MINIPROTEINS, CONJUGATES & USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of and priority to U.S. Provisional Patent Application Nos. 63/587,042, filed on September 29, 2023; 63/598,874, filed on November 14, 2023; 63/618,228, filed on January 5, 2024; and 63/636,078, filed on April 18, 2024, the disclosures of each of which are incorporated by reference herein in their entireties for all purposes.

SEQUENCE LISTING

[0002] This application contains a Sequence Listing that has been submitted electronically in XML format and is hereby incorporated by reference in its entirety. The XML file, created on September 26, 2024, is named AKT-031WO_SL.xml, and is 488,657 bytes in size.

BACKGROUND

[0003] Cancer is a leading cause of death worldwide. Classical cancer therapies such as radiotherapy, chemotherapy, and surgical procedures can be accompanied by severe side effects including those due to killing of healthy non-cancerous cells. Newer therapeutics enhance the targeting of cytotoxic drugs to tumor cells, relative to earlier therapies, including those that use biologics conjugates.

SUMMARY

[0004] The present disclosure describes technologies such as compositions and methods of use and manufacture thereof to address needs in the field of cancer. For example, in contrast to classical cancer diagnostics or therapies, targeted molecules can be designed to increase specificity and decrease toxicity of, *e.g.*, imaging or therapeutic modalities. For instance, delivery of a therapeutic having a chelator and/or radionuclide (*e.g.*, alpha emitter) using a polypeptide to specifically target the therapeutic to the tumor microenvironment describes focused treatment to tumor cells and avoids or reduces risk of toxicity to surrounding healthy tissues.

[0005] In contrast to classical cancer therapies, radionuclide therapies are more targeted and less toxic. For instance, delivery of a radionuclide specifically to a tumor microenvironment allows for selective radiation of tumor tissue, effectively killing malignant cells while preserving the surrounding healthy tissue. For example, a radionuclide can

employ a targeting molecule that specifically binds to an antigen expressed at an increased level and/or density on the surface of tumor cells relative to non-tumor cells. Binding of the radionuclide to the antigen-positive tumor cells targets radiation to those cells without targeting healthy tissue. Full-length antibodies have previously been evaluated as targeting moieties; however, due to considerations such as their large size, full-length antibodies, or even antibody fragments (*e.g.,* fragments larger than polypeptides provided herein) can have several challenges such as having poor tumor tissue penetration, longer circulating half-life which leads to normal tissue irradiation, as well as challenges such as manufacturing, and storage, among other things. Accordingly, a need remains for new approaches to specifically target tumors, and particularly solid tumors. The present disclosure describes technologies that meet this and other needs. Among other things, the present disclosure describes compositions comprising polypeptides and conjugates thereof with improved tumor penetration, decreased off-target toxicity and/or accumulation, and improved affinity for Nectin-4, as compared to currently existing technologies (*e.g.,* antibodies, *e.g.,* antibody-drug conjugates, polypeptides, *etc.*). In addition, the disclosure describes the insight that compositions and conjugates provided herein can be even further improved. Contemplated herein, in some examples, conjugates, such as radionuclide conjugates of the disclosure may be improved, such as improvement on one or more measures, such as efficacy and/or reduction in toxicity grade and/or off-target effects, by modification of one or more amino acids in a polypeptide sequence of the conjugate and/or addition of one or more decoys.

[0006] In some examples, the present disclosure describes polypeptides comprising certain amino acid sequences. In some examples, these polypeptides bind to Nectin-4 with certain affinities.

[0007] In one example, the disclosure describes a composition, comprising a polypeptide of at least 44 amino acids in length and having an amino sequence comprising that set forth in SEQ ID NO: 171, wherein X2 is E or D; X6 is E or Q; X17 is G or A; X21 is Q, Y, or E; X26 is Kme3, Kme2, Kme, K, Kipr, or S; X32 is A, G, or D; X41 is N or K; and X45 is S or absent.

[0008] In one example, the disclosure describes a composition, comprising a polypeptide of at least 44 amino acids in length and having an amino acid sequence comprising that set forth in SEQ ID NO: 176, wherein X2 is E or D; X6 is E or Q; X9 is T or A; X10 is A or G; X12 is A, Kme3, Kme2, Kme, Kipr or K; X13 is R or (Cit) X17 is G or A; X21 is Q, Y, or E; X24 is Q or K; X25 is A or K; X26 is Kme3, Kme2, Kme, K, Kipr, or S; X28 is Q or K; X29 is Y or K; X30 is L or V; X32 is A, G, or D; X41 is N or K; and X45 is S or absent.

[0009] In another example, the disclosure describes a composition, comprising a Nectin-4 binding polypeptide having an amino acid sequence comprising at least 44 amino acids, wherein the amino acids include (i) a cysteine at each of four positions corresponding to 1, 20, 34, and 44 of SEQ ID NO: 195; (ii) SEQ ID NO: 169 at positions corresponding to positions 9-15 of SEQ ID NO: 195; (iii) QKKme3 at positions corresponding to positions 24, 25, and 26 of SEQ ID NO: 195; and (iv) QYL at positions corresponding to positions 28, 29, and 30 of SEQ ID NO: 195.

[0010] In one example, the disclosure describes a composition, comprising a Nectin-4 binding polypeptide having an amino acid sequence comprising at least 44 amino acids, wherein the amino acids include (i) a cysteine at each of four positions corresponding to 1, 20, 34, and 44 of SEQ ID NO: 200; (ii) SEQ ID NO: 247 at positions corresponding to positions 9-15 of SEQ ID NO: 200; (iii) QKKme3 at positions corresponding to positions 24, 25, and 26 of SEQ ID NO: 200; and (iv) QYL at positions corresponding to positions 28, 29, and 30 of SEQ ID NO: 200.

[0011] In another example, the disclosure describes a composition, comprising a Nectin-4 binding polypeptide having an amino acid sequence, wherein the amino acid sequence comprises: at least four cysteines, which form two disulfide bonds; at least one modified lysine residue at a position corresponding to X12 and/or X26 of SEQ ID NO: 195, wherein the modification comprises at least one small alkyl group attached to the nitrogen of the lysine side chain, optionally comprising a methyl, dimethyl, trimethyl, or isopropyl group; at least 44 amino acids in length; and has a binding affinity for Nectin-4 stronger than 100 nM in a cell-based assay.

[0011a] In one aspect, the disclosure describes a composition, comprising: (a) a polypeptide of at least 44 amino acids in length and having an amino acid sequence comprising that set forth in SEQ ID NO: 176, wherein X2 is E or D; X6 is E or Q; X9 is T or A; X10 is A or G; X12 is A, Kme3, Kme2, Kme, Kipr or K; X13 is R or (Cit) X17 is G or A; X21 is Q, Y, or E; X24 is Q or K; X25 is A or K; X26 is Kme3, Kme2, Kme, K, Kipr, or S; X28 is Q or K; X29 is Y or K; X30 is L or V; X32 is A, G, or D; X41 is N or K; and X45 is S or absent; (b) a polypeptide of at least 44 amino acids in length and having an amino sequence comprising SEQ ID NO: 171, wherein X2 is E or D; X6 is E or Q; X17 is G or A; X21 is Q, Y, or E; X26 is Kme3, Kme2, Kme, K, Kipr, or S; X32 is A, G, or D; X41 is N or K; and X45 is S or absent; (c) a Nectin-4 binding polypeptide having an amino acid sequence comprising at least 44 amino acids comprising a cysteine at each of four positions

corresponding to 1, 20, 34, and 44 of SEQ ID NO: 195; (ii) SEQ ID NO: 169 at positions corresponding to positions 9-15 of SEQ ID NO: 195; (iii) QKKme3 at positions corresponding to positions 24, 25, and 26 of SEQ ID NO: 195; and (iv) QYL at positions corresponding to positions 28, 29, and 30 of SEQ ID NO: 195; (d) a Nectin-4 binding polypeptide having an amino acid sequence comprising at least 44 amino acids comprising a cysteine at each of four positions corresponding to 1, 20, 34, and 44 of SEQ ID NO: 200; (ii) SEQ ID NO: 247 at positions corresponding to positions 9-15 of SEQ ID NO: 200; (iii) QKKme3 at positions corresponding to positions 24, 25, and 26 of SEQ ID NO: 200; and (iv) QYL at positions corresponding to positions 28, 29, and 30 of SEQ ID NO: 200; or (e) a Nectin-4 binding polypeptide having an amino acid sequence comprising: (i) at least four cysteines, which form two disulfide bonds; (ii) at least one modified lysine residue at a position corresponding to X12 and/or X26 of SEQ ID NO: 195, wherein the modification comprises at least one small alkyl group attached to the nitrogen of the lysine side chain, optionally comprising a methyl, dimethyl, trimethyl, or isopropyl group; (iii) at least 44 amino acids in length; and (iv) has a binding affinity for Nectin-4 stronger than 100 nM in a cell-based assay.

[0012] In some examples, the polypeptide is at least 40 amino acids in length, but no greater than 100 amino acids in length.

[0013] In some examples, the polypeptide binds to Nectin-4 with an affinity of stronger than 10 nM in a cell-based assay.

[0014] In some examples, the amino acid sequence of the polypeptide shares at least 90% identity to any one of SEQ ID NOs: 3-158, 161-168, 177-208, or 212-215, but includes at least one lysine with at least one modification comprising at least one small alkyl group bonded to the nitrogen of the side chain, optionally selected from: trimethyl, dimethyl, monomethyl, and isopropyl.

[0015] In some examples, the amino acid sequence of the polypeptide shares at least 90% identity to at least 44 amino acids of a reference polypeptide, which reference polypeptide is longer than 44 amino acids in length and binds to Nectin-4 with a strength of

at least 10 nM on a cell-based assay, and/or has an inhibition constant of no greater than 10 nM.

[0016] In some examples, the amino acid sequence of the polypeptide shares at least 90% identity to at least 40 amino acids of any one of SEQ ID NOs: 3-158, 161-168, 177-208, or 212-215, provided that the 40 amino acids includes at least four cysteine residues that form two disulfide bridges.

[0017] In some examples, the amino acid sequence of the polypeptide shares at least 90% identity to at least 35 contiguous amino acids of any one of SEQ ID NOs: 3-158, 161-168, 177-208, or 212-215, provided that the 40 amino acids includes at least four cysteine residues that form two disulfide bridges.

[0018] In some examples, the amino acid sequence of the polypeptide shares 100% identity to at least 44 amino acids of a reference polypeptide, which reference polypeptide is longer than 44 amino acids in length.

[0019] In certain examples, the amino acid sequence shares 90% identity to at least 44 amino acids as set forth in any one of SEQ ID NO: 78, 83, 85, 99, 103, 162-168, 195, or 200.

[0020] In certain examples, the amino acid sequence shares 100% identity to at least 44 amino acids as set forth in any one of SEQ ID NO: 78, 83, 85, 99, 103, 162-168, 195, or 200.

[0021] In one aspect, the disclosure describes a composition comprising a polypeptide having an amino acid sequence comprising SEQ ID NO: 195.

[0021a] In another aspect, the disclosure describes a composition comprising a Nectin-4-binding miniprotein having an amino acid sequence comprising at least 44 amino acids, wherein the at least 44 amino acids comprise (i) a cysteine at each of four positions corresponding to 1, 20, 34, and 44 of SEQ ID NO: 195; (ii) TALX12RLR (SEQ ID NO: 169) at positions corresponding to positions 9-15 of SEQ ID NO: 195, wherein X12 is selected from A, Kme, Kme2, Kme3, or Kipr; (iii) QAX26, wherein X26 is selected from Kme3, Kme2, Kme, or Kipr at positions corresponding to positions 24, 25, and 26 of SEQ ID NO: 195; and (iv) QYL at positions corresponding to positions 28, 29, and 30 of SEQ ID NO: 195.

[0022] In another example, the disclosure describes a composition comprising a compound as set forth in C251 of Table 2A, having an amino acid sequence comprising SEQ ID NO: 195.

[0023] In one example, the disclosure describes a composition comprising a polypeptide having an amino acid sequence comprising SEQ ID NO: 200.

[0024] In one example, the disclosure describes a composition comprising a compound as set forth in C260 of Table 2A, having an amino acid sequence comprising SEQ ID NO: 200.

[0025] In some examples, the composition further comprises a radionuclide.

[0026] In some examples, the radionuclide is Ac-225, Cu-64, Ga-68, Lu-177, Pb-212, In-111, Cu-67, La-132, La-135, Ce-134, F-18, I-131, I-124, Pb-203, Th-232, Bi-123, Sm-153, Ra-225, Tb-165, or At-211.

[0027] In one example, the disclosure describes a composition comprising a polypeptide having an amino acid sequence of at least 44 amino acids in length, but with four amino acid

substitutions at positions corresponding to 12, 21, 26, and 32 of SEQ ID NO: 78, wherein the substitutions correspond to K12A, Y21Q, S26Kme3, and G32A.

[0028] In some examples, the C-terminus has an -OH or an -NH2.

[0029] In certain examples, the binding affinity for Nectin-4 is stronger than 100 nM.

[0030] In certain examples, the inhibition constant is no greater than 100 nM.

[0031] In some examples, the composition further comprises one or more of a linker, chelator, and radionuclide.

[0032] In some examples, the linker comprises or consists of a polyethylene glycol (PEG) linker of PEG4, PEG2, PEG, PEG6, PEG8, PEG12, PEG24, PEG36, lys(MPB)-PEG4, an ester linker, an amide linker, a maleimide linker, a succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC) linker, a propanoic acid linker, a dTyr-Gly-Phe (yGF) linker, a caproleic acid linker, or (Gly)n-(gGlu)n- or (PEG)n, wherein n is from 1 to 10, (Gly)1-10, or any fragment or combination via covalent bond thereof.

[0033] In some examples, the chelator comprises or consists of DOTA, Crown, NOPO, Macropa, lead specific chelator (PSC), N-succinimidyl 3-(tri-n-butylstannyl)benzoate (BuSTB), or N-succinimidyl 3-trimethylstannylbenzoate (MeSTB).

[0034] In some examples, the radionuclide is selected from Ac-225, Cu-64, Ga-68, Lu-177, Pb-212, In-111, Cu-67, La-132, La-135, Ce-134, F-18, I-131, I-124, Pb-203, Th-232, Bi-123, Sm-153, Ra-225, Tb-165, or At-211.

[0035] In some examples, if the polypeptide comprises any one of SEQ ID NO: 83, 85, 93, 99, 134, 138, 145, 155, 162-168, or 195 the polypeptide further comprises a linker, wherein the linker is PEG4, and an optional chelator, wherein the chelator is DOTA.

[0036] In certain examples, when present, the linker is attached to the N-terminus of the polypeptide. In certain examples, the C-terminal amino acid of the polypeptide is not a cysteine. In some examples, when present, the chelator is attached to either the polypeptide or the linker. In some examples, when present, the radionuclide is attached to the chelator.

[0037] In one example, the disclosure describes a composition comprising a formula selected from one or more of (M)x-L-C-R, (M)x-L-C, (M)x-C-R, (M)x-L-R, (M)x-C, (M)x-L, and (M)x-R, wherein M comprises a polypeptide (M), L comprises a linker (L), C comprises a chelator (C), R comprises a radionuclide (R), and x is 1, 2, 3, or 4, wherein M comprises an amino acid sequence of any one of SEQ ID NO: 162-176, 178-208, or 212-215.

[0038] In certain examples, the linker comprises or consists of a polyethylene glycol (PEG) linker of PEG4, PEG, PEG2, PEG6, PEG8, PEG12, PEG24, PEG36, lys(MPB)-

PEG4, an ester linker, an amide linker, a maleimide linker, a succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC) linker, a propanoic acid linker, a dTyr-Gly-Phe (yGF) linker, a caproleic acid linker, or (Gly)n-(gGlu)n- or (PEG)n, wherein n is from 1 to 10, (Gly)1-10, or any fragment or combination via covalent bond thereof.

[0039] In some examples, the chelator comprises or consists of DOTA, Crown, NOPO, Macropa, lead specific chelator (PSC), N-succinimidyl 3-(tri-n-butylstannyl)benzoate (BuSTB), or N-succinimidyl 3-trimethylstannylbenzoate (MeSTB).

[0040] In some examples, the radionuclide Ac-225, Cu-64, Ga-68, Lu-177, Pb-212, In-111, Cu-67, La-132, La-135, Ce-134, F-18, I-131, I-124, Pb-203, Th-232, Bi-123, Sm-153, Ra-225, Tb-165, or At-211.

[0041] In one example, the disclosure describes a composition comprising a formula selected from one or more of (M)x-L-C-R, (M)x-L-C, (M)x-C-R, (M)x-L-R, (M)x-C, (M)x-L, and (M)x-R, wherein M comprises a polypeptide (M), L comprises a linker (L), C comprises a chelator (C), R comprises a radionuclide (R), and x is 1, 2, 3, or 4, wherein M has an amino acid sequence comprising any one of those set forth in SEQ ID NOs: 162-176, 178-208, or 212-215.

[0041a] In one aspect, the disclosure describes a composition comprising a formula selected from one or more of (M)x-L-C-R, (M)x-L-C, (M)x-C-R, (M)x-L-R, (M)x-C, (M)x-L, and (M)x-R, wherein M comprises a polypeptide (M), L comprises a linker (L), C comprises a chelator (C), R comprises a radionuclide (R), and x is 1, 2, 3, or 4, wherein M has an amino acid sequence comprising any one of those set forth in SEQ ID NOs: 195, 3, 4, 6-47, 49-158, 161-168, 170-194, 196-208, 212-237, 243-246 or 248.

[0042] In some examples, when L is present, L comprises or consists of a polyethylene glycol (PEG) linker of PEG4, PEG, PEG2, PEG6, PEG8, PEG12, PEG24, lys(MPB)-PEG4, PEG36, an ester linker, an amide linker, a maleimide linker a valine-citrulline linker, a hydrazone linker, a N-succinimidyl-4-(2-pyridyldithio)butyrate (SPDB) linker, a succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SMCC) linker, a vinylsulfone-based linker, a propanoic acid linker, a dTyr-Gly-Phe (yGF) linker, a caproleic acid linker, or (Gly)n-(gGlu)n- or (PEG)n, wherein n is from 1 to 10, (Gly)1-10, or any fragment or combination via covalent bond thereof.

[0043] In some examples, when C is present, C comprises or consists of DOTA, Crown, NOPO, Macropa, lead-specific chelator (PSC), N-succinimidyl 3-(tri-n-butylstannyl)benzoate (BuSTB), or N-succinimidyl 3-trimethylstannylbenzoate (MeSTB).

[0044] In some examples, when R is present, R comprises or consists of Ac-225, Cu-64, Ga-68, Lu-177, Pb-212, In-111, Cu-67, La-132, La-135, Ce-134, F-18, I-131, I-124, Pb-203, Th-232, Bi-123, Sm-153, Ra-225, Tb-165, or At-211.

[0045] In certain examples, when present, the linker is attached to the N-terminus of the polypeptide. In certain examples, the C-terminal amino acid of the polypeptide is not a cysteine. In some examples, when present, the chelator is attached to either the

polypeptide or the linker. In some examples, when present, the radionuclide is attached to the chelator.

[0046] In some examples, the polypeptide comprises at least one disulfide bridge.

[0047] In some examples, the polypeptide comprises at least two disulfide bridges.

[0048] In some examples, the composition and/or polypeptide thereof selectively binds to Nectin-4 or a portion thereof.

[0049] In certain examples, the polypeptide has a binding affinity for Nectin-4 or a portion thereof of 10 pM to 200 nM, 10 pM to 100 nM, or 10 nM to 100 nM, *in vivo*, *ex vivo*, or *in vitro* and/or as measured in a cell-based assay.

[0050] In some examples, the polypeptide has a binding inhibition constant of no greater than 100 nM.

[0051] In one example, the disclosure describes a composition comprising a polypeptide-drug conjugate, comprising a polypeptide and at least one drug moiety, wherein the polypeptide comprises an amino acid sequence having at least 90% identity to at least 44 amino acids a polypeptide having an amino acid sequence set forth in any one of SEQ ID NOs: 3-158, 162-208, or 212-237.

[0051a] In one aspect, the disclosure describes a composition comprising a polypeptide-drug conjugate, comprising a polypeptide and at least one drug moiety, wherein the polypeptide comprises an amino acid sequence having at least 90% identity to at least 44 amino acids of an amino acid sequence set forth in any one of SEQ ID NOs: 3, 4, 6-47, 49-158, 161-168, 170-208, 212-237, 243-246 and 248.

[0052] In certain examples, the drug moiety is selected from a V-ATPase inhibitor, a pro-apoptotic agent, a Bcl2 inhibitor, an MCL1 inhibitor, a HSP90 inhibitor, an IAP inhibitor, an mTor inhibitor, a microtubule stabilizer, a microtubule destabilizer, an auristatin, a dolastatin, a maytansinoid, a MetAP (methionine aminopeptidase), an inhibitor of nuclear export of proteins CRMl, a DPPIV inhibitor, proteasome inhibitors, inhibitors of phosphoryl transfer reactions in mitochondria, a protein synthesis inhibitor, a kinase inhibitor, a CDK2 inhibitor, a CDK9 inhibitor, a kinesin inhibitor, an HDAC inhibitor, a DNA damaging agent, a DNA alkylating agent, a DNA intercalator, a DNA minor groove binder, a DHFR inhibitor, a topoisomerase inhibitor, an auristatin (*e.g.*, monomethyl auristatin E), and an immunotoxin.

[0053] In one example, the disclosure describes a composition comprising an isolated compound or pharmaceutically acceptable salt thereof comprising an optional linker (L), and one or more of a polypeptide (M), chelator (C) or radionuclide (R), wherein M has an amino acid sequence comprising any one of SEQ ID NOs: 3-158, 161-168, 171-208, 212-215, or 216-237, including amino acid substitutions as set forth in Table 1C, Table 1D, Table 2C, Table 2D, Table 2E, Table 2F, or Table 2G.

[0053a] In one aspect, the disclosure describes a composition comprising: (a) an isolated compound or pharmaceutically acceptable salt thereof comprising an optional linker (L), and one or more of a polypeptide (M), chelator (C) or radionuclide (R), wherein M has an amino acid sequence comprising any one of SEQ ID NOs: 3, 4, 6-47, 49-158, 161-168, 170-208, 212-237, 243-246 and 248, including amino acid substitutions as set forth in Table 1C, Table 1D, Table 2C, Table 2D, Table 2E, Table 2F, or Table 2G; (b) a compound designed to bind to Nectin-4, which compound comprises or consists of a polypeptide having an amino acid sequence comprising any one of SEQ ID NOs: 3, 4, 6-47, 49-158, 161-168, 170-208, 212-237, 243-246 and 248, including amino acid substitutions as set forth in Table 1C, Table 1D, Table 2C, Table 2D, Table 2E, Table 2F, or Table 2G, and further comprises a modified N and/or C-terminus; or (c) a compound comprising a miniprotein comprising an amino acid sequence having at least 90% identity to SEQ ID NO: 195, and further comprising one or more additional components according to a formula M-L-C-R, wherein L is a linker, C is a chelator, and R is a radionuclide.

[0054] In another example, the disclosure describes a composition comprising, a compound designed to bind to Nectin-4, which compound comprises or consists of a polypeptide having

an amino acid sequence comprising any one of SEQ ID NOs: 3-158, 161-168, 171-208, 212-215, or 216-237, including amino acid substitutions as set forth in Table 1C, Table 1D, Table 2C, Table 2D, Table 2E, Table 2F, or Table 2G, and further comprises a modified N and/or C-terminus.

[0055] In certain examples, the modified N-terminus comprises one or more of an NH2-, Acetyl-, PEGn-, wherein n=0-10, DOTA-, or Biotin-. In some examples, the C terminus comprises an -NH2 or an -OH. In some examples, the polypeptide selectively binds to Nectin-4 or a portion thereof. In some examples, the polypeptide has a binding affinity of stronger than about 100 nM to Nectin-4, or a portion thereof, *in vivo* or in a cell-based assay.

[0056] In one example, the disclosure describes a compound comprising a miniprotein having an amino acid sequence with 90% identity to SEQ ID NO: 195, and further comprising one or more additional components according to a formula M-L-C-R, wherein L is a linker, C is a chelator, and R is a radionuclide.

[0056a] In another aspect, the disclosure describes a conjugate comprising: a polypeptide (M) that specifically binds to Nectin-4; a chelator (C) conjugated to (M) through an optional linker (L), wherein (C) comprises DOTA, and (L), when present, comprises PEG, wherein the PEG is optionally PEG-4; and (iii) a radionuclide (R) chelated to (C), wherein (R) is Actinium-225, Copper-64, Gallium-68, Indium-111, Lead-212, or Lutetium-177.

[0056b] In yet another aspect, the disclosure describes a conjugate comprising a polypeptide (M) that specifically binds to Nectin-4 and a chelator (C) conjugated to the N-terminus of (M) through a linker (L), wherein the polypeptide has an amino acid sequence comprising: (i) at least 44 amino acids, wherein the amino acids comprise (a) a cysteine at each of four positions corresponding to 1, 20, 34, and 44 of SEQ ID NO: 195; (b) TALX12RLR at positions corresponding to positions 9-15 of SEQ ID NO: 195, wherein X12 is A, Kme, Kme2, Kme3, or Kipr; (c) QAX26 at positions corresponding to positions 24, 25, and 26 of SEQ ID NO: 195, wherein X26 is Kme3, Kme2, Kme, or Kipr; and (d) QYL at positions corresponding to positions 28, 29, and 30 of SEQ ID NO: 195; or (ii) SEQ ID NO: 195.

[0057] In some examples, L comprises or consists of a polyethylene glycol (PEG) linker of PEG4, PEG, PEG2, PEG6, PEG8, PEG12, PEG24, PEG36, lys(MPB)-PEG4, an ester linker, an amide linker, a maleimide linker, a succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC) linker, a propanoic acid linker, a

dTyr-Gly-Phe (yGF) linker, a caproleic acid linker, any linker set forth in Table 2A, or (Gly)n-(gGlu)n- or (PEG)n, wherein n is from 1 to 10, (Gly)1-10, or any fragment or combination via covalent bond thereof. In some examples, C comprises or consists of DOTA, Crown, NOPO, Macropa, lead specific chelator (PSC), N-succinimidyl 3-(tri-n-butylstannyl)benzoate (BuSTB), or N-succinimidyl 3-trimethylstannylbenzoate (MeSTB). In some examples, R comprises or consists of Ac-225, Cu-64, Ga-68, Lu-177, Pb-212, In-111, Cu-67, La-132, La-135, Ce-134, F-18, I-131, I-124, Pb-203, Th-232, Bi-123, Sm-153, Ra-225, Tb-165, or At-211.

[0058] In one example, the disclosure describes a compound comprising a miniprotein 90% identical to at least 40 amino acids of the amino acid sequence of SEQ ID NO: 195, wherein the N and/or C-terminus comprise between one and thirty additional amino acids, and/or wherein the C-terminus comprises one fewer amino acids or up to 30 additional amino acids, provided that the entire miniprotein is no greater than about 100 amino acids in length.

[0059] In one example, the disclosure describes a method of improving binding affinity strength of a polypeptide to Nectin-4, the improvement comprising modifying four amino

acid residues of a polypeptide, which polypeptide has at least 44 amino acids in length and has substitutions at positions corresponding to 12, 21, 26, and 32 of SEQ ID NO: 78, wherein the substitutions correspond to K12A, Y21Q, S26Kme3, and G32A.

[0060] In some examples, the disclosure describes a pharmaceutical composition comprising a polypeptide or compound as provided herein; and a pharmaceutically acceptable excipient.

[0060a] In one aspect, the disclosure describes a pharmaceutical composition comprising the composition as described herein, or the conjugate as described herein, and a pharmaceutically acceptable excipient

[0061] In one example, the disclosure describes a method of treating cancer, the method comprising administering to a subject in need thereof, a composition comprising a conjugate comprising a polypeptide having at least 90% identity to at least 40 amino acids of an amino acid sequence as set forth in any one of SEQ ID NOs: 3-158, 161-168, 171-208, 212-215, or 216-237 and a radionuclide.

[0061a] In one aspect, the disclosure describes use of the composition as described herein, or the conjugate as described herein, in the manufacture of a medicament for detecting presence of a Nectin-4 positive population of cancer cells or for the treatment of cancer.

[0062] In some examples, the radionuclide is associated with the polypeptide with a linker and/or chelator according to a formula M-L-C-R, wherein M is the polypeptide, L is a linker, C is a chelator, and R is the radionuclide. In some examples, the polypeptide has an amino acid sequence comprising or consisting of SEQ ID NO: 195 or SEQ ID NO: 200.

[0063] In some examples, L comprises or consists of a polyethylene glycol (PEG) linker of PEG4, PEG, PEG2, PEG6, PEG8, PEG12, PEG24, PEG36, lys(MPB)-PEG4, an ester linker, an amide linker, a maleimide linker, a succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC) linker, a propanoic acid linker, a dTyr-Gly-Phe (yGF) linker, a caproleic acid linker, any linker set forth in Table 2A, or (Gly)n-(gGlu)n- or (PEG)n, wherein n is from 1 to 10, (Gly)1-10, or any fragment or combination via covalent bond thereof. In some examples, C comprises or consists of DOTA, Crown, NOPO, Macropa, lead specific chelator (PSC), N-succinimidyl 3-(tri-n-butylstannyl)benzoate (BuSTB), or N-succinimidyl 3-trimethylstannylbenzoate (MeSTB). In some examples, R is Ac-225, Cu-64, Ga-68, Lu-177, Pb-212, In-

111, Cu-67, La-132, La-135, Ce-134, F-18, I-131, I-124, Pb-203, Th-232, Bi-123, Sm-153, Ra-225, Tb-165, or At-211.

[0064] In certain examples, R is a therapeutic agent and/or an imaging agent. In some such examples, R is Cu-64, Ga-68, Lu-177, In-111, Cu-67, La-132, or F-18.

[0065] In one example, the disclosure describes a method of reducing kidney cell uptake of a composition comprising administering to a subject a Nectin-4 binding protein having an amino acid sequence comprising at least one modified lysine residue at a position corresponding to X12 and/or X26 of SEQ ID NO: 195, wherein the modification comprises at least small alkyl group attached to the nitrogen of the lysine side chain, optionally comprising a monomethyl, dimethyl, trimethyl, or isopropyl group and the reduction is as

compared to administration to the subject or a control subject an otherwise identical composition but not comprising the modified lysine residue at the position corresponding to X12 and/or X26.

[0066] In another example, the disclosure describes a method of treating cancer, the improvement comprising administering a composition comprising a Nectin-4 binding protein having an amino acid sequence comprising at least one modified lysine residue at a position corresponding to X12 and/or X26 of SEQ ID NO: 195, wherein the modification comprises at least one carbon attached to the nitrogen of the lysine side chain, optionally comprising a methyl, dimethyl, trimethyl, or isopropyl group as compared to a composition not comprising a modified lysine residue at a position corresponding to X12 and/or X26.

[0067] In one example, the disclosure describes a method of treating a subject with refractory or recurrent cancer comprising administering a composition, compound, or pharmaceutical composition as provided herein, wherein the treatment treats the cancer.

[0068] In one example, the disclosure describes a method of improving biodistribution of a pharmaceutical composition for a Nectin-4 positive population of cancer cells in a subject having a Nectin-4-positive cancer, comprising contacting the population with a polypeptide that has a modified lysine at a position corresponding to X12 and/or X26 of SEQ ID NO: 195, wherein the lysine is modified by adding at least one small alkyl group to a lysine side chain and wherein the biodistribution is improved as compared to contacting the population without the modified lysine at a position corresponding to X12 and/or X26 of SEQ ID NO: 195.

[0069] In another example, the disclosure describes a method of diagnosing presence of a Nectin-4 positive population of cancer cells comprising:

[0070] contacting a population of cells with a composition, compound, or pharmaceutical composition as provided herein; detecting the presence of the composition, compound, or pharmaceutical composition of step (a) by measuring a signal; and comparing the detection in step (b) to a control signal; and diagnosing cancer if the composition, compound, or pharmaceutical composition of step (a) is detected above the control.

[0071] In some examples, the contacting is performed by administering to a subject in need thereof. In some examples, the administering is intravenous or subcutaneous. In some examples, the contacting is outside of the subject, optionally in vitro with a biopsy sample.

[0072] In one example, the disclosure describes a method of treating a cancer in a subject using an immunotherapy, the method comprising administering to the subject a composition comprising a composition, compound, or pharmaceutical composition as provided herein.

[0073] In another example, the disclosure describes a use of a composition, compound, or pharmaceutical composition as provided herein to treat cancer in a subject.

[0074] In one example, the disclosure describes a method of treating a subject in need thereof comprising administering to the subject in need thereof a composition, compound, or pharmaceutical composition as provided herein.

[0075] In some examples, the subject is diagnosed as having cancer. In some examples, a cancer cell from the subject expresses Nectin-4, or a portion thereof. In some examples, the expression of Nectin-4 is higher in the cancer cell than in a non-cancer cell, which expression can be measured by protein and/or nucleic acid levels.

[0076] In some examples, the composition, compound, or pharmaceutical composition is not taken up and/or retained in the kidney as compared to a compound that does not comprise a composition, compound, or pharmaceutical composition as provided herein. In some examples, the composition, compound, or pharmaceutical composition is internalized in a cell expressing human Nectin-4.

[0077] In some examples, the cancer is selected from breast cancer, ovarian cancer, melanoma, pancreatic cancer, peripheral neuroma, glioblastoma, adrenocortical carcinoma, AIDS-related lymphoma, anal cancer, urothelial cancer, bladder cancer, meningioma, glioma, astrocytoma, cervical cancer, chronic myeloproliferative disorders, colon cancer, endometrial cancer, ependymoma, esophageal cancer, Ewing's sarcoma, extracranial germ cell tumors, extrahepatic bile duct cancer, gallbladder cancer, gastric cancer, gastrointestinal carcinoid tumors, gestational trophoblastic tumors, hairy cell leukemia, Hodgkin lymphoma, non-Hodgkin lymphoma, hypopharyngeal cancer, islet cell carcinoma, Kaposi sarcoma, laryngeal cancer, leukemia, lip cancer, oral cavity cancer, liver cancer, male breast cancer, malignant mesothelioma, medulloblastoma, Merkel cell carcinoma, metastatic squamous neck cell carcinoma, multiple myeloma and other plasma cell neoplasms, mycosis fungoides and Sezary syndrome, myelodysplastic syndromes, nasopharyngeal cancer, neuroblastoma, non-small cell lung cancer, small cell lung cancer, head and neck cancer, skin cancer, oropharyngeal cancer, bone cancers, including osteosarcoma and malignant fibrous histiocytoma of bone, paranasal sinus cancer, parathyroid cancer, penile cancer, pheochromocytoma, pituitary tumors, prostate cancer, rectal cancer, renal cell cancer, retinoblastoma, rhabdomyosarcoma, salivary gland cancer, small intestine cancer, soft tissue

sarcoma, supratentorial primitive neuroectodermal tumors, pineoblastoma, testicular cancer, thymoma, thymic carcinoma, thyroid cancer, transitional cell cancer of the renal pelvis and ureter, urethral cancer, uterine sarcoma, vaginal cancer, vulvar cancer, and Wilms tumor and other childhood kidney tumors.

[0078] In some examples, the composition, compound, or pharmaceutical composition is administered intravenously or subcutaneously.

[0079] In one example, the disclosure describes a method of targeting cancer cells expressing Nectin-4, the method comprising: determining or having determined a level of expression of Nectin-4 in a population of cancer cells; administering to a subject in need thereof a composition comprising a composition, compound, or pharmaceutical composition as provided herein, wherein the polypeptide of the composition, compound, or pharmaceutical composition is designed to specifically bind to human Nectin-4 and (iii) wherein the composition, compound, or pharmaceutical composition is attached to the surface and/or internalized into one or more Nectin-4 expressing cancer cells.

[0080] In some examples, the subject is treated after the administering as compared to prior to the administering.

[0081] In one example, the disclosure describes a method of targeting a population of cancer cells expressing Nectin-4, the improvement comprising contacting the population with a composition, compound, or pharmaceutical composition as provided herein, wherein X12 and/or X26 comprise a lysine with at least one additional small alkyl group attached to the nitrogen in the side chain, wherein the composition is taken up less by kidney cells than in a composition comprising a polypeptide that does not have a small alkyl group attached to a nitrogen on the side chain of a lysine at positions X12 and/or X26, wherein, optionally, the small alkyl group is part of a monomethyl, dimethyl, trimethyl, or isopropyl group.

[0082] In one example, the disclosure describes a conjugate comprising: a polypeptide (M) that specifically binds to Nectin-4; a chelator (C) conjugated to (M) through an optional linker (L), wherein (C) comprises DOTA, and (L), when present, comprises PEG, wherein the PEG is optionally PEG-4; and (iii) a radionuclide (R) chelated to (C), wherein (R) is Actinium-225.

[0083] In one example, the disclosure describes a conjugate comprising: a polypeptide (M) that specifically binds to Nectin-4; a chelator (C) conjugated to (M) through an optional linker (L), wherein (C) comprises DOTA, and (L), when present, comprises PEG, wherein the PEG is optionally PEG-4; and (iii) a radionuclide (R) chelated to (C), wherein (R) is Copper-64.

[0084] In one example, the disclosure describes a conjugate comprising: a polypeptide (M) that specifically binds to Nectin-4; a chelator (C) conjugated to (M) through an optional linker (L), wherein (C) comprises DOTA, and (L), when present, comprises PEG, wherein the PEG is optionally PEG-4; and (iii) a radionuclide (R) chelated to (C), wherein (R) is Gallium-68.

[0085] In another example, the disclosure describes a conjugate comprising: a polypeptide (M) that specifically binds to Nectin-4; a chelator (C) conjugated to (M) through an optional linker (L), wherein (C) comprises DOTA, and (L), when present, comprises PEG, wherein the PEG is optionally PEG-4; and (iii) a radionuclide (R) chelated to (C), wherein (R) is Indium-111.

[0086] In one example, the disclosure describes a conjugate comprising: a polypeptide (M) that specifically binds to Nectin-4; a chelator (C) conjugated to (M) through an optional linker (L), wherein (C) comprises DOTA, and (L), when present, comprises PEG, wherein the PEG is optionally PEG-4; and (iii) a radionuclide (R) chelated to (C), wherein (R) is Lead-212.

[0087] In another example, the disclosure describes a conjugate comprising: a polypeptide (M) that specifically binds to Nectin-4; a chelator (C) conjugated to (M) through an optional linker (L), wherein (C) comprises DOTA, and (L), when present, comprises PEG, wherein the PEG is optionally PEG-4; and (iii) a radionuclide (R) chelated to (C), wherein (R) is Lutetium-177.

[0088] In one example, the disclosure describes a conjugate comprising: a miniprotein (M) that specifically binds to Nectin-4; an N-terminal modification, conjugated to (M) through an optional linker (L), wherein (L), when present, comprises PEG, wherein the PEG is optionally PEG-4; and the N-terminal modification comprises a biotin.

[0089] In some examples, M of the conjugate has an amino acid sequence comprising any one of the amino acid sequences set forth in SEQ ID NOs: 3-158, 161-168, 171-208, or 212-215. In some examples, the amino acid sequence has at least 90% identity to that of at least 40 amino acids of SEQ ID NO: 176, wherein X2 is E or D; X6 is E or Q; X9 is T or A; X10 is A or G; X12 is A, Kme3, Kme2, Kme, Kipr or K; X13 is R or (Cit) X17 is G or A; X21 is Q, Y, or E; X24 is Q or K; X25 is A or K; X26 is Kme3, Kme2, Kme, K, Kipr, or S; X28 is Q or K; X29 is Y or K; X30 is L or V; X32 is A, G, or D; X41 is N or K; and X45 is S or absent.

[0090] In certain examples, M has an amino acid sequence comprising or consisting of SEQ ID NO: 195.

[0091] In certain examples, M has an amino acid sequence comprising or consisting of SEQ ID NO: 200.

[0092] In one example, the disclosure describes an isolated polynucleotide comprising one or more nucleic acid sequences encoding a polypeptide selected from any one of SEQ ID NOs: 3-158, 161-208, and 212-215; or a nucleic acid sequence encoding a polypeptide comprising at least 90%, 95%, 96%, 97%, 98%, 99% or greater identity to any one of SEQ ID NOs: 3-158, 161-168, 171-208, or 212-215.

[0093] In some examples, the disclosure describes a vector comprising an isolated polynucleotide provided herein. In some examples, the disclosure describes a host cell transformed with an isolated polynucleotide provided herein or a vector provided herein.

[0094] In one example, the disclosure describes a method of evaluating locations of one or more populations of cancerous cells in a subject, the method comprising administering to the subject a composition, compound, or pharmaceutical composition as provided herein and detecting to determine location of the composition in the subject.

[0095] In one example, the disclosure describes a method of decreasing kidney uptake of a composition administered to detect and/or treat one or more populations of cancer cells, the improvement comprising administering to a subject in need thereof a composition, compound, or pharmaceutical composition as provided herein, wherein X12 and/or X26 comprise a lysine with at least one additional small alkyl group attached to the nitrogen in the side chain, wherein the composition is taken up less by kidney cells than in a composition comprising a polypeptide that does not have a small alkyl group attached to a nitrogen on the side chain of a lysine at positions X12 and/or X26, wherein, optionally, the small alkyl group is part of a monomethyl, dimethyl, trimethyl, or isopropyl group.

[0096] In some examples, the detecting comprises an imaging procedure allows for selecting subjects, monitoring subjects, and/or treating subjects with a therapeutic comprising a miniprotein designed to bind to Nectin-4 expressed on one or more cancer cells in the one or more populations of cancer cells. In some examples, the therapeutic comprises a composition, compound, pharmaceutical composition, or conjugate as provided herein.

[0097] In one example, the disclosure describes a method of improving delivery of a radionuclide to a population of cancer cells in a subject, the method comprising administering a composition, compound, pharmaceutical composition, or conjugate as provided herein, wherein the amino acid sequences of the polypeptide comprise amino acids corresponding to positions X12 and/or X26 of SEQ ID NO: 195, and wherein X12 and/or X26 comprise a lysine with at least one additional small alkyl group attached to the nitrogen

in the side chain, wherein uptake by kidney cells is less than with a polypeptide having an amino acid sequence that does not comprise an additional small alkyl group attached to a nitrogen on the side chain of a lysine at positions X12 and/or X26.

[0098] In some examples, the small alkyl group comprises a monomethyl, dimethyl, trimethyl, or isopropyl group.

[0099] In one example, the disclosure describes a method of treating an individual with cancer, the improvement comprising reducing one or more off-target effects or toxicity measures by administering composition, compound, pharmaceutical composition, or conjugate as provided herein, wherein the amino acid sequences of the polypeptide comprise amino acids corresponding to positions X12 and/or X26 of SEQ ID NO: 195, and wherein X12 and/or X26 comprise a lysine with at least one additional small alkyl group attached to the nitrogen in the side chain, wherein uptake by kidney cells is less than with a polypeptide having an amino acid sequence that does not comprise an additional small alkyl group attached to a nitrogen on the side chain of a lysine at positions X12 and/or X26.

[0100] In another example, the disclosure describes a method of treating an individual with cancer, the improvement comprising achieving a reduction in concentration of R in a kidney tissue in the presence of composition, compound, pharmaceutical composition, or conjugate as provided herein, wherein the amino acid sequences of the polypeptide comprise amino acids corresponding to positions X12 and/or X26 of SEQ ID NO: 195, and wherein X12 and/or X26 comprise a lysine with at least one additional small alkyl group attached to the nitrogen in the side chain, wherein uptake by kidney cells is less than with a polypeptide having an amino acid sequence that does not comprise a small alkyl group attached to a nitrogen on the side chain of a lysine at positions X12 and/or X26. as compared to the concentration of R in the kidney tissue in the absence the composition, compound, pharmaceutical composition, or conjugate.

[0101] In some examples, the reduction in concentration of R in the kidney tissue is measured by urine output of R as measured by percent of administered radiation recovered or by detection as measured by a cell-based in vitro assay, or an in vivo detection assay.

[0102] In some examples, the administration of the composition can be repeated at least 2, 3, 4, 5, 6, or 7 times more in the presence of the composition having 90% identity to at least 40 amino acids of SEQ ID NO: 195 including a modified lysine at positions corresponding to X12 and/or X26 of SEQ ID NO: 195 than in the presence of an A or K at positions corresponding to X12 and/or X26.

[0103] In one example, the disclosure describes a method of reducing uptake by a kidney tissue of a composition, the improvement comprising administering a composition comprising (a) a radionuclide therapeutic comprising at least a polypeptide and a radionuclide (R); wherein the polypeptide has at least 90% identity to 40 amino acids of SEQ ID NO: 195 and/or has a modified lysine at positions corresponding to X12 and/or X26 of SEQ ID NO: 195, such that in the presence of the modified lysine, the radionuclide is less concentrated in the kidney tissue than in the absence of the polypeptide.

[0104] In another example, the disclosure describes a method comprising administering to a subject in need thereof a compound that binds to Nectin-4 and includes one or two modified lysines at positions corresponding to X12 and/or X26, respectively, of SEQ ID NO: 195, wherein administration of the compound having a miniprotein with the one or two modified lysines reduces one or more off target effects, toxicity grades, and/or uptake and/or retention in a kidney tissue as compared to a compound having an alanine or unmodified lysine at a position corresponding to X12 and an unmodified lysine at a position corresponding to X26.

[0105] In one example, the disclosure describes a method of treating an individual having or suspected of having a Nectin-4-positive cancer, the method comprising administering to the individual: a means for blocking uptake and/or retention of a radiotherapeutic to kidney tissue, and a linker, a chelator, and a radionuclide.

[0106] In some examples, the means for blocking uptake and/or retention of a radiotherapeutic to kidney tissue binds to Nectin-4 and includes one or two modified lysines at positions corresponding to X12 and/or X26, respectively, of SEQ ID NO: 195 and/or has at least 90% identity to 40 amino acids of SEQ ID NO: 195 and/or has a modified lysine at positions corresponding to X12 and/or X26 of SEQ ID NO: 195.

[0107] In some examples, the means for blocking uptake and/or retention of a radiotherapeutic to kidney tissue binds to Nectin-4 and includes one or two modified lysines at positions corresponding to X12 and/or X26, respectively, of SEQ ID NO: 195 and/or has at least 90% identity to 35 contiguous amino acids of SEQ ID NO: 195 and/or has a modified lysine at positions corresponding to X12 and/or X26 of SEQ ID NO: 195.

[0108] In some examples, the means for blocking uptake and/or retention of a radiotherapeutic to the kidney tissue blocks uptake and/or retention to the kidney tissue greater than as compared to the blocking of uptake and/or retention to the kidney tissue by a means that does not include one or two modified lysines at positions corresponding to X12 and/or X26, respectively, of SEQ ID NO: 195 and/or has at least 90% identity to 40 amino

acids of SEQ ID NO: 195 and/or has a modified lysine at positions corresponding to X12 and/or X26 of SEQ ID NO: 195.

[0109] In some examples, the means for blocking uptake and/or retention of a radiotherapeutic to the kidney tissue blocks uptake and/or retention to the kidney tissue greater than as compared to the blocking of uptake and/or retention to the kidney tissue by a means that does not include one or two modified lysines at positions corresponding to X12 and/or X26, respectively, of SEQ ID NO: 195 and/or has at least 90% identity to 35 contiguous amino acids of SEQ ID NO: 195 and/or has a modified lysine at positions corresponding to X12 and/or X26 of SEQ ID NO: 195.

[0110] In some examples, the means for blocking uptake and/or retention of a radiotherapeutic to the kidney tissue is a radiotherapeutic. In some examples, the radiotherapeutic is targeted to a tumor or a population of cancer cells. In some examples, the radiotherapeutic targeted to the tumor or the population of cancer cells is at a greater concentration than in the absence of the means for binding to kidney tissue. In some examples, the radiotherapeutic comprises a polypeptide that targets Nectin-4.

[0111] In some examples, the radiotherapeutic comprises or consists of a compound selected from C3-C293 or C298-C307. In some examples, the radionuclide of the radiotherapeutic is selected from Ac-225, Cu-64, Ga-68, In-111, Lu-177, or Pb-212.

[0112] In one example, the disclosure describes a kit comprising a polypeptide and instructions for use, wherein the polypeptide has an amino acid sequence as set forth in a polypeptide of any one of a composition, compound, pharmaceutical composition, or conjugate as described herein.

[0112a] In one aspect, the disclosure describes a kit comprising a polypeptide and instructions for use, wherein the polypeptide has an amino acid sequence as set forth in a polypeptide of any one of a composition, conjugate, pharmaceutical composition, as described herein.

[0113] In some examples, the kit further comprises one or more of a linker, chelator, and radionuclide. In some examples, the linker comprises or consists of a polyethylene glycol (PEG) linker of PEG4, PEG, PEG2, PEG6, PEG8, PEG12, PEG24, PEG36, lys(MPB)-PEG4, an ester linker, an amide linker, a maleimide linker, a succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC) linker, a propanoic acid linker, a dTyr-Gly-Phe (yGF) linker, a caproleic acid linker, or (Gly)n- (gGlu)n- or (PEG)n, wherein n is from 1 to 10, (Gly)1-10, or any fragment or combination via covalent bond thereof.

In some examples, the chelator comprises or consists of DOTA, NOPO, Crown, Macropa, lead specific chelator (PSC), N-succinimidyl 3-(tri-n-butylstannyl)benzoate (BuSTB), or N-succinimidyl 3-trimethylstannylbenzoate (MeSTB).

[0114] In some examples, prior to use, the compound is labeled with a radionuclide, wherein the radionuclide is chelated to the chelator to produce a composition with a formula M-L-C-R.

[0115] In some examples, the radionuclide is selected from Ac-225, Cu-64, Ga-68, Lu-177, Pb-212, In-111, Cu-67, La-132, La-135, Ce-134, F-18, I-131, I-124, Pb-203, Th-232, Bi-123, Sm-153, Ra-225, Tb-165, or At-211. In some examples, the radionuclide is Ac-225, Cu-64, Ga-68, In-111, Lu-177, or Pb-212.

[0116] In some examples, if the polypeptide has an amino acid comprising any of those set forth in any one of SEQ ID NOs: 83, 85, 93, 99, 134, 138, 145, 155, 161-176, 195, or 200 the polypeptide further comprises a linker, wherein the linker is PEG4, and a chelator, wherein the chelator is DOTA. In some examples, when present, the linker is attached to the N-terminus amino acid of the polypeptide. In some examples, the C-terminal amino acid of the polypeptide is not cysteine. In some examples, when present, the chelator is attached to either the polypeptide or the linker. In some examples, when present, the radionuclide is attached to the chelator. In some examples, when present, the radionuclide is attached to the N-terminus amino acid of the polypeptide.

BRIEF DESCRIPTION OF FIGURES

[0117] **FIGs. 1A and 1B** are fitted sensorgrams (SPR) of an exemplary Nectin-4 peptide binding to mouse and human Nectin-4, respectively.

[0118] **FIGs. 2A and 2B** are fitted sensorgrams (SPR) of an exemplary Nectin-4 peptide binding to mouse and human Nectin-4, respectively.

[0119] **FIGs. 3A and 3B** are fitted sensorgrams (SPR) of an exemplary conjugate comprising an exemplary Nectin-4 peptide binding to mouse and human Nectin-4, respectively.

[0120] **FIGs. 4A and 4B** are graphs showing association (FIG. 4A) and dissociation (FIG. 4B) kinetics for an exemplary compound comprising a radioconjugate miniprotein provided herein, on a human Nectin-4-expressing cancer line.

[0121] **FIGs. 5A and 5B** are graphs showing association (FIG. 5A) and dissociation (FIG. 5B) kinetics of an exemplary compound comprising a radioconjugate miniprotein provided herein, on a human Nectin-4-expressing cancer line.

[0122] **FIGs. 6A and 6B** are graphs showing internalization of an exemplary radioconjugate in HT-1376 (FIG. 6A) and MCF7 (FIG. 6B) cell lines at 37°C and 4°C. TB = total binding, NSB = non-specific binding.

CLAIMS

What is claimed is:

1. A composition, comprising:

(a) a polypeptide of at least 44 amino acids in length and having an amino acid sequence comprising that set forth in SEQ ID NO: 176, wherein X2 is E or D; X6 is E or Q; X9 is T or A; X10 is A or G; X12 is A, Kme3, Kme2, Kme, Kipr or K; X13 is R or (Cit) X17 is G or A; X21 is Q, Y, or E; X24 is Q or K; X25 is A or K; X26 is Kme3, Kme2, Kme, K, Kipr, or S; X28 is Q or K; X29 is Y or K; X30 is L or V; X32 is A, G, or D; X41 is N or K; and X45 is S or absent;

(b) a polypeptide of at least 44 amino acids in length and having an amino sequence comprising SEQ ID NO: 171, wherein X2 is E or D; X6 is E or Q; X17 is G or A; X21 is Q, Y, or E; X26 is Kme3, Kme2, Kme, K, Kipr, or S; X32 is A, G, or D; X41 is N or K; and X45 is S or absent;

(c) a Nectin-4 binding polypeptide having an amino acid sequence comprising at least 44 amino acids comprising a cysteine at each of four positions corresponding to 1, 20, 34, and 44 of SEQ ID NO: 195; (ii) SEQ ID NO: 169 at positions corresponding to positions 9-15 of SEQ ID NO: 195; (iii) QKKme3 at positions corresponding to positions 24, 25, and 26 of SEQ ID NO: 195; and (iv) QYL at positions corresponding to positions 28, 29, and 30 of SEQ ID NO: 195;

(d) a Nectin-4 binding polypeptide having an amino acid sequence comprising at least 44 amino acids comprising a cysteine at each of four positions corresponding to 1, 20, 34, and 44 of SEQ ID NO: 200; (ii) SEQ ID NO: 247 at positions corresponding to positions 9-15 of SEQ ID NO: 200; (iii) QKKme3 at positions corresponding to positions 24, 25, and 26 of SEQ ID NO: 200; and (iv) QYL at positions corresponding to positions 28, 29, and 30 of SEQ ID NO: 200; or

(e) a Nectin-4 binding polypeptide having an amino acid sequence comprising:

(i) at least four cysteines, which form two disulfide bonds;

(ii) at least one modified lysine residue at a position corresponding to X12 and/or X26 of SEQ ID NO: 195, wherein the modification comprises at least one small alkyl group attached to the nitrogen of the lysine side chain, optionally comprising a methyl, dimethyl, trimethyl, or isopropyl group;

(iii) at least 44 amino acids in length; and

(iv) has a binding affinity for Nectin-4 stronger than 100 nM in a cell-based assay.

2. The composition of claim 1, wherein at least one population of cells is to be contacted with the composition, and wherein the at least one population of cells includes kidney cells.

3. The composition of any one of claims 1 or 2, wherein the polypeptide has one or more of the following characteristics:

(a) is at least 40 amino acids in length, but no greater than 100 amino acids in length;

(b) binds to Nectin-4 with an affinity that is stronger than 100nM;

(c) binds to Nectin-4 with an affinity that is stronger than 10 nM in a cell-based assay; and/or

(d) has an inhibition constant that is no greater than 100 nM.

4. The composition of any one of claims 1 to 3, wherein the amino acid sequence of the polypeptide:

(a) shares at least 90% identity to any one of SEQ ID NOs: 3, 4, 6-47, 49-158, 161-168, 177-208, or 212-215, and includes at least one lysine with at least one modification comprising at least one small alkyl group bonded to the nitrogen of the side chain, optionally selected from: trimethyl, dimethyl, monomethyl, and isopropyl;

(b) shares at least 90% identity to at least 44 amino acids of a reference polypeptide, which reference polypeptide is longer than 44 amino acids in length, wherein the polypeptide binds to Nectin-4 with a strength of at least 10 nM on a cell-based assay, and/or wherein the polypetide has an inhibition constant of no greater than 10 nM;

(c) shares at least 90% identity to at least 40 amino acids of any one of SEQ ID NOs: 3, 4, 6-47, 49-158, 161-168, 177-208, or 212-215, provided that the 40 amino acids includes at least four cysteine residues that form two disulfide bridges;

(d) shares at least 90% identity to at least 35 contiguous amino acids of any one of SEQ ID NOs: 3, 4, 6-47, 49-158, 161-168, 177-208, or 212-215, provided that the 40 amino acids includes at least four cysteine residues that form two disulfide bridges;

(e) shares 90% identity to at least 44 amino acids as set forth in any one of SEQ ID NO: 78, 83, 85, 99, 103, 162-168, 195, or 200; and/or

(f) shares 100% identity to at least 44 amino acids as set forth in any one of SEQ ID NO: 78, 83, 85, 99, 103, 162-168, 195, or 200.

5. The composition of any one of claims 1 to 4, wherein the amino acid comprises the amino acid sequence as set forth in SEQ ID NO: 195, 78, 83, 85, 99, 103, 162-168, or 200.

6. The composition of any one of claims 1-5, wherein:
   (a) a C-terminal amino acid of the polypeptide is not a cysteine; and/or
   (b) a C-terminus of the polypeptide has an -OH or an -NH2

7. The composition of any one of claims 1 to 6, further comprising one or more of a linker, a chelator, and/or a radionuclide.

8. The composition of claim 7, wherein:
   (a) the radionuclide is Ac-225, Cu-64, Ga-68, Lu-177, Pb-212, In-111, Cu-67, La-132, La-135, Ce-134, F-18, I-131, I-124, Pb-203, Th-232, Bi-123, Sm-153, Ra-225, Tb-165, or At-211;
   (b) the linker comprises or consists of a polyethylene glycol (PEG) linker of PEG4, PEG2, PEG, PEG6, PEG8, PEG12, PEG24, PEG36, lys(MPB)-PEG4, an ester linker, an amide linker, a maleimide linker, a succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC) linker, a propanoic acid linker, a dTyr-Gly-Phe (yGF) linker, a caproleic acid linker, or (Gly)n-(gGlu)n- or (PEG)n, wherein n is from 1 to 10, (Gly)1-10, or any fragment or combination via covalent bond thereof; and/or
   (c) the chelator comprises or consists of DOTA, Crown, NOPO, Macropa, lead specific chelator (PSC), N-succinimidyl 3-(tri-n-butylstannyl)benzoate (BuSTB), or N-succinimidyl 3-trimethylstannylbenzoate (MeSTB).

9. The composition of any one of claims 7 or 8, wherein:
   (a) when present, the linker is attached to the N-terminus of the polypeptide;
   (b) when present, the chelator is attached to either the polypeptide or the linker; and/or
   (c) when present, the radionuclide is attached to the chelator.

10. A composition comprising a Nectin-4-binding miniprotein having an amino acid sequence comprising at least 44 amino acids, wherein the at least 44 amino acids comprise (i) a cysteine at each of four positions corresponding to 1, 20, 34, and 44 of SEQ ID NO: 195; (ii) TALX12RLR (SEQ ID NO: 169) at positions corresponding to positions 9-15 of SEQ ID NO: 195, wherein X12 is selected from A, Kme, Kme2, Kme3, or Kipr; (iii) QAX26, wherein X26 is selected from Kme3, Kme2, Kme, or Kipr at positions corresponding to positions 24, 25, and 26 of SEQ ID NO: 195; and (iv) QYL at positions corresponding to positions 28, 29, and 30 of SEQ ID NO: 195.

11. The composition of claim 10, wherein:

(a) the miniprotein binds to Nectin-4 with an affinity that is stronger than 100nM, or stronger than 10 nM in a cell-based assay;

(b) the inhibition constant is no greater than 100 nM;

(c) the C-terminus of the miniprotein has an -OH or an -NH2; and/or

(d) the miniprotein comprises a modified N-terminus, and the modified N-terminus comprises one or more of an NH2-, Acetyl-, PEGn-, wherein n=0-10, DOTA-, or Biotin

12. The composition of claim 10, further comprising one or more of a linker, chelator, and radionuclide.

13. The composition of claim 12, wherein the linker is attached to the N-terminus of the miniprotein and the linker comprises a polyethylene glycol (PEG) linker selected from PEG4, PEG2, PEG, PEG6, PEG8, PEG12, PEG24, PEG36, lys(MPB)-PEG4, an ester linker, an amide linker, a maleimide linker, a succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC) linker, a propanoic acid linker, a dTyr-Gly-Phe (yGF) linker, a caproleic acid linker, and (Gly)n-(gGlu)n- or (PEG)n, wherein n is from 1 to 10, (Gly)1-10, or any fragment or combination via covalent bond thereof.

14. The composition of claim 12, wherein the chelator comprises DOTA, Crown, NOPO, Macropa, lead specific chelator (PSC), N-succinimidyl 3-(tri-n-butylstannyl)benzoate (BuSTB), or N-succinimidyl 3-trimethylstannylbenzoate (MeSTB).

15. The composition of claim 12, wherein the radionuclide is Ac-225, Cu-64, Ga-68, Lu-177, Pb-212, In-111, Cu-67, La-132, La-135, Ce-134, F-18, I-131, I-124, Pb-203, Th-232, Bi-123, Sm-153, Ra-225, Tb-165, or At-211.

16. The composition of claim 12, wherein:

(a) when present, the linker is attached to the N-terminus of the miniprotein;

(b) when present, the chelator is attached to either the miniprotein or the linker; and/or

(c) when present, the radionuclide is attached to the chelator.

17. A composition comprising a formula selected from one or more of: (M)x-L-C-R, (M)x-L-C, (M)x-C-R, (M)x-L-R, (M)x-C, (M)x-L, and (M)x-R, wherein M comprises a polypeptide (M), L comprises a linker (L), C comprises a chelator (C), R comprises a radionuclide (R), and x is 1, 2, 3, or 4, wherein M comprises an amino acid sequence of any one of SEQ ID NO: 195, 3, 4, 6-47, 49-158, 161-168, 170-194, 196-208, 212-237, 243-246 or 248.

18. The composition of claim 17, wherein the linker (L) comprises or consists of a polyethylene glycol (PEG) linker of PEG4, PEG, PEG2, PEG6, PEG8, PEG12, PEG24, lys(MPB)-PEG4, PEG36, an ester linker, an amide linker, a maleimide linker, a valine-citrulline linker, a hydrazone linker, a N-succinimidyl-4-(2-pyridyldithio)butyrate (SPDB) linker, a succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC) linker, a vinylsulfone-based linker, a propanoic acid linker, a dTyr-Gly-Phe (yGF) linker, a caproleic acid linker, or (Gly)n-(gGlu)n- or (PEG)n, wherein n is from 1 to 10, (Gly)1-10, or any fragment or combination via covalent bond thereof.

19. The composition of any one of claims 17 or 18, wherein the chelator (C) comprises or consists of DOTA, Crown, NOPO, Macropa, lead specific chelator (PSC), N-succinimidyl 3-(tri-n-butylstannyl)benzoate (BuSTB), or N-succinimidyl 3-trimethylstannylbenzoate (MeSTB).

20. The composition of any one of claims 17 to 19, wherein the radionuclide (R) Ac-225, Cu-64, Ga-68, Lu-177, Pb-212, In-111, Cu-67, La-132, La-135, Ce-134, F-18, I-131, I-124, Pb-203, Th-232, Bi-123, Sm-153, Ra-225, Tb-165, or At-211.

21. The composition of any one of claims 17 to 20, wherein:

(a) when present, the linker (L) is attached to the N-terminus of the polypeptide (M);

(b) when present, the chelator (C) is attached to either the polypeptide (M) or the linker (L); and/or

(c) when present, the radionuclide (R) is attached to the chelator (C).

22. The composition of any one of claims 17 to 21, wherein the polypeptide (M) comprises at least one disulfide bridge.

23. The composition of any one of claims 17 to 22, wherein the polypeptide (M) binds to Nectin-4 with affinity of 10 pM to 200 nM, 10 pM to 100 nM, or 10 nM to 100 nM, *in vivo, ex vivo,* or *in vitro* and/or as measured in a cell-based assay; and/or has a binding inhibition constant of no greater than 100 nM.

24. A composition comprising a polypeptide-drug conjugate, comprising a polypeptide and at least one drug moiety, wherein the polypeptide comprises an amino acid sequence having at least 90% identity to at least 44 amino acids of an amino acid sequence set forth in any one of SEQ ID NOs: 3, 4, 6-47, 49-158, 161-168, 170-208, 212-237, 243-246 and 248.

25. The composition of claim 24, wherein the drug moiety is selected from a V-ATPase inhibitor, a pro-apoptotic agent, a Bcl2 inhibitor, an MCL1 inhibitor, a HSP90 inhibitor, an IAP inhibitor, an mTor inhibitor, a microtubule stabilizer, a microtubule destabilizer, an auristatin, a dolastatin, a maytansinoid, a MetAP (methionine aminopeptidase), an inhibitor of nuclear export of proteins CRMl, a DPPIV inhibitor, proteasome inhibitors, inhibitors of phosphoryl transfer reactions in mitochondria, a protein synthesis inhibitor, a kinase inhibitor, a CDK2 inhibitor, a CDK9 inhibitor, a kinesin inhibitor, an HDAC inhibitor, a DNA damaging agent, a DNA alkylating agent, a DNA intercalator, a DNA minor groove binder, a DHFR inhibitor, a

topoisomerase inhibitor, an auristatin (*e.g.*, monomethyl auristatin E), and an immunotoxin.

26. A composition comprising:

(a) an isolated compound or pharmaceutically acceptable salt thereof comprising an optional linker (L), and one or more of a polypeptide (M), chelator (C) or radionuclide (R), wherein M has an amino acid sequence comprising any one of SEQ ID NOs: 3, 4, 6-47, 49-158, 161-168, 170-208, 212-237, 243-246 and 248, including amino acid substitutions as set forth in Table 1C, Table 1D, Table 2C, Table 2D, Table 2E, Table 2F, or Table 2G;

(b) a compound designed to bind to Nectin-4, which compound comprises or consists of a polypeptide having an amino acid sequence comprising any one of SEQ ID NOs: 3, 4, 6-47, 49-158, 161-168, 170-208, 212-237, 243-246 and 248, including amino acid substitutions as set forth in Table 1C, Table 1D, Table 2C, Table 2D, Table 2E, Table 2F, or Table 2G, and further comprises a modified N and/or C-terminus; or

(c) a compound comprising a miniprotein comprising an amino acid sequence having at least 90% identity to SEQ ID NO: 195, and further comprising one or more additional components according to a formula M-L-C-R, wherein L is a linker, C is a chelator, and R is a radionuclide.

27. The composition of claim 26, wherein:

(a) the modified N-terminus comprises one or more of an NH2-, Acetyl-, PEGn-, wherein n=0-10, DOTA-, or Biotin-;

(b) the C terminus comprises an -NH2 or an -OH;

(c) the polypeptide selectively binds to Nectin-4 or a portion thereof;

(d) the polypeptide has a binding affinity of stronger than 100 nM to Nectin-4, or a portion thereof, *in vivo* or in a cell-based assay.

28. The composition of any one of claims 26 or 27, wherein:

(a) L comprises or consists of a polyethylene glycol (PEG) linker of PEG4, PEG, PEG2, PEG6, PEG8, PEG12, PEG24, PEG36, lys(MPB)-PEG4, an ester linker, an amide linker, a maleimide linker, a succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC) linker, a propanoic

acid linker, a dTyr-Gly-Phe (yGF) linker, a caproleic acid linker, any linker set forth in Table 2A, or (Gly)n-(gGlu)n- or (PEG)n, wherein n is from 1 to 10, (Gly)1-10, or any fragment or combination via covalent bond thereof;

(b) C comprises or consists of DOTA, Crown, NOPO, Macropa, lead specific chelator (PSC), N-succinimidyl 3-(tri-n-butylstannyl)benzoate (BuSTB), or N-succinimidyl 3-trimethylstannylbenzoate (MeSTB); and/or

(c) R comprises or consists of Ac-225, Cu-64, Ga-68, Lu-177, Pb-212, In-111, Cu-67, La-132, La-135, Ce-134, F-18, I-131, I-124, Pb-203, Th-232, Bi-123, Sm-153, Ra-225, Tb-165, or At-211.

29. A composition, comprising a polypeptide having an amino acid sequence comprising SEQ ID NO: 195.

30. The composition of claim 29, further comprising a linker comprising PEG4 and a chelator comprising DOTA.

31. A conjugate comprising: a polypeptide (M) that specifically binds to Nectin-4; a chelator (C) conjugated to (M) through an optional linker (L), wherein (C) comprises DOTA, and (L), when present, comprises PEG, wherein the PEG is optionally PEG-4; and (iii) a radionuclide (R) chelated to (C), wherein (R) is Actinium-225, Copper-64, Gallium-68, Indium-111, Lead-212, or Lutetium-177.

32. The conjugate of claim 31, wherein M has an amino acid sequence comprising:

(a) any one amino acid sequence set forth in SEQ ID NOs: 3, 4 , 6-47, 49-158, 161-168, 170-208, 212-237, 243-246 and 248;

(b) at least 90% identity to at least 40 amino acids of SEQ ID NO: 176, wherein X2 is E or D; X6 is E or Q; X9 is T or A; X10 is A or G; X12 is A, Kme3, Kme2, Kme, Kipr or K; X13 is R or (Cit) X17 is G or A; X21 is Q, Y, or E; X24 is Q or K; X25 is A or K; X26 is Kme3, Kme2, Kme, K, Kipr, or S; X28 is Q or K; X29 is Y or K; X30 is L or V; X32 is A, G, or D; X41 is N or K; and X45 is S or absent;

(c) SEQ ID NO: 195; or

(d) SEQ ID NO: 200.

33. A conjugate comprising a polypeptide (M) that specifically binds to Nectin-4 and a chelator (C) conjugated to the N-terminus of (M) through a linker (L), wherein the polypeptide has an amino acid sequence comprising: (i) at least 44 amino acids, wherein the amino acids comprise (a) a cysteine at each of four positions corresponding to 1, 20, 34, and 44 of SEQ ID NO: 195; (b) TALX12RLR at positions corresponding to positions 9-15 of SEQ ID NO: 195, wherein X12 is A, Kme, Kme2, Kme3, or Kipr; (c) QAX26 at positions corresponding to positions 24, 25, and 26 of SEQ ID NO: 195, wherein X26 is Kme3, Kme2, Kme, or Kipr; and (d) QYL at positions corresponding to positions 28, 29, and 30 of SEQ ID NO: 195; or (ii) SEQ ID NO: 195.

34. The conjugate of claim 33, wherein (C) comprises DOTA and (L) comprises PEG-4.

35. The conjugate of claim 33, further comprising a radionuclide (R) chelated to (C).

36. The conjugate of claim 33, wherein M is a miniprotein that has an amino acid sequence comprising SEQ ID NO: 195.

37. The conjugate of claim 35, wherein R comprises Ac-225, Cu-64, Ga-68, Lu-177, Pb-212, In-111, Cu-67, La-132, La-135, Ce-134, F-18, I-131, I-124, Pb-203, Bi-123, Sm-153, Ra-225, or At-211.

38. The conjugate of claim 37, wherein R is Ac-225 or Cu-64.

39. A pharmaceutical composition comprising the composition of any one of claims 1-30, or the conjugate of any one of claims 31-38, and a pharmaceutically acceptable excipient.

40. Use of the composition of any one of claims 1-30, or the conjugate of any one of claims 31 to 38, in the manufacture of a medicament for detecting presence of a Nectin-4 positive population of cancer cells or for the treatment of cancer.

41. The use of claim 40, wherein the cancer is selected from breast cancer, ovarian cancer, melanoma, pancreatic cancer, peripheral neuroma, glioblastoma,

adrenocortical carcinoma, AIDS-related lymphoma, anal cancer, urothelial cancer, bladder cancer, meningioma, glioma, astrocytoma, cervical cancer, chronic myeloproliferative disorders, colon cancer, endometrial cancer, ependymoma, esophageal cancer, Ewing's sarcoma, extracranial germ cell tumors, extrahepatic bile duct cancer, gallbladder cancer, gastric cancer, gastrointestinal carcinoid tumors, gestational trophoblastic tumors, hairy cell leukemia, Hodgkin lymphoma, non-Hodgkin lymphoma, hypopharyngeal cancer, islet cell carcinoma, Kaposi sarcoma, laryngeal cancer, leukemia, lip cancer, oral cavity cancer, liver cancer, male breast cancer, malignant mesothelioma, medulloblastoma, Merkel cell carcinoma, metastatic squamous neck cell carcinoma, multiple myeloma and other plasma cell neoplasms, mycosis fungoides and Sezary syndrome, myelodysplastic syndromes, nasopharyngeal cancer, neuroblastoma, non-small cell lung cancer, small cell lung cancer, head and neck cancer, skin cancer, oropharyngeal cancer, bone cancers, including osteosarcoma and malignant fibrous histiocytoma of bone, paranasal sinus cancer, parathyroid cancer, penile cancer, pheochromocytoma, pituitary tumors, prostate cancer, rectal cancer, renal cell cancer, retinoblastoma, rhabdomyosarcoma, salivary gland cancer, small intestine cancer, soft tissue sarcoma, supratentorial primitive neuroectodermal tumors, pineoblastoma, testicular cancer, thymoma, thymic carcinoma, thyroid cancer, transitional cell cancer of the renal pelvis and ureter, urethral cancer, uterine sarcoma, vaginal cancer, vulvar cancer, and Wilms tumor and other childhood kidney tumors.

42. The use of any one of claims 40 or 41, wherein the cancer is refractory, recurrent, or metastatic.

43. The use of any one of claims 40 to 42, wherein the medicament is to be administered intravenously or subcutaneously.

44. A kit comprising a polypeptide and instructions for use, wherein the polypeptide has an amino acid sequence as set forth in a polypeptide of any one of the composition of any one of claims 1 to 30, or the conjugate according to any one of claims 31 to 38, or the pharmaceutical composition of claim 39.

45. The kit of claim 44, further comprising one or more of a linker, chelator, and radionuclide.