, ROR 1, mesothelin, CD33/IL3Ra, cMet, PSMA, Glycolipid F77, EGFRvIII, GD2, NY-ESO- 1, MAGE A3, and combinations thereof,
 - (d) the intracellular signaling domain comprises a costimulatory signaling region, and

- (e) the intracellular signaling domain comprises a costimulatory signaling region comprising the intracellular domain of a costimulatory molecule selected from the group consisting of CD27, CD28, 4-1BB, OX40, CD30, CD40, PD-1, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, a ligand that specifically binds with CD83, and any combination thereof.
19. An isolated nucleic acid molecule comprising a sequence encoding the CAR of claim 17 or claim 18.
20. A cell comprising a nucleic acid molecule comprising a sequence encoding the CAR of claim 17 or claim 18, optionally wherein the cell is a T cell or a natural killer (NK) cell, and optionally wherein the cell exhibits an anti-tumor immunity when the target-binding polypeptide, DBDpp, or fusion protein binds to its corresponding tumor antigen..
21. Use of an immune cell comprising the CAR of claim 17 or claim 18 for the manufacture of a medicament for the treatment of cancer.
22. Use of an immune cell comprising a chimeric antigen receptor (CAR) for the manufacture of a medicament for the treatment of cancer, wherein the CAR comprises:
- (a) a target binding domain comprising a target-binding polypeptide having an amino acid sequence selected from the group consisting of
- MGSWX₅X₆FKX₉X₁₀LAX₁₃IKX₁₆X₁₇LEALGGSEAELAX₃₀FEX₃₃X₃₄IAX₃₇FEX₄₀X₄₁LQX₄₄YKGKGNPEVEALRKEAAAIRDELQAYRHN (SEQ ID NO:2),
- MGSWAEFKQRLAAIKTRLEALGGSEAELAAFX₃₂X₃₃EIX₃₆AFX₃₉X₄₀ELX₄₃AYKGKGNPEVEALX₅₇X₅₈EAX₆₁AIX₆₄X₆₅ELX₆₈AYRHN (SEQ ID NO:3),
- MGSWX₅EFX₈X₉RLX₁₂AIX₁₅X₁₆RLX₁₉ALGGSEAELAAFEKEIAAFESELQAYKGKGNPEVEX₅₅LRX₅₈X₅₉AAX₆₂IRX₆₅X₆₆LQAYRHN (SEQ ID NO:4),
- MGSWX₅X₆FKX₉X₁₀LAX₁₃IKX₁₆X₁₇LEALGGSEAELAAFX₃₂X₃₃EIX₃₆AFX₃₉X₄₀ELX₄₃AYKGKGNPEVEX₅₅LRX₅₈X₅₉AAX₆₂IRX₆₅X₆₆LQAYRHN (SEQ ID NO:5), and
- MGSWX₅EFX₈X₉RLX₁₂AIX₁₅X₁₆RLX₁₉ALGGSEAELAX₃₀FEX₃₃X₃₄IAX₃₇FEX₄₀X₄₁LQX₄₄YKGKGNPEVEALX₅₇X₅₈EAX₆₁AIX₆₄X₆₅ELX₆₈AYRHN (SEQ ID NO:6), wherein X_n is a

natural or non-natural amino acid, wherein X_n is not a cysteine or a proline residue, wherein the target-binding polypeptide specifically binds a target of interest expressed by a cancer cell, and wherein the specific binding of the target-binding polypeptide to the target of interest is greater than binding of a polypeptide according to SEQ ID NO:1 to the target of interest,

- (b) a transmembrane domain selected from 41BB and CD28, and
- (c) an intracellular domain, wherein the intracellular domain comprises a signaling domain selected from an alpha, beta, or zeta chain of the T cell receptor.

23. The use according to claim 21 or 22, wherein the immune cell is a T cell or a natural killer (NK) cell.

24. A method for transforming a reference polypeptide into a target-binding polypeptide capable of specifically binding to a target of interest, the method comprising:

- (a) modifying a plurality of amino acid residues from the reference polypeptide to generate a plurality of candidate target-binding polypeptides; wherein the candidate target-binding polypeptides comprise a variant of the amino acid sequence of SEQ ID NO: 1, wherein the candidate target-binding polypeptides comprise three anti-parallel alpha helices joined by linker peptides, wherein the amino acid residues modified are solvent accessible or solvent inaccessible, and wherein the modification comprises one or more conservative or non-conservative amino acid substitutions and does not include a substitution with a cysteine or a proline;
- (b) packaging the plurality of candidate target-binding polypeptides in a plurality of vectors to generate a candidate library; and
- (c) screening the candidate library for candidate target-binding polypeptides that exhibit specific binding to the target of interest,

wherein the candidate target-binding polypeptides comprise an amino acid sequence selected from the group consisting of

MGSWX₅X₆FKX₉X₁₀LAX₁₃IKX₁₆X₁₇LEALGGSEAELAX₃₀FEX₃₃X₃₄IAX₃₇FEX₄₀X₄₁LQ

X_{44} YKGGKNPEVEALRKEAAAIRDELQAYRHN (SEQ ID NO:2),
 MGSWAEFKQRLAAIKTRLEALGGSEAELAAFX₃₂X₃₃EIX₃₆AFX₃₉X₄₀ELX₄₃AYKGGKNPE
 VEALX₅₇X₅₈EAX₆₁AIX₆₄X₆₅ELX₆₈AYRHN (SEQ ID NO:3),
 MGSWX₅EFX₈X₉RLX₁₂AIX₁₅X₁₆RLX₁₉ALGGSEAELAAFEKEIAAFESELQAYKGGKNPEV
 EX₅₅LRX₅₈X₅₉AAX₆₂IRX₆₅X₆₆LQAYRHN (SEQ ID NO:4),
 MGSWX₅X₆FKX₉X₁₀LAX₁₃IKX₁₆X₁₇LEALGGSEAELAAFX₃₂X₃₃EIX₃₆AFX₃₉X₄₀ELX₄₃AYK
 GGGKNPEVEX₅₅LRX₅₈X₅₉AAX₆₂IRX₆₅X₆₆LQAYRHN (SEQ ID NO:5),
 MGSWX₅EFX₈X₉RLX₁₂AIX₁₅X₁₆RLX₁₉ALGGSEAELAX₃₀FEX₃₃X₃₄IAX₃₇FEX₄₀X₄₁LQ
 X_{44} YKGGKNPEVEALX₅₇X₅₈EAX₆₁AIX₆₄X₆₅ELX₆₈AYRHN (SEQ ID NO:6),
 MGSWX₅X₆FKX₉X₁₀LAX₁₃IKX₁₆X₁₇LEALZ₁EAELAX₂₈FEX₃₁X₃₂IAX₃₅FEX₃₈X₃₉LQX₄₂YZ₂
 NPEVEALRKEAAAIRDELQAYRHN (SEQ ID NO:7),
 MGSWAEFKQRLAAIKTRLEALZ₁EAELAAFX₃₀X₃₁EIX₃₄AFX₃₇X₃₈ELX₄₁AYZ₂NPEVEALX₅₂
 X₅₃EAX₅₆AIX₅₉X₆₀ELX₆₃AYRHN (SEQ ID NO:8),
 MGSWX₅EFX₈X₉RLX₁₂AIX₁₅X₁₆RLX₁₉ALZ₁EAELAAFEKEIAAFESELQAYZ₂NPEVE
 X₅₀LRX₅₃X₅₄AAX₅₇IRX₆₀X₆₁LQAYRHN (SEQ ID NO:9),
 MGSWX₅X₆FKX₉X₁₀LAX₁₃IKX₁₆X₁₇LEALZ₁EAELAAFX₃₀X₃₁EIX₃₄AFX₃₇X₃₈ELX₄₁AYZ₂NP
 EVEX₅₀LRX₅₃X₅₄AAX₅₇IRX₆₀X₆₁LQAYRHN (SEQ ID NO:10) and
 MGSWX₅EFX₈X₉RLX₁₂AIX₁₅X₁₆RLX₁₉ALZ₁EAELAX₂₈FEX₃₁X₃₂IAX₃₅FEX₃₈X₃₉LQX₄₂YZ₂N
 PEVEALX₅₂X₅₃EAX₅₆AIX₅₉X₆₀ELX₆₃AYRHN (SEQ ID NO:11), wherein X_n is a natural or
 non-natural amino acid and Z₁ and Z₂ comprise between about 2 to about 30 natural or non-
 natural amino acids.

25. The method of claim 24, wherein the method further comprises identifying potentially immunogenic amino acid residues in a candidate target-binding polypeptide and modifying at least one of the potentially immunogenic amino acid residues in the candidate target-binding polypeptide, wherein the modification comprises an amino acid substitution.
26. Virus-like particles (VLPs) comprising a fusion polypeptide comprising the DBDpp of any one of claims 5 to 7, wherein the VLPs are suitable for use as immunogens for antibody generation, wherein said antibodies are directed against the DBDpp.