HUMAN SERUM ALBUMIN LINKERS AND CONJUGATES THEREOF

FIELD OF THE INVENTION

The invention provides a human serum albumin (HSA) linker and binding, diagnostic, and therapeutic conjugates thereof. In one embodiment, the HSA linker includes two amino acid substitutions. The invention further provides methods for the manufacture and administration of diagnostic and therapeutic HSA linker conjugates.

BACKGROUND OF THE INVENTION

The serum albumins belong to a family of proteins that includes alpha-fetoprotein and human group-specific component, also known as vitamin-D binding protein. The serum albumins are the major soluble proteins of the circulatory system and contribute to many vital physiological processes. Serum albumin generally comprises about 50% of the total blood component by dry weight. The albumins and their related blood proteins also play an important role in the transport, distribution, and metabolism of many endogenous and exogenous ligands in the human body, including a variety of chemically diverse molecules, such as fatty acids, amino acids, steroids, calcium, metals such as copper and zinc, and various pharmaceutical agents. The albumin family of molecules are generally thought to facilitate transfer of many of these ligands across organ-circulatory interfaces, such as the liver, intestines, kidneys, and the brain. The albumins are thus involved in a wide range of circulatory and metabolic functions.

Human serum albumin (HSA) is a protein of about 66,500 kD and is comprised of 585 amino acids including at least 17 disulphide bridges. As with many of the members of the albumin family, human serum albumin plays an important role in human physiology and is located in virtually every human tissue and bodily secretion. As indicated above, HSA has theability to bind and transport a wide spectrum of ligands throughout the circulatory system, including the long-chain fatty acids, which are otherwise insoluble in circulating plasma.

SUMMARY OF THE INVENTION

In a first aspect, the present invention refers to an agent comprising a human serum albumin (HSA) linker comprising an amino acid sequence having at least 95% sequence identity to the sequence set forth in SEQ ID NO: 1 and comprising a first binding moiety bonded to the amino terminus of said HAS linker and a second binding moiety bonded to the carboxy terminus of said HSA linker, wherein each of said first and second binding moieties specifically binds ErbB2 or ErbB3 and wherein: i) if said first binding moiety specifically binds ErbB3, said second binding moiety specifically binds ErbB2; and ii) if said first binding moiety specifically binds ErbB2, said second binding moiety specifically binds ErbB3.

In an example, the invention features an agent that includes a human serum albumin (HSA) linker having an amino acid sequence set forth in any one of SEQ ID NOS:6-10 and first and second

binding moieties selected from antibodies, single-chain Fv molecules, bispecific single chain Fv ($(scFv')_2$) molecules, domain antibodies, diabodies, triabodies, hormones, Fab fragments, $F(ab')_2$ molecules, tandem scFv (taFv) fragments, receptors (e.g., cell surface receptors), ligands, aptamers, and biologically-active fragments thereof, in which the first binding moiety is bonded to the amino terminus of the HSA linker and the second binding moiety is bonded to the carboxy terminus of the HSA linker. In one example, the first binding moiety specifically binds ErbB3 and the second binding moiety specifically binds ErbB2. In other examples, the HSA linker has the amino acid sequences set forth in SEQ ID NOS:9 or 10.

In a further example, the invention features an agent that includes an HSA linker that has an amino acid sequence set forth in any one SEQ ID NOS:11-15 and at least first and second binding moieties, which can be selected from antibodies, single-chain Fv molecules, bispecific single chain Fv ($(scFv')_2$) molecules, domain antibodies, diabodies, triabodies, hormones, Fab fragments, $F(ab')_2$ molecules, tandem scFv (taFv) fragments, receptors (e.g., cell surface receptors), ligands, aptamers, and biologically-active fragments thereof, wherein the first binding moiety is bonded to the amino terminus and the second binding moiety is bonded to the carboxy terminus of the sequence. In an example, three or more binding moieties (e.g., 4, 5, 6, 7, 8, 9, 10, or more) can be included in the agent; these additional binding moities can be added to the agent, e.g., in tandem (e.g., 2, 3, 4, or 5 or more in tandem) with the first or second binding moiety. In one example, the first binding moiety specifically binds ErbB3 and the second binding moiety specifically binds ErbB2. In other examples, the HSA linker has the amino acid sequences set forth in SEQ ID NOS:14 or 15.

In a further example, the invention provides an HSA linker that has an amino acid sequence having at least 90% sequence identity to the sequence set forth in SEQ ID NO:1, and a serine residue at position 34 and a glutamine residue at position 503 of the amino acid sequence set forth in SEQ ID NO:1. In one example, the amino acid sequence has at least 95% sequence identity to the sequence set forth in SEQ ID NO:1. In another example, the HSA linker has the amino acid sequence set forth in SEQ ID NO:1.

In a further example, the invention features an agent that includes an HSA linker having at least 90% amino acid sequence identity to the sequence set forth in SEQ ID NO:1 and at least a first binding moiety. In one example, the agent includes a first polypeptide connector that binds the first binding moiety to the HSA linker.

In an example, the invention features an agent that includes an HSA linker having an amino acid sequence set forth in any one of SEQ ID NOS:11-15, or a fragment or variant of any one of these sequences, and at least a first binding moiety.

In one example of the invention, the agent further includes a first polypeptide connector (e.g., AAS, AAQ, or AAAL) that binds the first binding moiety to the amino or carboxy terminus of the HSA linker. In an example, the first connector covalently binds the first binding moiety to the HSA linker.

In one example of the invention, the agent further includes at least a second binding moiety. In an example, the agent further includes a second polypeptide connector (e.g., AAS, AAQ, or AAAL) that binds the second binding moiety to the HSA linker. In other examples, the second connector binds the second binding moiety to the amino or carboxy terminus of the HSA linker. In a further example, the second connector covalently binds the second binding moiety to the HSA linker. In other examples, the agent further includes three or more binding moieties which are included in tandem with the first or second binding moiety; the three or more binding moieties can further include a connector sequence that joins the three or more binding moieties to the first or second binding moiety and to each other.

In one example of the invention, the agent includes a first polypeptide connector that covalently binds a first binding moiety to the amino terminus of the HSA linker and a second polypeptide connector that covalently binds a second binding moiety to the carboxy terminus of the HSA linker. In one example, the first connector has the amino acid sequence AAS or AAQ and the second connector has the amino acid sequence set forth in SEQ ID NO:5.

In one example of the invention, the first or second binding moiety (or third or more binding moiety) is an antibody, single-chain Fv molecule, bispecific single chain Fv ($(scFv')_2$) molecule, domain antibody, diabody, triabody, hormone, Fab fragment, $F(ab')_2$ molecule, tandem scFv (taFv) fragment, receptor (e.g., cell surface receptor), ligand, aptamer, or biologically-active fragment thereof. In other examples, the agents of the invention include combinations of these different types of binding moities. In one example, at least the first or second binding moiety is a human or humanized single-chain Fv molecule.

In any example of the invention, one or more of the first or second binding moiety (or, if present, the third or further binding moiety) is or specifically binds to a protein selected from the group consisting of an insulin-

like growth factor 1 receptor (IGF1R), IGF2R, insulin-like growth factor (IGF), mesenchymal epithelial transition factor receptor (c-met; also known as hepatocyte growth factor receptor (HGFR)), hepatocyte growth factor (HGF), epidermal growth factor receptor (EGFR), epidermal growth factor (EGF), heregulin, fibroblast growth factor receptor (FGFR), platelet-derived growth factor receptor (PDGFR), platelet-derived growth factor (PDGF), vascular endothelial growth factor receptor (VEGFR), vascular endothelial growth factor (VEGF), tumor necrosis factor receptor (TNFR), tumor necrosis factor alpha (TNF-α), TNF-β, folate receptor (FOLR), folate, transferrin receptor (TfR), mesothelin, Fc receptor, c-kit receptor, c-kit, an integrin (e.g., an α4 integrin or a β-1 integrin), P-selectin, sphingosine-1-phosphate receptor-1 (S1PR), hyaluronate receptor, leukocyte function antigen-1 (LFA-1), CD4, CD11, CD18, CD20, CD25, CD27, CD52, CD70, CD80, CD85, CD95 (Fas receptor), CD106 (vascular cell adhesion molecule 1 (VCAM1), CD166 (activated leukocyte cell adhesion molecule (ALCAM)), CD178 (Fas ligand), CD253 (TNF-related apoptosis-inducing ligand (TRAIL)), ICOS ligand, CCR2, CXCR3, CCR5, CXCL12 (stromal cell-derived factor 1 (SDF-1)), interleukin 1 (IL-1), CTLA-4, MART-1, gp100, MAGE-1, ephrin (Eph) receptor, mucosal addressin cell adhesion molecule 1 (MAdCAM-1), carcinoembryonic antigen (CEA), $Lewis^Y$, MUC-1, epithelial cell adhesion molecule (EpCAM), cancer antigen 125 (CA125), prostate specific membrane antigen (PSMA), TAG-72 antigen, and fragments thereof. In a further example, one or more of the first or second binding moiety (or, if present, the third or further binding moiety) is or specifically binds to erythroblastic leukemia viral oncogene homolog (ErbB) receptor (e.g., ErbB1 receptor; ErbB2 receptor; ErbB3 receptor; and ErbB4 receptor). In another example, one or more of the first or second binding moiety (or, if present, the third or further binding moiety) is or specifically binds to alpha-fetoprotein (AFP) or an interferon, or a biologically-active fragment thereof. In a further example, one or more of the first or second binding moiety (or, if present, the third or further binding moiety) is natalizumab, infliximab, adalimumab, rituximab, alemtuzumab, bevacizumab, daclizumab, efalizumab, golimumab, certolizumab, trastuzumab, abatacept, etanercept, pertuzumab, cetuximab, panitumumab, or anakinra.

In any example of the invention, the agent is conjoined to a diagnostic, a therapeutic agent, or both. In one example, the diagnostic agent is a detectable label, such as a radioactive, fluorescent, or heavy metal label. In another example, the therapeutic agent is a cytotoxic agent, cytostatic, or immunomodulatory agent. Cytotoxic agents include alkylating agents, antibiotics, antineoplastic agents, antiproliferative agents, antimetabolites,

tubulin inhibitors, topoisomerase I or II inhibitors, hormonal agonists or antagonists, immunomodulators, DNA minor groove binders, and radioactive agents, or any agent capable of binding to and killing a tumor cell or inhibiting tumor cell proliferation. Antineoplastic agents include cyclophosphamide, camptothecin, homocamptothecin, colchicine, combrestatin, combrestatin, rhizoxin, dolistatin, ansamitocin p3, maytansinoid, auristatin, caleachimicin, methotrexate, 5-fluorouracil (5-FU), doxorubicin, paclitaxel, docetaxel, cisplatin, carboplatin, tamoxifen, raloxifene, letrozole, epirubicin, bevacizumab, pertuzumab, trastuzumab, and their derivatives.

In a second aspect, the present invention refers to a medicament comprising the agent as defined above admixed with a pharmaceutically acceptable carrier, excipient, or diluent.

In one example of the invention, the agent is admixed with a pharmaceutically acceptable carrier, excipient, or diluent. In one example, the agent exhibits an *in vivo* half-life of between 6 hours and 7 days. In another example, the agent exhibits an *in vivo* half-life greater than 8 hours.

In a third aspect, the present invention refers to the use of the agent as defined above in the manufacture of a medicament for treating a mammal having a proliferative disease.

In an example, the invention features a method for treating a mammal having a disease or disorder by administering any one of the HSA agents described in the first five aspects. In one example, the disease or disorder is associated with cellular signaling through a cell surface receptor. In another example, the mammal is a human. In a further example, the disease or disorder is a proliferative or autoimmune disease. Proliferative diseases include melanoma, clear cell sarcoma, head and neck cancer, bladder cancer, breast cancer, colon cancer, ovarian cancer, endometrial cancer, gastric cancer, pancreatic cancer, renal cancer, prostate cancer, salivary gland cancer, lung cancer, liver cancer, skin cancer, and brain cancer. Autoimmune diseases include multiple sclerosis, psoriasis, myasthenia gravis, uveitis, systemic lupus erythematosus, and rheumatoid arthritis. In one example, the HSA agent is administered in combination with one or more therapeutic agents, such as an antineoplastic agent.

In an example, the invention features a method for making an HSA linker agent by bonding at least a first binding moiety to the amino terminus and a second binding moiety to the carboxy terminus of the amino acid sequence set forth in any one of SEQ ID NOS:1, 3, or 6-15, or a sequence having at least 90%, 95%, 97%, or 100% sequence identity to a sequence set forth in any one of SEQ ID NOS:1, 3, or 6-15. In one example, the first or second binding moiety is covalently joined to the amino or carboxy terminus of an HSA linker. In other examples, a third or additional binding moiety (e.g., a fourth, fifth, sixth, seventh, eighth, ninth, or tenth binding moiety) is covalently joined in tandem with the first or second binding moiety to the amino or

carboxy terminus of the HSA linker. In another example, one or more of the first or second binding moiety (or, if present, the third or further binding moiety) is an antibody, single-chain Fv molecule, bispecific single chain Fv ($(scFv')_2$) molecule, domain antibody, diabody, triabody, hormone, Fab fragment, $F(ab')_2$ molecule, tandem scFv (taFv) fragment, receptor (e.g., cell surface receptor), ligand, or aptamer. In another example, the first or second binding moiety (or, if present, the third or further binding moiety) is a human or humanized single-chain Fv molecule. In yet another example, one or more of the first or second binding moiety (or, if present, the third or further binding moiety) is or specifically binds to insulin-like growth factor 1 receptor (IGF1R), IGF2R, insulin-like growth factor (IGF), mesenchymal epithelial transition factor receptor (c-met; also known as hepatocyte growth factor receptor (HGFR)), hepatocyte growth factor (HGF), epidermal growth factor receptor (EGFR), epidermal growth factor (EGF), heregulin, fibroblast growth factor receptor (FGFR), platelet-derived growth factor receptor (PDGFR), platelet-derived growth factor (PDGF), vascular endothelial growth factor receptor (VEGFR), vascular endothelial growth factor (VEGF), tumor necrosis factor receptor (TNFR), tumor necrosis factor alpha (TNF-α), TNF-β, folate receptor (FOLR), folate, transferrin receptor (TfR), mesothelin, Fc receptor, c-kit receptor, c-kit, an integrin (e.g., an α4 integrin or a β-1 integrin), P-selectin, sphingosine-1-phosphate receptor-1 (S1PR), hyaluronate receptor, leukocyte function antigen-1 (LFA-1), CD4, CD11, CD18, CD20, CD25, CD27, CD52, CD70, CD80, CD85, CD95 (Fas receptor), CD106 (vascular cell adhesion molecule 1 (VCAM1), CD166 (activated leukocyte cell adhesion molecule (ALCAM)), CD178 (Fas ligand), CD253 (TNF-related apoptosis-inducing ligand (TRAIL)), ICOS ligand, CCR2, CXCR3, CCR5, CXCL12 (stromal cell-derived factor 1 (SDF-1)), interleukin 1 (IL-1), CTLA-4, MART-1, gp100, MAGE-1, ephrin (Eph) receptor, mucosal addressin cell adhesion molecule 1 (MAdCAM-1), carcinoembryonic antigen (CEA), $Lewis^Y$, MUC-1, epithelial cell adhesion molecule (EpCAM), cancer antigen 125 (CA125), prostate specific membrane antigen (PSMA), TAG-72 antigen, and fragments thereof. In a further example, one or more of the first or second binding moiety (or, if present, the third or further binding moiety) is or specifically binds to erythroblastic leukemia viral oncogene homolog (ErbB) receptor (e.g., ErbB1 receptor; ErbB2 receptor; ErbB3 receptor; and ErbB4 receptor). In another example, one or more of the first or second binding moiety (or, if present, the third or further binding moiety) is or specifically binds to alpha-fetoprotein (AFP) or an interferon, or a biologically-active fragment thereof. In a further example, one or more of the first or second binding moiety (or, if present, the third or further binding moiety) is

natalizumab, infliximab, adalimumab, rituximab, alemtuzumab, bevacizumab, daclizumab, efalizumab, golimumab, certolizumab, trastuzumab, abatacept, etanercept, pertuzumab, cetuximab, panitumumab, or anakinra. In another example, the agent is conjoined to a diagnostic or therapeutic agent. In one example, the diagnostic agent is a detectable label, such as a radioactive, bioluminescent, fluorescent, heavy metal, or epitope tag. In another example, the therapeutic agent is a cytotoxic agent, cytostatic, or immunomodulatory agent. Cytotoxic agents include alkylating agents, antibiotics, antineoplastic agents, antiproliferative agents, antimetabolites, tubulin inhibitors, topoisomerase I and II inhibitors, hormonal agonists or antagonists, immunomodulators, DNA minor groove binders, and radioactive agents, or any agent capable of binding to and killing a tumor cell or inhibiting tumor cell proliferation. Antineoplastic agents include cyclophosphamide, camptothecin, homocamptothecin, colchicine, combrestatin, combrestatin, rhizoxin, dolistatin, ansamitocin p3, maytansinoid, auristatin, caleachimicin, methotrexate, 5-fluorouracil (5-FU), doxorubicin, paclitaxel, docetaxel, cisplatin, carboplatin, tamoxifen, raloxifene, letrozole, epirubicin, bevacizumab, pertuzumab, trastuzumab, and their derivatives. In a further example, the agent is admixed with a pharmaceutically acceptable carrier, excipient, or diluent.

In a fourth aspect, the present invention refers to a method for making the agent as defined above, comprising expressing a polynucleotide molecule encoding said agent in a bacterial, insect, yeast, or mammalian cell, or in a non-human mammalian tissue, organ, or organism.

In a further example, the invention features a method for making an HSA agent by substituting one or more surface-exposed amino acid residues in the amino acid sequences set forth in any one of SEQ ID NOS:1, 3, and 6-15 with a substitute amino acid capable of chemical modification that allows conjugation of a diagnostic or therapeutic agent. In one example, the substitute amino acid is cysteine and the surface exposed amino acid residues are serine or threonine. In another example, the chemical modification results in a covalent bond between the substitute amino acid and the diagnostic or therapeutic agent. In a further example, the surface-exposed amino acid residues is threonine at position 496, serine at position 58, threonine at position 76, threonine at position 79, threonine at position 83, threonine at position 125, threonine at position 236, serine at position 270, serine at position 273, serine at position 304, serine at position 435, threonine at position 478, threonine at position 506, or threonine at position 508.

In a further example, the invention features a method for making an HSA agent by substituting one or more of the residues in the amino acid sequences set forth in any one of SEQ ID NOS:1, 3, and 6-15 with an asparagine, serine, or threonine, thereby incorporating a glycosylation site within the HSA agent.

In a further example, the invention features a method for making an HSA agent by substituting one or more of the asparagine, serine, or threonine residues in the amino acid sequences set forth in any one of SEQ ID NOS:1, 3, and 6-15 with any amino acid other than asparagine, serine, or threonine, thereby removing a glycosylation site from the HSA agent.

In a further example, the invention features an agent that has at least 90% sequence identity to one of the amino acid sequences set forth in SEQ ID NOS:16-25. In one example, the agent has at least 95% sequence identity to one of the amino acid sequences set forth in SEQ ID NOS:16-25. In another example, the agent has one of the amino acid sequences set forth in SEQ ID NOS:16-25. In a further example, the agent is conjoined to a diagnostic or therapeutic agent. Diagnostic agents include detectable labels, such as a radioactive, bioluminescent, fluorescent, or heavy metal labels, or epitope tags. Fluorescent molecules that can serve as detectable labels include green fluorescent protein (GFP), enhanced GFP (eGFP), yellow fluorescent protein (YFP), cyan fluorescent protein (CFP), red fluorescent protein (RFP), and dsRed. In one example, the bioluminescent molecule is luciferase. In another example, the epitope tag is c-myc, hemagglutinin, or a histidine tag. In a further example, the therapeutic agent is a cytotoxic polypeptide such as cytochrome c, caspase 1-10, granzyme A or B, tumor necrosis factor-alpha (TNF-α), TNF-β, Fas, Fas ligand, Fas-associated death doman-like IL-1β converting enzyme (FLICE), TRAIL/APO2L, TWEAK/APO3L, Bax, Bid, Bik, Bad, Bak, RICK, vascular apoptosis inducing proteins 1 and 2 (VAP1 and VAP2), pierisin, apoptosis-inducing protein (AIP), IL-1α propiece polypeptide, apoptin, apoptin-associated protein 1 (AAP-1), endostatin, angiostatin, and biologically-active fragments thereof. An agent of the invention can be combined with one or more therapeutic agents such as cyclophosphamide, camptothecin, homocamptothecin, colchicine, combrestatin, combrestatin, rhizoxin, dolistatin, ansamitocin p3, maytansinoid, auristatin, caleachimicin, methotrexate, 5-fluorouracil (5-FU), doxorubicin, paclitaxel, docetaxel, cisplatin, carboplatin, tamoxifen, raloxifene, letrozole, epirubicin, bevacizumab, pertuzumab, trastuzumab, and derivatives thereof.

In an example of any aspect of the invention, the first and second binding moieties (and, if present, one or more of the third or further binding moiety) specifically bind the same target molecule. In another example of any aspect of the invention, the first and second binding moieties (and, if present, one or more of the third or further binding moiety) specifically bind different target molecules. In a further example of any aspect of the invention, the first

and second binding moieties (and, if present, one or more of the third or further binding moiety) specifically bind different epitopes on the same target molecule.

In a further example, the invention features a polypeptide linker that has amino acid residues 25-44 and 494-513 of the amino acid sequence set forth in SEQ ID NO:1. In one example, the polypeptide linker has amino acid residues 25-70 and 450-513 of the amino acid sequence set forth in SEQ ID NO:1. In another example, the polypeptide linker has amino acid residues 15-100 and 400-520 of the amino acid sequence set forth in SEQ ID NO:1. In a further example, the polypeptide linker has amino acid residues 10-200 and 300-575 of the amino acid sequence set forth in SEQ ID NO:1. In another example, the polypeptide linker has amino acid residues 5-250 and 275-580 of the amino acid sequence set forth in SEQ ID NO:1.

In a further example of the invention, the polypeptide linker includes at least a first binding moiety. In one example, the polypeptide linker includes at least a first polypeptide connector that binds the first binding moiety to the amino or carboxy terminus of the polypeptide linker. In another example, the first polypeptide connector covalently binds the first binding moiety to the polypeptide linker. In a further example, the polypeptide linker includes a second binding moiety. In one example, the polypeptide linker includes a second polypeptide connector that binds the second binding moiety to the polypeptide linker. In other examples, the second connector binds the second binding moiety to the amino or carboxy terminus of the polypeptide linker. In a further example, the second connector covalently binds the second binding moiety to the polypeptide linker. In other examples, the polypeptide linker includes a third, fourth, fifth, sixth, seventh, eighth, ninth, or tenth binding moiety. In other examples, these additional binding moieties are present in tandem with one or both of the first or second binding moiety. In yet other examples, a polypeptide connector (e.g., AAS, AAQ, or AAAL) separates one or more of these additional binding moities from each other, the first or second binding moiety, or the polypeptide linker.

In a further example of the invention, the polypeptide linker includes a first polypeptide connector that covalently binds a first binding moiety to the amino terminus of the polypeptide linker and a second polypeptide connector that covalently binds a second binding moiety to the carboxy terminus of the polypeptide linker. In one example, the first connector has the amino acid

sequence AAS or AAQ and the second connector has the amino acid sequence set forth in SEQ ID NO:5.

In a further example of the invention, one or more of the first or second binding moiety (or, if present, the third or further binding moiety) is an antibody, single-chain Fv molecule, bispecific single chain Fv ($(scFv')_2$) molecule, domain antibody, diabody, triabody, hormone, Fab fragment, $F(ab')_2$ molecule, tandem scFv (taFv) fragment, receptor (e.g., cell surface receptor), ligand, aptamer, or biologically-active fragment thereof. In one example, one or more of the first or second binding moiety (or, if present, the third or further binding moiety) is a human or humanized single-chain Fv molecule.

In a further example of the invention, one or more of the first or second binding moiety (or, if present, the third or further binding moiety) is or specifically binds to a protein selected from the group consisting of an insulin-like growth factor 1 receptor (IGF1R), IGF2R, insulin-like growth factor (IGF), mesenchymal epithelial transition factor receptor (c-met; also known as hepatocyte growth factor receptor (HGFR)), hepatocyte growth factor (HGF), epidermal growth factor receptor (EGFR), epidermal growth factor (EGF), heregulin, fibroblast growth factor receptor (FGFR), platelet-derived growth factor receptor (PDGFR), platelet-derived growth factor (PDGF), vascular endothelial growth factor receptor (VEGFR), vascular endothelial growth factor (VEGF), tumor necrosis factor receptor (TNFR), tumor necrosis factor alpha (TNF-α), TNF-β, folate receptor (FOLR), folate, transferrin receptor (TfR), mesothelin, Fc receptor, c-kit receptor, c-kit, an integrin (e.g., an α4 integrin or a β-1 integrin), P-selectin, sphingosine-1-phosphate receptor-1 (S1PR), hyaluronate receptor, leukocyte function antigen-1 (LFA-1), CD4, CD11, CD18, CD20, CD25, CD27, CD52, CD70, CD80, CD85, CD95 (Fas receptor), CD106 (vascular cell adhesion molecule 1 (VCAM1), CD166 (activated leukocyte cell adhesion molecule (ALCAM)), CD178 (Fas ligand), CD253 (TNF-related apoptosis-inducing ligand (TRAIL)), ICOS ligand, CCR2, CXCR3, CCR5, CXCL12 (stromal cell-derived factor 1 (SDF-1)), interleukin 1 (IL-1), CTLA-4, MART-1, gp100, MAGE-1, ephrin (Eph) receptor, mucosal addressin cell adhesion molecule 1 (MAdCAM-1), carcinoembryonic antigen (CEA), $Lewis^Y$, MUC-1, epithelial cell adhesion molecule (EpCAM), cancer antigen 125 (CA125), prostate specific membrane antigen (PSMA), TAG-72 antigen, and fragments thereof. In a further example, the first or second binding moiety is or specifically binds to erythroblastic leukemia viral oncogene homolog (ErbB) receptor (e.g., ErbB1 receptor; ErbB2 receptor; ErbB3 receptor; and ErbB4 receptor). In another example, one or more of the

first or second binding moiety (or, if present, the third or further binding moiety) is or specifically binds to alpha-fetoprotein (AFP) or an interferon, or a biologically-active fragment thereof. In a further example, one or more of the first or second binding moiety (or, if present, the third or further binding moiety) is natalizumab, infliximab, adalimumab, rituximab, alemtuzumab, bevacizumab, daclizumab, efalizumab, golimumab, certolizumab, trastuzumab, abatacept, etanercept, pertuzumab, cetuximab, panitumumab, or anakinra.

In a further example of the invention, the polypeptide linker is conjoined to a diagnostic, a therapeutic agent, or both. In one example, the diagnostic agent is a detectable label, such as a radioactive, fluorescent, or heavy metal label. In another example, the therapeutic agent is a cytotoxic agent, cytostatic, or immunomodulatory agent. Cytotoxic agents include alkylating agents, antibiotics, antineoplastic agents, antiproliferative agents, antimetabolites, tubulin inhibitors, topoisomerase I or II inhibitors, hormonal agonists or antagonists, immunomodulators, DNA minor groove binders, and radioactive agents, or any agent capable of binding to and killing a tumor cell or inhibiting tumor cell proliferation. Antineoplastic agents include cyclophosphamide, camptothecin, homocamptothecin, colchicine, combrestatin, combrestatin, rhizoxin, dolistatin, ansamitocin p3, maytansinoid, auristatin, caleachimicin, methotrexate, 5-fluorouracil (5-FU), doxorubicin, paclitaxel, docetaxel, cisplatin, carboplatin, tamoxifen, raloxifene, letrozole, epirubicin, bevacizumab, pertuzumab, trastuzumab, and their derivatives. In one example, the polypeptide linker is admixed with a pharmaceutically acceptable carrier, excipient, or diluent. In another example, the polypeptide linker exhibits an *in vivo* half-life of between 6 hours and 7 days. In a further example, the polypeptide linker exhibits an *in vivo* half-life greater than 8 hours.

An example of the invention features an agent of any of the prior aspects of the invention (one through twelve), in which the HSA polypeptide linker sequence is replaced by another polypeptide linker sequence. For example, the polypeptide linker sequence could be a mammalian, non-human serum albumin polypeptide sequence, such as, e.g., a bovine, murine, feline, and canine serum albumin (BSA) polypeptide sequence. In other examples this polypeptide linker sequence is between 5 and 1,000 amino acids in length, e.g., 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, or 900 amino acids in length, or any number of amino acids within this range. In other examples, the polypeptide linker sequence includes a single amino acid (including, but not limited to, e.g., glycine, alanine, serine, glutamine, leucine, and valine), or combinations of amino acids.

In another example, the HSA polypeptide linker sequence is replaced by an alpha-fetoprotein polypeptide (AFP) polypeptide sequence, e.g., mammalian AFP polypeptide sequence, such as a human, murine, bovine, or canine AFP polypeptide sequence. In an example, the AFP linker sequence of the agent can correspond to the full-length human AFP polypeptide sequence (a.a. 1-609; SEQ ID NO: 58), the mature human AFP polypeptide sequence lacking amino acids 1-18 of the signal sequence (a.a., 19-609 of SEQ ID NO: 58), or fragments thereof. In other examples, the AFP polypeptide linker sequence contains at least 5 to 8 contiguous amino acids, preferably at least 10, 20, or 50 contiguous amino acids, more preferably at least 100 contiguous amino acids, and most preferably at least 200, 300, 400, or more contiguous amino acids of SEQ ID NO: 58, or has at least 90% sequence identity (e.g., at least 95%, 97%, 99%, or more sequence identity) to a continguous polypeptide sequence of SEQ ID NO: 58 having one or more of these lengths. For example, an AFP polypeptide linker sequence having 90% sequence identity to a 34-mer human AFP peptide corresponding to amino acids 446-479 of SEQ ID NO: 58

(LSEDKLLACGEGAADIIIGHLCIRHEMTPVNPGV; SEQ ID NO: 59) may contain up to 3 amino acids altered from the 446-479 segment of SEQ ID NO: 58. One such example of sequence deviation in biologically active human AFP fragments is found in, e.g., U.S. Patent No. 5,707,963 (incorporated by reference herein), which discloses a 34-amino acid fragment of human AFP (SEQ ID NO: 59) with flexibility at two amino acid residues (amino acid 9 and 22 of SEQ ID NO: 59). Other examples of AFP polypeptide linker sequences include, e.g., amino acids 19-198 of SEQ ID NO: 58 (human AFP Domain I), amino acids 217-408 of SEQ ID NO: 58 (human AFP Domain II), amino acids 409-609 of SEQ ID NO: 58 (human AFP Domain III), amino acids 19-408 of SEQ ID NO: 58 (human AFP Domain I+II), amino acids 217-609 of SEQ ID NO: 58 (human AFP Domain II+III), and amino acids 285-609 of SEQ ID NO: 58 (human AFP Fragment I). In another embodiment, the human AFP polypeptide linker sequence is an 8-amino acid sequence that includes amino acids 489-496 (i.e., EMTPVNPG) of SEQ ID NO: 58.

In a fifth aspect, the present invention refers to a kit comprising the agent as defined above and instructions for administering said agent to a patient.

A further example of the invention features kits that include any of the HSA linkers, HSA linker agents, or any other agents described in previous examples of the invention. The kits further include instructions to allow a practitioner (e.g., a physician, nurse, or patient) to administer the compositions and agents contained therein. In an example, the kits include multiple packages of a single- or multi-dose pharmaceutical composition containing an effective amount of an agent of the invention, e.g,. an HSA linker of the

invention that includes, e.g., one or more binding moieties (e.g., antibodies or antibody fragments (e.g., scFv)), diagnostic agents (e.g., radionuclide or chelating agents), and/or therapeutic agents (e.g., cytotoxic or immunomodulatory agents). Optionally, instruments or devices necessary for administering the pharmaceutical composition(s) may be included in the kits. For instance, a kit of this invention may provide one or more pre-filled syringes containing an effective amount of an HSA linker of the invention, or any binding, diagnostic, and/or therapeutic agent conjugated thereto. Furthermore, the kits may also include additional components such as instructions or administration schedules for a patient suffering from a disease or condition (e.g., a cancer, autoimmune disease, or cardiovascular disease) to use the pharmaceutical composition(s) containing, e.g., an HSA linker of the invention, or any binding, diagnostic, and/or therapeutic agent conjugated thereto.

DEFINITIONS

The term "antibody" as used interchangeably herein, includes whole antibodies or immunoglobulins and any antigen-binding fragment or single chains thereof. Antibodies, as used herein, can be mammalian (e.g., human or mouse), humanized, chimeric, recombinant, synthetically produced, or naturally isolated. In most mammals, including humans, antibodies have at least two heavy (H) chains and two light (L) chains connected by disulfide bonds. Each heavy chain consists of a heavy chain variable region (abbreviated herein as V_H) and a heavy chain constant region. The heavy chain constant region consists of three domains, C_H1, C_H2, and C_H3 and a hinge region between C_H1 and C_H2. Each light chain consists of a light chain variable region (abbreviated herein as V_L) and a light chain constant region. The light chain constant region consists of one domain, C_L. The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (*e.g.*, effector cells) and the first component (Clq) of the classical complement system. Antibodies of the present invention include all known forms of antibodies and other protein scaffolds with antibody-like properties. For example, the antibody can be a human antibody, a humanized

antibody, a bispecific antibody, a chimeric antibody, or a protein scaffold with antibody-like properties, such as fibronectin or ankyrin repeats. The antibody also can be a Fab, Fab'2, scFv, SMIP, diabody, nanobody, aptamers, or a domain antibody. The antibody can have any of the following isotypes: IgG (e.g., IgG1, IgG2, IgG3, and IgG4), IgM, IgA (e.g., IgA1, IgA2, and IgAsec), IgD, or IgE. Antibodies that can be used as binding moieties, as defined herein, in combination with an HSA linker of the invention include, but are not limited to, natalizumab, infliximab, adalimumab, rituximab, alemtuzumab, bevacizumab, daclizumab, efalizumab, golimumab, certolizumab, trastuzumab, abatacept, etanercept, pertuzumab, cetuximab, and panitumumab.

The term "antibody fragment," as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to an antigen (*e.g.*, ErbB2). The antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Examples of binding fragments encompassed within the term "antigen-binding portion" of an antibody include but are not limited to: (i) a Fab fragment, a monovalent fragment consisting of the V_L, V_H, C_L, and C_H1 domains; (ii) a $F(ab')_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the V_H and C_H1 domains; (iv) a Fv fragment consisting of the V_L and V_H domains of a single arm of an antibody, (v) a dAb including V_H and V_L domains; (vi) a dAb fragment (Ward *et al., Nature* 341:544-546 (1989)), which consists of a V_H domain; (vii) a dAb which consists of a V_H or a V_L domain; (viii) an isolated complementarity determining region (CDR); and (ix) a combination of two or more isolated CDRs which may optionally be joined by a synthetic linker. Furthermore, although the two domains of the Fv fragment, V_L and V_H, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the V_L and V_H regions pair to form monovalent molecules (known as single chain Fv (scFv); see *e.g.*, Bird *et al., Science* 242:423-426 (1988) and Huston *et al., Proc. Natl. Acad. Sci. USA* 85:5879-5883 (1988)). These antibody fragments are obtained using conventional techniques known to those with skill in the art, and the fragments are screened for utility in the same manner as are intact antibodies. Antibody fragments can be produced by recombinant DNA techniques, or by enzymatic or chemical cleavage of intact immunoglobulins.

By "autoimmune disease" is meant a disease in which an immune system response is generated against self epitopes or antigens. Examples of autoimmune diseases include, but are not

limited to, alopecia areata, ankylosing spondylitis, antiphospholipid syndrome, autoimmune Addison's disease, autoimmune hemolytic anemia, autoimmune hepatitis, Behcet's disease, bullous pemphigoid, cardiomyopathy, celiac Sprue-dermatitis, chronic fatigue immune dysfunction syndrome (CFIDS), chronic inflammatory demyelinating polyneuropathy, Churg-Strauss syndrome, cicatricial pemphigoid, CREST syndrome, cold agglutinin disease, Crohn's disease, discoid lupus, essential mixed cryoglobulinemia, fibromyalgia-fibromyositis, Grave's disease, Guillain-Barré syndrome, Hashimoto's thyroiditis, hypothyroidism, idiopathic pulmonary fibrosis, idiopathic thrombocytopenia purpura (ITP), IgA nephropathy, insulin dependent diabetes, juvenile arthritis, lichen planus, lupus, Ménière's disease, mixed connective tissue disease, multiple sclerosis, pemphigus vulgaris, pernicious anemia, polyarteritis nodosa, polychondritis, polyglandular syndromes, polymyalgia rheumatica, polymyositis and dermatomyositis, primary agammaglobulinemia, primary biliary cirrhosis, psoriasis, Raynaud's phenomenon, Reiter's syndrome, rheumatic fever, rheumatoid arthritis, sarcoidosis, scleroderma, Sjögren's syndrome, Stiff-Man syndrome, systemic lupus erythematosus (SLE), Takayasu arteritis, temporal arteritis/giant cell arteritis, ulcerative colitis, uveitis (e.g., birdshot retinochoroidopathy uveitis and sarcoid uveitis), vasculitis, vitiligo, Wegener's granulomatosis, and myasthenia gravis.

By "binding moiety" is meant any molecule that specifically binds to a target epitope, antigen, ligand, or receptor. Binding moieties include but are not limited to antibodies (e.g., monoclonal, polyclonal, recombinant, humanized, and chimeric antibodies), antibody fragments (e.g., Fab fragments, Fab'2, scFv antibodies, SMIP, domain antibodies, diabodies, minibodies, scFv-Fc, affibodies, nanobodies, and domain antibodies), receptors, ligands, aptamers, and other molecules having a known binding partner.

By "biologically-active" is meant that a molecule, including biological molecules, such as nucleic acids, peptides, polypeptides, and proteins, exerts a physical or chemical activity on itself or other molecule. For example, a "biologically-active" molecule may possess, e.g., enzymatic activity, protein binding activity (e.g., antibody interactions), or cytotoxic activities are "biologically-active."

The term "chimeric antibody" refers to an immunoglobulin or antibody whose variable regions derive from a first species and whose constant regions derive from a second species. Chimeric antibodies can be constructed, for example, by genetic engineering, from immunoglobulin gene segments belonging to different species (e.g., from a mouse and a human).

By "connector" or "polypeptide connector" is meant an amino acid sequence of 2 to 20 residues in length that is covalently attached to one or both of the amino or carboxy termini of an HSA linker of the invention, or is covalently attached to one or more residues of an HSA linker of the invention (e.g., a residue between the amino and carboxy terminal residues). In preferred embodiments, the polypeptide connector attached to the amino terminus of an HSA linker has the amino acid sequence "AAS" or "AAQ" and the connector attached to the carboxy terminus has the amino acid sequence "AAAL" (SEQ ID NO:5).

The terms "effective amount" or "amount effective to" or "therapeutically effective amount" means an amount of an agent of the invention (e.g., an HSA linker bonded with one or more binding moieties or diagnostic or therapeutic agents with or without a connector sequence) sufficient to produce a desired result, for example, killing a cancer cell, reducing tumor cell proliferation, reducing inflammation in a diseased tissue or organ, or labeling a specific population of cells in a tissue, organ, or organism (e.g., a human).

The term "human antibody," as used herein, is intended to include antibodies, or fragments thereof, having variable regions in which both the framework and CDR regions are derived from human germline immunoglobulin sequences as described, for example, by Kabat *et al.*, (*Sequences of proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242 (1991)). Furthermore, if the antibody contains a constant region, the constant region also is derived from human germline immunoglobulin sequences. The human antibodies may include amino acid residues not encoded by human germline immunoglobulin sequences (e.g., mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*). However, the term "human antibody", as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences (i.e., a humanized antibody or antibody fragment).

The term "humanized antibody" refers to any antibody or antibody fragment that includes at least one immunoglobulin domain having a variable region that includes a variable framework region substantially derived from a human immunoglobulin or antibody and complementarity determining regions (e.g., at least one CDR) substantially derived from a non-human immunoglobulin or antibody.

As used herein, an "inflammatory signaling inhibitor" or "ISI" is an agent that decreases the binding between a pro-inflammatory cytokine (e.g., TNF-alpha, TNF-beta, or IL-1) and its receptor (e.g., TNF receptor 1 or 2, or IL-1 receptor, respectively); decreases the binding of activating molecules to pro-inflammatory cell surface signaling molecules (e.g., CD20, CD25, CTLA-4, CD80/CD86, or CD28); or decreases the downstream activation of, or activity of, intracellular signaling molecules that are activated following the binding of pro-inflammatory cytokines to their receptors or the binding of activating molecules to pro-inflammatory cell surface signaling molecules (e.g., an agent that decreases the activation of, or activity of, signaling molecules in the p38 MAPK signaling pathway). Preferably, the decrease in binding between a pro-inflammatory cytokine and its receptor, the decrease in binding of an activating molecule to a pro-inflammatory cell surface signaling molecule, or the decrease in intracellular signaling which occurs following the binding of pro-inflammatory cytokines to their receptors or activating molecules to pro-inflammatory cell surface signaling molecules is by at least about 10%, preferably by 20%, 30%, 40%, or 50%, more preferably by 60%, 70%, 80%, 90% or more (up to 100%). An ISI may act by reducing the amount of pro-inflammatory cytokine (e.g., TNF-alpha, TNF-beta, or IL-1) freely available to bind the receptor. For example, an ISI may be a soluble pro-inflammatory cytokine receptor protein (e.g., a soluble TNF receptor fusion protein such as etanercept (ENBREL®) or lenercept), or a soluble pro-inflammatory cell surface signaling molecule (e.g., a soluble CTLA-4 (abatacept)), or an antibody directed against a pro-inflammatory cytokine or a pro-inflammatory cell surface signaling molecule (e.g., an anti-TNF antibody, such as adalimumab, certolizumab, inflixamab, or golimumab; an anti-CD20 antibody, such as rituximab; or TRU-015 (TRUBION®)). In addition, an ISI may act by disrupting the ability of the endogenous wild-type pro-inflammatory cytokine or the pro-inflammatory cell surface signaling molecule to bind to its receptor (e.g., TNF receptor 1 or 2, IL-1 receptor, or CD11a (e.g., efalizumab (RAPTIVA®, Genentech))). Examples of dominant-negative TNF-alpha variants are XENP345 (a pegylated version of TNF variant A145R/I97T) and XproTM1595, and further variants disclosed in U.S. Patent Application Publication Nos. 20030166559 and 20050265962, herein incorporated by reference. An example of a dominant negative IL-1 variant is anakinra (KINERET®), which is a soluble form of IL-1 that binds to the IL-1 receptor without activating intracellular signaling pathways. Inflammatory signaling inhibitors, which can be used in the present invention, are also small molecules which inhibit or reduce the signaling pathways downstream of pro-inflammatory cytokine or pro-inflammatory cell surface

signaling molecules (e.g., DE 096). Examples of ISIs of this kind include inhibitors of p38 MAP kinase, e.g., 5-amino-2-carbonylthiopene derivatives (as described in WO 04/089929, herein incorporated); ARRY-797; BIRB 796 BS, (1-5-tert-butyl-2-p-tolyl-2H-pyrazol-3-yl)-3-[4-2(morpholin-4-yl-ethoxy)-naphtalen-1-yl]-urea); CHR-3620; CNI-1493; FR-167653 (Fujisawa Pharmaceutical, Osaka, Japan); ISIS 101757 (Isis Pharmaceuticals); ML3404; NPC31145; PD169316; PHZ1112; RJW67657, (4-(4-(4-fluorophenyl)-1-(3-phenylpropyl)-5-(4-pyridinyl)-1H-imidazol-2-yl)-3-butyn-1-ol; SCIO-469; SB202190; SB203580, (4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)1H-imidazole); SB239063, trans-1-(4-hydroxycyclohexyl)-4-(4-fluorophenyl-methoxypyridimidin-4-yl)imidazole; SB242235; SD-282; SKF-86002; TAK 715; VX702; and VX745. Furthermore, an ISI may interfere with the processing of a pro-inflammatory cytokine (e.g., TNF-alpha and TNF-beta) from its membrane bound form to its soluble form. Inhibitors of TACE are ISIs of this class. Examples of inhibitors of TACE include BB-1101, BB-3103, BMS-561392, butynyloxyphenyl β-sulfone piperidine hydroxomates, CH4474, DPC333, DPH-067517, GM6001, GW3333, Ro 32-7315, TAPI-1, TAPI-2, and TMI 005. Additional examples of ISIs include short peptides derived from the *E. coli* heat shock proteins engineered for disease-specific immunomodulatory activity (e.g., dnaJP1).

By "integrin antagonist" is meant any agent that reduces or inhibits the biological activity of an integrin molecule (e.g., a reduction or inhibition of at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99%, or more relative to the biological activity in the absence of the integrin antagonist), such as the α4 subunit of an integrin molecule. The agent may act directly or indirectly on the α4 integrin subunit (NCBI Accession No. P13612; Takada *et al., EMBO J.* 8:1361-1368 (1989)) by inhibiting the activity or expression of the α4 integrin subunit, or may act on a target to which the intact integrin containing an α4 subunit binds. For example, an antibody or blocking peptide that binds to vascular cell adhesion molecule-1 (VCAM-1), thus preventing the binding of α4β1 integrin to VCAM-1 is considered an integrin antagonist for purposes of the present invention. Non-limiting exemplary integrin antagonists suitable for use with the present invention may include proteins, blocking peptides, antibodies, such as natalizumab (TYSABRI®), and small molecule inhibitors. Examples of α4 integrin antagonists include, but are not limited to, natalizumab (Elan/Biogen Idec; see, e.g., U.S. Patent Nos. 5,840,299; 6,033,665; 6,602,503; 5,168,062; 5,385,839; and 5,730,978; incorporated by reference herein), oMEPUPA-V (Biogen; U.S. Patent No. 6,495,525; incorporated by reference herein), alefacept, CDP-323 (Celltech); firategrast (SB-68399;

GlaxoSmithKline); TR-9109 (Pfizer); ISIS-107248 (Antisense Therapeutics); R-1295 (Roche); and TBC-4746 (Schering-Plough). Additional non-limiting examples of α4 integrin antagonists include the small molecules described in U.S. Patent Nos. 5,821,231; 5,869,448; 5,936,065; 6,265,572; 6,288,267; 6,365,619; 6,423,728; 6,426,348; 6,458,844; 6.479,666; 6,482,849; 6,596,752; 6,667,331; 6,668,527; 6,685,617; 6,903,128; and 7,015,216 (each herein incorporated by reference); in U.S. Patent Application Publication Nos. 2002/0049236; 2003/0004196; 2003/0018016; 2003/0078249; 2003/0083267; 2003/0100585; 2004/0039040; 2004/0053907; 2004/0087574; 2004/0102496; 2004/0132809; 2004/0229858; 2006/0014966; 2006/0030553; 2006/0166866; 2006/0166961; 2006/0241132; 2007/0054909; and 2007/0232601 (each herein incorporated by reference); in European Patent Nos. EP 0842943; EP 0842944; EP 0842945; EP 0903353; and EP 0918059; and in PCT Publication Nos. WO 95/15973; WO 96/06108; WO 96/40781; WO 98/04247; WO 98/04913; WO 98/42656; WO 98/53814; WO 98/53817; WO 98/53818; WO 98/54207; WO 98/58902; WO 99/06390; WO 99/06431; WO 99/06432; WO 99/06433; WO 99/06434; WO 99/06435; WO 99/06436; WO 99/06437; WO 99/10312; WO 99/10313; WO 99/20272; WO 99/23063; WO 99/24398; WO 99/25685; WO 99/26615; WO 99/26921; WO 99/26922; WO 99/26923; WO 99/35163; WO 99/36393; WO 99/37605; WO 99/37618; WO 99/43642; WO 01/42215; and WO 02/28830; all of which are incorporated by reference herein. Additional examples of α4 integrin antagonists include the phenylalanine derivatives described in: U.S. Patent Nos. 6,197,794; 6,229,011; 6,329,372; 6,388,084; 6,348,463; 6,362,204; 6,380,387; 6,445,550; 6,806,365; 6,835,738; 6,855,706; 6,872,719; 6,878,718; 6,911,451; 6,916,933; 7,105,520; 7,153,963; 7,160,874; 7,193,108; 7,250,516; and 7,291,645 (each herein incorporated by reference). Additional amino acid derivatives that are α4 integrin antagonists include those described in, e.g., U.S. Patent Application Publication Nos. 2004/0229859 and 2006/0211630 (herein incorporated by reference), and PCT Publication Nos. WO 01/36376; WO 01/47868; and WO 01/70670; all of which are incorporated by reference herein. Other examples of α4 integrin antagonists include the peptides, and the peptide and semi-peptide compounds described in, e.g., PCT Publication Nos. WO 94/15958; WO 95/15973; WO 96/00581; WO 96/06108; WO 96/22966 (Leu-Asp-Val tripeptide; Biogen, Inc.); WO 97/02289; WO 97/03094; and WO 97/49731. An additional example of an α4 integrin antagonist is the pegylated molecule described in U.S. Patent Application Publication No. 2007/066533 (herein incorporated by reference). Examples of antibodies that are α4 integrin antagonists include those described in, e.g.,

PCT Publication Nos. WO 93/13798; WO 93/15764; WO 94/16094; and WO 95/19790. Additional examples of α4 integrin antagonists are described herein.

By "interferon" is meant a mammalian (e.g., a human) interferon -alpha, -beta, -gamma, or –tau polypeptide, or biologically-active fragment thereof, e.g., IFN-α (e.g., IFN-α-1a; see U.S. Patent Application No. 20070274950, incorporated herein by reference in its entirety), IFN-α-1b, IFN-α-2a (see PCT Application No. WO 07/044083, herein incorporated by reference in its entirety), and IFN-α-2b), IFN-β (e.g., described in U.S. Patent No. 7,238,344, incorporated by reference in its entirety; IFN-b-1a (AVONEX® and REBIF®), as described in U.S. Patent No. 6,962,978, incorporated by reference in its entirety, and IFN-β-1b (BETASERON®, as described in U.S. Patent Nos. 4,588,585; 4,959,314; 4,737,462; and 4,450,103; incorporated by reference in their entirety), IFN-g, and IFN-t (as described in U.S. Patent No. 5,738,845 and U.S. Patent Application Publication Nos. 20040247565 and 20070243163; incorporated by reference in their entirety).

By "HSA linker conjugate" is meant a human serum albumin (HSA) linker protein of the invention in combination with one or more binding moieties, polypeptide connectors, diagnostic agents, or therapeutic agents.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to conventional (polyclonal) antibody preparations which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. Monoclonal antibodies can be prepared using any art recognized technique and those described herein such as, for example, a hybridoma method, as described by Kohler *et al.*, *Nature* 256:495 (1975), a transgenic animal (*e.g.*, Lonberg *et al.*, *Nature* 368(6474):856-859 (1994)), recombinant DNA methods (*e.g.*, U.S. Pat. No. 4,816,567), or using phage, yeast, or synthetic scaffold antibody libraries using the techniques described in, for example, Clackson *et al.*, *Nature* 352:624-628 (1991) and Marks *et al.*, *J. Mol. Biol.* 222:581-597 (1991).

By "pharmaceutically acceptable carrier" is meant a carrier which is physiologically acceptable to the treated mammal while retaining the therapeutic properties of the compound with which it is administered. One exemplary pharmaceutically acceptable carrier is physiological saline.

Other physiologically acceptable carriers and their formulations are known to one skilled in the art and described, for example, in *Remington's Pharmaceutical Sciences*, (18th edition), ed. A. Gennaro, 1990, Mack Publishing Company, Easton, PA incorporated herein by reference.

By "proliferative disease" or "cancer" is meant any condition characterized by abnormal or unregulated cell growth. Examples of proliferative diseases include, for example, solid tumors such as: sarcomas (e.g., clear cell sarcoma), carcinomas (e.g., renal cell carcinoma), and lymphomas; tumors of the breast, colon, rectum, lung, oropharynx, hypopharynx, esophagus, stomach, pancreas, liver, bilecyst, bile duct, small intestine, urinary system (including the kidney, bladder, and epithelium of the urinary tract), female genital system (including the uterine neck, uterus, ovary, chorioma, and gestational trophoblast), male genital system (including the prostate, seminal vesicle, and testicles), endocrine glands (including the thyroid gland, adrenal gland, and pituitary body), skin (including angioma, melanoma, sarcoma originating from bone or soft tissue, and Kaposi's sarcoma), brain and meninges (including astrocytoma, neuroastrocytoma, spongioblastoma, retinoblastoma, neuroma, neuroblastoma, neurinoma and neuroblastoma), nerves, eyes, hemopoietic system (including chloroleukemia, plasmacytoma and dermal T lymphoma/leukemia), and immune system (including lymphoma, e.g., Hodgkin's lymphoma and non-Hodgkin's lymphoma). An example of a non-solid tumor proliferative disease is leukemia (e.g., acute lymphoblastic leukemia).

The term "recombinant antibody," refers to an antibody prepared, expressed, created, or isolated by recombinant means, such as (a) antibodies isolated from an animal (e.g., a mouse) that is transgenic or transchromosomal for immunoglobulin genes (e.g., human immunoglobulin genes) or a hybridoma prepared therefrom, (b) antibodies isolated from a host cell transformed to express the antibody, e.g., from a transfectoma, (c) antibodies isolated from a recombinant, combinatorial antibody library (e.g., containing human antibody sequences) using phage, yeast, or synthetic scaffold display, and (d) antibodies prepared, expressed, created, or isolated by any other means that involve splicing of immunoglobulin gene sequences (e.g., human immunoglobulin genes) to other DNA sequences.

By "specifically bind" is meant the preferential association of a binding moiety (e.g., an antibody, antibody fragment, receptor, ligand, or small molecule portion of an agent of the invention) to a target molecule (e.g., a secreted target molecule, such as a cytokine, chemokine, hormone, receptor, or ligand) or to a cell or tissue bearing the target molecule (e.g., a cell surface antigen, such as a receptor or ligand) and not to non-target cells or tissues lacking the target molecule. It is

recognized that a certain degree of non-specific interaction may occur between a binding moiety and a non-target molecule (present alone or in combination with a cell or tissue). Nevertheless, specific binding may be distinguished as mediated through specific recognition of the target molecule. Specific binding results in a stronger association between the binding moiety (e.g., an antibody) and e.g., cells bearing the target molecule (e.g., an antigen) than between the binding moiety and e.g., cells lacking the target molecule. Specific binding typically results in greater than 2-fold, preferably greater than 5-fold, more preferably greater than 10-fold and most preferably greater than 100-fold increase in amount of bound binding moiety (per unit time) to e.g., a cell or tissue bearing the target molecule or marker as compared to a cell or tissue lacking that target molecule or marker. Binding moieties bind to the target molecule or marker with a dissociation constant of e.g., less than 10^{-6}M, more preferably less than 10^{-7}M, 10^{-8}M, 10^{-9}M, 10^{-10}M, 10^{-11}M, or 10^{-12}M, and most preferably less than 10^{-13}M, 10^{-14}M, or 10^{-15}M. Specific binding to a protein under such conditions requires a binding moiety that is selected for its specificity for that particular protein. A variety of assay formats are appropriate for selecting binding moieties (e.g., antibodies) capable of specifically binding to a particular target molecule. For example, solid-phase ELISA immunoassays are routinely used to select monoclonal antibodies specifically immunoreactive with a protein. See Harlow & Lane, Antibodies, A Laboratory Manual, Cold Spring Harbor Publications, New York (1988), for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity.

The term "substantial identity" or "substantially identical," when used in the context of comparing a polynucleotide or polypeptide sequence to a reference sequence, means that the polynucleotide or polypeptide sequence is the same as the reference sequence or has a specified percentage of nucleotides or amino acid residues that are the same at the corresponding locations within the reference sequence when the two sequences are optimally aligned. For instance, an amino acid sequence that is "substantially identical" to a reference sequence has at least about 60% identity, preferably 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or higher percentage identity (up to 100%) to the reference sequence (e.g., the HSA amino acid sequence as set forth in SEQ ID NO:1, or a fragment thereof), when compared and aligned for maximum correspondence over the full length of the reference sequence as measured using a BLAST or BLAST 2.0 sequence comparison algorithms with default parameters, or by manual alignment and visual inspection (see, e.g., NCBI web site).

By "surface-exposed amino acid residue" or "surface-exposed" is meant an amino acid residue that is present on the exterior face of the folded and conformationally-correct tertiary structure of a HSA polypeptide. Such residues can be substituted with e.g., other, chemically-reactive, amino acids (e.g., cysteine) to allow for site-specific conjugation of diagnostic or therapeutic agents. Additionally, surface-exposed amino acid residues can be substituted to allow (e.g., by addition of serine, threonine, or asparagine residues, or glycosylation motifs) or prevent (e.g., by removal of serine, threonine, or asparagine residues, or glycosylation motifs) glycosylation. Surface-exposed amino acid residues include, but are not limited to, threonine at position 496, serine at position 58, threonine at position 76, threonine at position 79, threonine at position 83, threonine at position 125, threonine at position 236, serine at position 270, serine at position 273, serine at position 304, serine at position 435, threonine at position 478, threonine at position 506, and threonine at position 508 (amino acid numbering is relative to e.g., the sequence of the HSA linker set forth in SEQ ID NO:1). Other surface-exposed residues can be identified by the skilled artisan using the HSA crystal structure (Sugio *et al.*, "Crystal structure of human serum albumin at 2.5 A resolution," *Protein Eng.* 12:439-446 (1999)).

A "target molecule" or "target cell" is meant a molecule (e.g., a protein, epitope, antigen, receptor, or ligand) or cell to which a binding moiety (e.g., an antibody), or an HSA conjugate that contains one or more binding moieties (e.g., an HSA linker bonded to one or more antibodies or antibody fragments) can specifically bind. A target molecule can be present on the exterior of a target cell (e.g., a cell-surface or secreted protein) or in the interior of a target cell.

By "treating" is meant the reduction (e.g., by at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 60%, 70%, 80%, 90%, 95%, 99%, or even 100%) in the progression or severity of a human disease or disorder (e.g., an autoimmune or proliferative disease), or in the progression, severity, or frequency of one or more symptoms of the human disease or disorder in a human patient.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is an illustration showing the schematic representation of an exemplary HSA linker conjugate. The connector between the amino terminal binding moiety and the HSA linker has a sequence of alanine, alanine and serine. The connector between the HSA linker and the carboxy terminal binding moiety has a sequence of alanine, alanine, alanine, leucine (SEQ ID NO:5).

Figure 2 is a graph showing that B2B3 variants inhibit HRG-induced pErbB3 in ZR75-1 breast cancer cells.

Figures 3A-D are graphs showing the inhibition of phosphorylated ErbB3 in BT474 breast cancer cells following 24 hour pre-treatment with the B2B3 variants A5-HSA-B1D2 (Figure 3A), H3-HSA-B1D2 (Figure 3B), H3-HSA-F5B6H2 (Figure 3C), and F4-HSA-F5B6H2 (Figure 3D).

Figures 4A-D are graphs showing the inhibition of phosphorylated AKT in BT474 breast cancer cells following 24 hour pre-treatment with the B2B3 variants A5-HSA-B1D2 (Figure 4A), H3-HSA-B1D2 (Figure 4B), H3-HSA-F5B6H2 (Figure 4C), and F4-HSA-F5B6H2 (Figure 4D).

Figures 5A-D are graphs showing that inhibition of phosphorylated ERK in BT474 breast cancer cells following 24 hour pre-treatment with the B2B3 variants A5-HSA-B1D2 (Figure 5A), H3-HSA-B1D2 (Figure 5B), H3-HSA-F5B6H2 (Figure 5C), and F4-HSA-F5B6H2 (Figure 5D).

Figure 6 is a graph showing that treatment of BT474 breast cancer cells with B2B3-1 variants causes G1 arrest and a decrease in the number of cells in S phase.

Figure 7 is a flow cytometry histogram showing that pre-incubation of BT-474-M3 cells with 1 μM B2B3-1 substantially blocks binding of HRG.

Figures 8A-D are graphs showing that B2B3-1 inhibits ErbB3 and AKT phosphorylation in B-T474-M3 and ZR75-30 cell lines. Breast cancer cell lines BT-474-M3 (Figures 8A and 8C) and ZR75-3 0 (Figures 8B and 8D) were pre-treated with a dose titration of B2B3-1 for 24 hours and then stimulated for 10 minutes with 5 nM of HRG 1β EGF domain. The phosphorylation status of ErbB3 and AKT was then examined using an ELISA assay.

Figure 9 is a photograph of a Western blot that shows the effect of B2B3-1 treatment on signaling proteins in BT474 breast cancer cells.

Figure 10 is a photograph of a Western blot that shows the immunoprecipitation of B2B3-1 treated BT474 breast cancer cells.

Figures 11A-C are graphs showing B2B3-1 treatment of BT-474 cell line causes G1 arrest and a decrease in the population of cells in S phase (Figure 11A), and inhibits colony formation in both BT-474 and SKBr3 cells compared to untreated cells (Figure 11B). In addition, B2B3-1 inhibits proliferation of BT-474-M3 cells in a cell impedance assay (Figure 11C).

Figure 12 is a graph showing that B2B3-1 does not stimulate ErbB3 phosphorylation in ZR75-1 cells.

Figures 13A-B are graphs showing that B2B3-1 binds specifically to ErbB3 (Figure 13A) and ErbB2 (Figure 13B).

Figure 14 is a graph showing that avidity binding of B2B3-1 to MALME-3 cells results in a significant increase in apparent binding affinity compared to ErbB2-only binding variant, SKO-3, and ErbB3-only binding variant, SKO-2.

Figures 15A-C are graphs showing the stability of B2B3-1 in mouse, Cynomolgus monkey, and human serum. 100 nM B2B3-1 was incubated in mouse (Figure 15A), Cynomolgus monkey (Figure 15B), or human (Figure 15C) serum at 37°C for a period of 120 hours. Samples were removed at 0, 24, 48, 72, 96 and 120 hours and the ability of B2B3-1 to bind both ErbB2 and ErbB3 was measured by ELISA (RLU = relative light units).

Figures 16 is a graph showing B2B3-1 dose response in a BT-474-M3 breast cancer xenograft model. The relationship of B2B3-1 dose on tumor response was assessed in the BT-474-M3 breast tumor line at the doses indicated. HSA was given at 52.5 mg/kg, which is an equimolar dose to the 90 mg/kg B2B3-1 dose.

Figures 17A-E are graphs showing that B2B3-1 is effective in multiple xenograft models in an ErbB2 dependent manner. Calu-3 (human lung adenocarcinoma; Figure 17A), SKOV-3 (human ovarian adenocarcinoma; Figure 17B), NCI-N87 (human gastric carcinoma; Figure 17C), ACHN (human kidney adenocarcinoma; Figure 17D), and MDA-MB-361 (human breast adenocarcinoma; Figure 17E) xenograft model are shown. Mice were treated with 30 mg/kg of B2B3-1 every 3 days or HAS control at an equimolar dose to B2B3-1.

Figures 18A-B are graphs showing that the over-expression of ErbB2 converts B2B3-1 non-responder ADRr breast cancer xenograft model into a responder. ErbB2 was over-expressed in wild type ADRr xenografts (Figure 18A) and ADRr-E2 xenografts (Figure 18B) using a retroviral expression system.

Figures 19A-B are graphs showing that B2B3-1 activity correlates positively with ErbB2 expression levels *in vitro* (Figure 19A) and *in vivo* (Figure 19B).

Figure 20A-B show that B2B3-1 treatment modifies tumor cell cycling. Figure 20A includes fluorescent micrographs showing that B2B3-1 treatment of BT474-M3 breast tumor cells for 6 hours results in translocation of cell cycle inhibitor $p27^{kip1}$ to the nucleus. Hoechst stain was used to identify the nucleus. Figure 20B is a Western blot of BT-474-M3 cells treated with B2B3-1 for 72

hours, which resulted in a decrease in the levels of the cell cycle regulator Cyclin D1. The cytoskeleton protein vinculin was probed as a protein loading control in this experiment.

Figures 21A-B are micrographs showing that B2B3-1 treatment of BT474 breast tumor xenografts results in translocation of $p27^{kip1}$ to the nucleus. BT474 breast tumor xenografts were treated with B2B3-1 (Figure 21A) at a dose of 30 mg/kg or an equimolar dose of HSA (Figure 21B) every 3 days for a total of 4 doses and stained for $p27^{kip1}$.

Figure 22A-B are fluorescent micrographs showing that B2B3-1 treatment results in a reduction of the proliferation marker Ki67 in BT474-M3 breast cancer xenograft. BT474-M3 breast tumor xenografts were treated with B2B3-1 (Figure 22A) at a dose of 30 mg/kg or an equimolar dose of HSA (Figure 22B) every 3 days for a total of 4 doses.

Figures 23A-B are fluorescent micrographs showing that B2B3-1 treatment results in a reduction of vessel density in BT474-M3 breast cancer xenograft tumors (as determined by a reduction in CD31 staining). BT474-M3 breast tumor xenografts were treated with B2B3-1 (Figure 23A) at a dose of 30 mg/kg or an equimolar dose of HSA (Figure 23B) every 3 days for a total of 4 doses.

Figures 24A-B are graphs showing that B2B3-1 inhibits phosphorylation of ErbB3 *in vivo*. Lysates from individual BT-474-M3 xenograft tumors treated with B2B3-1 (M1-M5) or control HSA (H1-H2) were subjected to SDS-PAGE and probed for pErbB3 and beta tubulin using Western blot analysis (Figure 24A). Normalization of the mean pErbB3 signal to the mean beta tubulin signal demonstrated that B2B3-1 treated tumors contained significantly less pErbB3 than HSA tumors (Figure 24B).

Figures 25A and B are graphs showing the *in vivo* activity of B2B3-1 in BT-474-M3 xenografts which have reduced PTEN activity. Cultured BT-474-M3 tumor cells were transfected with a control vector (Figure 25A) or with a retroviral vector encoding shPTEN (Figure 25B), which knocks out PTEN activity. BT-474-M3 breast cancer cells engineered to knock out PTEN activity were injected into the right flank of mice, while cells transfected with control vector were injected into the left flank of the same mouse. Mice were treated with 30mg/kg B2B3-1 every 3 days or 10mg/kg Herceptin every week and HSA was injected as a control at an equimolar dose to B2B3-1. B2B3-1 and Herceptin promoted a reduction in the size of tumors formed by control BT-474-M3 breast cancer cells (Figure 25A), whereas only B2B3-1

(and not Herceptin) promoted a reduction in the size of tumors formed by BT-474-M3 breast cancer cells lacking expression of PTEN (Figure 25B).

Figures 26A-B show that B2B3-1 inhibits phosphorylation of AKT in BT-474-M3 xenografts that have reduced PTEN activity. Tumors were lysed following the completion of treatment (q3dx11) and tested for PTEN, pErbB3, and pAKT expression levels by Western blot analysis (Figure 26A). Densitometry on the band intensity for pAKT normalized to total AKT and total protein demonstrate that B2B3-1 was able to inhibit phosphorylation of this protein, when Herceptin did not (Figure 26B).

Figures 27A-D are graphs showing the single dose pharmacokinetic properties of 5 (Figure 27A), 15 (Figure 27B), 30 (Figure 27C), and 45 (Figure 27D) mg/kg bolus dose of B2B3-1 in nu/nu mice. B2B3-1 serum concentrations are comparable measured by the HSA assay or ErbB2/ErbB3 assay, indicating that the antigen binding activity of B2B3-1 is retained in circulation.

Figure 28 is a graph showing the dose-exposure relationship for 5, 15, 30, and 45 mg/kg bolus doses of B2B3-1 in nude mice. Increases in dose result in a linear increase in overall exposure to B2B3-1.

Figure 29 is a graph showing the B2B3-1 serum concentrations measured in Cynomolgus monkeys dosed every three days for 4 doses with 4 mg/kg (n=2), 20 mg/kg (n=2) and 200 mg/kg (up to 336 hour n=4, for 384, 552 and 672 hour time points n=2).

Figure 30 is an illustration of the B2B3-1 expression plasmid pMP10k4H3-mHSA-B1D2.

Figure 31 is an illustration of the neomycin resistance plasmid pSV2-neo.

Figure 32 is an illustration of the hygromycin resistance plasmid pTK-Hyg.

DETAILED DESCRIPTION OF THE INVENTION

This invention provides a human serum albumin (HSA) linker which, when used to produce an agent of the invention (e.g., a binding, diagnostic, or therapeutic agent) exhibits, for example, an increased *in vivo* half-life of between 6 hours and 7 days and which does not induce significant humoral or cell-mediated immune responses when administered *in vivo* to a mammal (e.g., a human). In one aspect, the invention provides a mutated HSA linker that has two defined amino acid substitutions (i.e., the "C34S" and "N503Q" substitutions, as set forth in SEQ ID NO:1). In another aspect, the invention provides an HSA linker bonded to one or more binding moieties (e.g.,

antibodies, antibody fragments, receptor/ligands, or small molecules) for diagnostic or therapeutic applications in a mammal (e.g., a human) *in vivo* or for use *in vitro* in connection with mammalian cells, tissues, or organs. In a further aspect, the HSA linker may be coupled to one or more immunomodulatory agents, cytotoxic or cytostatic agents, detectable labels, or radioactive agents for diagnostic or therapeutic applications in a mammal (or in connection with a mammalian cell, tissue, or organ). An agent of the invention, which includes the HSA linker, can be optionally combined with one or more pharmaceutically acceptable carriers or excipients and can be formulated to be administered intravenously, intramuscularly, orally, by inhalation, parenterally, intraperitoneally, intraarterially, transdermally, sublingually, nasally, through use of suppositories, transbuccally, liposomally, adiposally, opthalmically, intraocularly, subcutaneously, intrathecally, topically, or locally. An agent of the invention which includes the HSA linker can be combined or coadministered with one or more biologically-active agents (e.g., biological or chemical agents, such as chemotherapeutics and antineoplastic agents). In a further aspect, the invention provides a kit, with instructions, for the conjugation of binding moieties (e.g., antibodies, antibody fragments, receptors or ligands), immunomodulatory agents, cytotoxic or cytostatic agents, detectable labels, or radioactive agents to the HSA linker of the invention to prepare agents of the invention that can be used for diagnostic or therapeutic applications.

A Mutated Human Serum Albumin (HSA) Linker

A mutated HSA linker of the invention contains two amino acid mutations, at positions 34 and 503, relative to the wild-type HSA amino acid sequence set forth in SEQ ID NO:3. The cysteine residue at position 34 (i.e., C34) can be mutated to any amino acid residue other than cysteine (e.g., serine, threonine, or alanine). Likewise, the asparagine residue at position 503 (i.e., N503) can be mutated to any amino acid residue other than asparagine (e.g., glutamine, serine, histidine, or alanine). In one embodiment, the HSA linker of the invention has the the amino acid and corresponding nucleotide sequence set forth in SEQ ID NOS:1 and 2, respectively. This mutated HSA linker of the invention contains two amino acid substitutions (i.e., serine for cysteine at amino acid residue 34 ("C34S") and glutamine for asparagine at amino acid residue 503 ("N503Q")). The HSA linker, when bonded to one or more binding moieties (e.g., antibodies, antibody fragments (e.g., single chain antibodies), or other targeting or biologically active agents (e.g., receptors and ligands)), confers several beneficial pharmacologic properties to those conjugates and to additional

diagnostic or therapeutic agents also conjoined (e.g., immunomodulatory agents, cytotoxic or cytostatic agents, detectable labels, or radioactive agents)) relative to the pharmacologic properties of these agents in the absence of the HSA linker of the invention. These benefits can include decreased immunogenicity (e.g., decreased host antibody neutralization of linker-antibody conjugates), increased detection of HSA linker conjugates (e.g., by mass spectroscopy) and increased pharmacologic half-life (e.g., a half-life greater than 6 hours, 8 hours, 12 hours, 24 hours, 36 hours, 2 days, 3 days, 4 days, 5 days, 6 days, or 7 days) when administered to a mammal (e.g., a human). Specifically, the substitution of serine for cysteine at amino acid residue 34 results in reduced oxidation and protein heterogeneity of the HSA linker. In wild-type HSA, the asparagine at amino acid residue 503 is sensitive to deamination, likely resulting in reduced pharmacologic half-life. The substitution of glutamine for asparagine at amino acid residue 503 can result in increased pharmacologic half-life of the HSA linker of the invention, and correspondingly, of conjugate agents that include the HSA linker when administered to a mammal (e.g., a human) or cells, tissues, or organs thereof.

In other embodiments, the mutated HSA linker of the invention includes domain I of HSA (SEQ ID NO:53; residues 1-197 of SEQ ID NO:1), domain III of HSA (SEQ ID NO:55; residues 381-585 of SEQ ID NO:1), combination of domains I and III of HSA, or a combination of domain I or III of HSA with domain II of HSA (SEQ ID NO:54; residues 189-385 of SEQ ID NO:1). For example, an HSA linker of the invention can include domains I and II, I and III, or II and III. In addition, the cysteine residue at position 34 (i.e., C34) of domain I (SEQ ID NO:53) can be mutated to any amino acid residue other than cysteine (e.g., serine, threonine, or alanine). Likewise, the asparagine residue at position 503 (i.e., N503) of domain III (SEQ ID NO:55) can be mutated to any amino acid residue other than asparagine (e.g., glutamine, serine, histidine, or alanine). These HSA linkers can be incorporated into an HSA linker conjugate of the invention, which includes one or more of a polypeptide connector, a binding moiety, and therapeutic or diagnostic agents, each of which is described in detail below.

Polypeptide Connectors

To facilitate the conjugation of binding moieties, as defined herein, to the HSA linker of the invention, short (e.g., 2-20 amino acids in length) polypeptide connectors that can be bonded (e.g, covalently (e.g., a peptidic bond), ionically, or hydrophobically bonded, or via a high-affinity

protein-protein binding interaction (e.g., biotin and avidin)) to the amino or carboxy termini of an HSA linker. These connectors provide flexible tethers to which any of the binding moieties described herein can be attached. A polypeptide connector may be 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more amino acids in length. In one embodiment, the connector is a sequence of, e.g., glycine, alanine, serine, glutamine, leucine, or valine residues. Although not specifically enumerated herein, the connector of the invention can be solely glycine, alanine, serine, glutamine, leucine, or valine residues, or it may be any combination of these residues up to about 20 amino acids in length. In a preferred embodiment, the connector attached to the amino terminus of an HSA linker has the amino acid sequence "AAS" or "AAQ" and the connector attached to the carboxy terminus has the amino acid sequence "AAAL" (SEQ ID NO:5). The connector can be covalently bound to the amino or carboxy terminal residue of the HSA linker, to an amino acid residue within the HSA linker, or can be included between one or more binding moieties, if present.

HSA Linker Manufacture

The HSA linker of the invention, which may optionally include the peptide connectors described above, one or more of the binding moieties described below, polypeptide-based detectable labels, and other polypeptide-based therapeutic agents, can be produced recombinantly. For example, a nucleotide sequence encoding the HSA linker (and one or more of the optional elements) may be expressed (e.g., in a plasmid, viral vector, or transgenically) in a bacterial (e.g., *E. coli*), insect, yeast, or mammalian cell (e.g., a CHO cell), or a mammalian tissue, organ, or organism (e.g., a transgenic rodent, ungulate (e.g., a goat), or non-human primate). After expression of the HSA linker in the host cell, tissue, or organ, the skilled artisan may isolate and purify the HSA linker using standard protein purification methods (e.g., FPLC or affinity chromatography). A recombinant expression system for the production of an HSA linker in combination with two binding moieties is illustrated in Figure 1.

The HSA linker of the invention, which may include one or more of the optional elements described above, can be synthetically produced. For example, the HSA linker can be prepared by techniques generally established in the art of peptide synthesis, such as the solid-phase approach. Solid-phase synthesis involves the stepwise addition of amino acid residues to a growing peptide chain that is linked to an insoluble support or matrix, such as polystyrene. The C-terminus residue of the peptide is first anchored to a commercially available support with its amino group protected with

an N-protecting agent such as a t-butyloxycarbonyl group (tBoc) or a fluorenylmethoxycarbonyl (FMOC) group. The amino-protecting group is removed with suitable deprotecting agents such as TFA in the case of tBOC or piperidine for FMOC and the next amino acid residue (in N-protected form) is added with a coupling agent such as dicyclocarbodiimide (DCC). Upon formation of a peptide bond, the reagents are washed from the support. After addition of the final residue, the agent is cleaved from the support with a suitable reagent, such as trifluoroacetic acid (TFA) or hydrogen fluoride (HF). If desired, the HSA linker, which may include one or more of the optional elements described above, can be manufactured in one, two, three, or more segments, which can then be ligated to form the whole HSA linker construct.

HSA Linker Binding Moieties

The HSA linker of the invention can also be prepared as a construct that includes one or more binding moieties, such as antibodies, antibody fragments (as defined herein, e.g., a single chain Fv (scFv)) or receptor/ligands (i.e., protein or glycoprotein ligands or receptors) that allow selective and specific binding of the HSA linker construct to a target cell, tissue, or organ. The binding moieties can be bonded to the HSA linker (e.g., via a covalent (e.g., a peptide bond), ionic, or hydrophobic bond, or via a high-affinity protein-protein binding interaction (e.g., biotin and avidin)). As is discussed above, an HSA linker of the invention that includes one or more polypeptide connectors or binding moieties can be recombinantly or synthetically produced.

One or more binding moieties can be bonded to an HSA linker of the invention. In one embodiment, two or more identical binding moieties (i.e., moieties having the same structure and binding affinities) are bonded to an HSA linker, one or more (e.g., in tandem) each at the amino and carboxy termini, the HSA linker thereby affording improved avidity of the binding moieties for their target antigen. Alternatively, two or more different binding moieties (e.g., an antibody, such as a scFv, with binding affinities for two or more different target molecules, or scFv with binding affinities for two or more different epitopes on the same target molecule) can be bonded to an HSA linker (e.g., a bispecific HSA linker conjugate) to allow multiple target antigens or epitopes to be bound by the HSA linker conjugate. In another embodiment, different species of binding moieties can also be bonded to an HSA linker to bestow, for example, two or more different binding specificities or agonistic/antagonistic biological properties on the linker conjugate. Useful combinations of binding moiety pairs for use in the preparation of bispecific HSA linker conjugates

are disclosed in, e.g., International Patent Application Publications WO 2006/091209 and WO 2005/117973, herein incorporated by reference. In other embodiments, more than two binding moieties (e.g., the same or different binding moieties) can be bonded to an HSA linker to form an agent of the invention.

The invention features an agent having at least first and second binding moieties, each of which can be bound at either the amino or carboxy terminus of the HSA linker protein, or to polypeptide connectors, as defined herein, present at either or both termini. Figure 1 illustrates an exemplary mutated HSA linker of the invention in which two binding moieties ("arm 1" and "arm 2") are bonded to the mutated HSA linker by the amino terminal polypeptide connector AAS and carboxy terminal polypeptide connector AAAL (SEQ ID NO:5). Binding moieties (e.g., an antibody or scFv) can also be bound to other loci (e.g., internal amino acid residues of the HSA linker), for example, covalently or ionically, e.g., using biotin-avidin interactions. Biotinylation of amine (e.g., lysine residues) and sulfhydryl (e.g., cysteine residues) amino acid side chains is known in the art and can be used to attach binding moieties to the HSA linker.

Binding moieties that can be included in an HSA linker conjugate of the invention include antibodies, antibody fragments, receptors, and ligands. Binding moieties bound to an HSA linker of the invention may be recombinant (e.g., human, murine, chimeric, or humanized), synthetic, or natural. The invention features complete antibodies, domain antibodies, diabodies, bi-specific antibodies, antibody fragments, Fab fragments, F(ab')2 molecules, single chain Fv (scFv) molecules, tandem scFv molecules, antibody fusion proteins, and aptamers.

Antibodies

Antibodies of the invention include the IgG, IgA, IgM, IgD, and IgE isotypes. Antibodies or antibody fragments of the invention, as used herein, contain one or more complementarity determining regions (CDR) or binding peptides that bind to target proteins, glycoproteins, or epitopes present on the exterior or in the interior of target cells.

Many of the antibodies, or fragments thereof, described herein can undergo non-critical amino-acid substitutions, additions or deletions in both the variable and constant regions without loss of binding specificity or effector functions, or intolerable reduction of binding affinity (e.g., below about 10^{-7} M). Usually, an antibody or antibody fragment incorporating such alterations exhibits substantial sequence identity to a reference antibody or antibody fragment from which it is derived.

Occasionally, a mutated antibody or antibody fragment can be selected having the same specificity and increased affinity compared with a reference antibody or antibody fragment from which it was derived. Phage-display technology offers powerful techniques for selecting such antibodies. See, e.g., Dower *et al.*, WO 91/17271 McCafferty *et al.*, WO 92/01047; and Huse, WO 92/06204, incorporated by reference herein.

The HSA linker of the invention can also be bonded to one or more fragments of an antibody that retain the ability to bind with specificity to a target antigen. Antibody fragments include separate variable heavy chains, variable light chains, Fab, Fab', $F(ab')_2$, Fabc, and scFv. Fragments can be produced by enzymatic or chemical separation of intact immunoglobulins. For example, a $F(ab')_2$ fragment can be obtained from an IgG molecule by proteolytic digestion with pepsin at pH 3.0-3.5 using standard methods such as those described in Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Pubs., N.Y. (1988). Fab fragments may be obtained from $F(ab')_2$ fragments by limited reduction, or from whole antibody by digestion with papain in the presence of reducing agents. Fragments can also be produced by recombinant DNA techniques. Segments of nucleic acids encoding selected fragments are produced by digestion of full-length coding sequences with restriction enzymes, or by *de novo* synthesis. Often fragments are expressed in the form of phage-coat fusion proteins. This manner of expression is advantageous for affinity-sharpening of antibodies.

Humanized Antibodies

The invention further provides for humanized antibodies in combination with the HSA linker of the invention, in which one or more of the antibody CDRs are derived from a non-human antibody sequence, and one or more, but preferably all, of the CDRs bind specifically to an antigen (e.g., a protein, glycoprotein, or other suitable epitope).

A humanized antibody contains constant framework regions derived substantially from a human antibody (termed an acceptor antibody), as well as, in some instances, a majority of the variable region derived from a human antibody. One or more of the CDRs (all or a portion thereof, as well as discreet amino acids surrounding one or more of the CDRs) are provided from a non-human antibody, such as a mouse antibody. The constant region(s) of the antibody, may or may not be present.

The substitution of one or more mouse CDRs into a human variable domain framework is most likely to result in retention of their correct spatial orientation if the human variable domain framework adopts the same or a similar conformation as the mouse variable framework from which the CDRs originated. This is achieved by obtaining the human variable domains from human antibodies whose framework sequences exhibit a high degree of sequence and structural identity with the murine variable framework domains from which the CDRs were derived. The heavy and light chain variable framework regions can be derived from the same or different human antibody sequences. The human antibody sequences can be the sequences of naturally occurring human antibodies, consensus sequences of several human antibodies, or can be human germline variable domain sequences. See, e.g., Kettleborough *et al.*, *Protein Engineering* 4:773 (1991); Kolbinger *et al.*, *Protein Engineering* 6:971 (1993).

Suitable human antibody sequences are identified by alignments of the amino acid sequences of the mouse variable regions with the sequences of known human antibodies. The comparison is performed separately for heavy and light chains but the principles are similar for each.

Methods of preparing chimeric and humanized antibodies and antibody fragments are described in, e.g., U.S. Patent Nos. 4,816,567, 5,530,101, 5,622,701, 5,800,815, 5,874,540, 5,914,110, 5,928,904, 6,210,670, 6,677,436, and 7,067,313 and U.S. Patent Application Nos. 2002/0031508, 2004/0265311, and 2005/0226876. Preparation of antibody or fragments thereof is further described in, e.g., U.S. Patent Nos. 6,331,415, 6,818,216, and 7,067,313.

Receptors and Ligands

The invention provides for protein or glycoprotein receptors or ligands bound to an HSA linker of the invention. HSA linkers bonded with a receptor or ligand can be used, for example, to specifically target a secreted protein, a cell (e.g., a cancer cell), tissue, or organ. Furthermore, the specific binding of the HSA linker-receptor or ligand conjugate to cognate target receptors or ligands can cause agonistic or antagonistic biological activity in intracellular or intercellular signaling pathways. As with the other binding moieties described herein, receptors and ligands, or fragments thereof, can be conjoined to the amino and/or carboxy termini of an HSA linker of the invention, or to an amino acid residue within the HSA linker.

Exemplary receptors and ligands that can be joined to an HSA linker of the invention include, but are not limited to, insulin-like growth factor 1 receptor (IGF1R), IGF2R, insulin-like

growth factor (IGF), mesenchymal epithelial transition factor receptor (c-met; also known as hepatocyte growth factor receptor (HGFR)), hepatocyte growth factor (HGF), epidermal growth factor receptor (EGFR), epidermal growth factor (EGF), heregulin, fibroblast growth factor receptor (FGFR), platelet-derived growth factor receptor (PDGFR), platelet-derived growth factor (PDGF), vascular endothelial growth factor receptor (VEGFR), vascular endothelial growth factor (VEGF), tumor necrosis factor receptor (TNFR), tumor necrosis factor alpha (TNF-α), TNF-β, folate receptor (FOLR), folate, transferrin receptor (TfR), mesothelin, Fc receptor, c-kit receptor, c-kit, α4 integrin, P-selectin, sphingosine-1-phosphate receptor-1 (S1PR), hyaluronate receptor, leukocyte function antigen-1 (LFA-1), CD4, CD11, CD18, CD20, CD25, CD27, CD52, CD70, CD80, CD85, CD95 (Fas receptor), CD106 (vascular cell adhesion molecule 1 (VCAM1), CD166 (activated leukocyte cell adhesion molecule (ALCAM)), CD178 (Fas ligand), CD253 (TNF-relatied apoptosis-inducing ligand (TRAIL)), ICOS ligand, CCR2, CXCR3, CCR5, CXCL12 (stromal cell-derived factor 1 (SDF-1)), interleukin 1 (IL-1), CTLA-4, receptors alpha and beta, MART-1, gp100, MAGE-1, ephrin (Eph) receptor, mucosal addressin cell adhesion molecule 1 (MAdCAM-1), carcinoembryonic antigen (CEA), $Lewis^Y$, MUC-1, epithelial cell adhesion molecule (EpCAM), cancer antigen 125 (CA125), prostate specific membrane antigen (PSMA), TAG-72 antigen, and biologically-active fragments thereof.

Receptors and ligands can be expressed, isolated, or joined to an HSA linker of the invention using any of the methods described *supra* relating to antibodies or antibody fragments.

Diagnostic Agents

The HSA linker of the invention, or any binding moiety conjugated thereto (e.g., antibody, antibody fragment, receptor, or ligand), can be coupled to a chelating agent or to a detectable label to form a diagnostic agent of the invention. Also contemplated are HSA linker conjugates that include a detectable label, as described herein, as well as one or more of the therapeutic agents or binding moieties described herein.

The HSA linker and chelator components can be coupled to form a conjugate by reacting the free amino group of a threonine residue of the HSA linker with an appropriate functional group of the chelator, such as a carboxyl group or activated ester. For example, a conjugate may be formed by incorporating the chelator ethylenediaminetetraacetic acid (EDTA), which is common in the art of coordination chemistry, when functionalized with a carboxyl substituent on the ethylene chain.

Synthesis of EDTA derivatives of this type are reported in Arya *et al.* (*Bioconjugate Chemistry* 2:323 (1991)), which describes blocking each of the four coordinating carboxyl groups with a t-butyl group while the carboxyl substituent on the ethylene chain is free to react with the amino group of a peptide portion of the agent of the invention, thereby forming a conjugate.

An HSA linker or an HSA linker conjugate may incorporate a metal chelator component that is peptidic, i.e., compatible with solid-phase peptide synthesis. In this case, the chelator may be coupled to the HSA linker of the invention in the same manner as EDTA described above or, more conveniently, the chelator and HSA linker or HSA linker conjugate of the invention are synthesized *in toto* starting from the C-terminal residue of the HSA linker or HSA linker conjugate and ending with the N-terminal residue of the chelator.

An HSA linker or an HSA linker conjugate may further incorporate a linking group component that serves to couple the HSA linker of the invention to the chelator while not adversely affecting the biological properties of the HSA linker, the targeting function of the binding moiety portion(s) of the HSA linker conjugate, or the metal binding function of the chelator. Suitable linking groups include amino acid chains and alkyl chains functionalized with reactive groups for coupling to the HSA linker or the HSA linker conjugate and to the chelator. An amino acid chain is the preferred linking group when the chelator is peptidic so that the HSA linker or HSA linker conjugate can be synthesized *in toto* by solid-phase techniques. An alkyl chain-linking group may be incorporated in the HSA linker or HSA linker conjugate by reacting the amino group of a threonine residue of a peptide portion of an HSA linker of the invention with a first functional group on the alkyl chain, such as a carboxyl group or an activated ester. Subsequently the chelator is attached to the alkyl chain to complete the formation of the HSA linker or HSA linker conjugate by reacting a second functional group on the alkyl chain with an appropriate group on the chelator. The second functional group on the alkyl chain is selected from substituents that are reactive with a functional group on the chelator while not being reactive with a threonine residue of the mutated HSA linker protein. For example, when the chelator incorporates a functional group such as a carboxyl group or an activated ester, the second functional group of the alkyl chain-linking group can be an amino group. It will be appreciated that formation of the HSA linker or HSA linker conjugate may require protection and deprotection of the functional groups present in order to avoid formation of undesired products. Protection and deprotection are accomplished using protecting groups, reagents, and

protocols common in the art of organic synthesis. Particularly, protection and deprotection techniques employed in solid phase peptide synthesis described above may be used.

An alternative chemical linking group to an alkyl chain is polyethylene glycol (PEG), which is functionalized in the same manner as the alkyl chain described above for incorporation in the HSA linker or HSA linker conjugate. It will be appreciated that linking groups may alternatively be coupled first to the chelator and then to the HSA linker or HSA linker conjugate of the invention.

In accordance with one aspect of the invention, HSA linker-chelator conjugates of the invention incorporate a diagnostically useful metal capable of forming a complex. Suitable metals include, e.g., radionuclides, such as technetium and rhenium in their various forms (e.g., $^{99m}TcO^{3+}$, $^{99m}TcO_2^{+}$, ReO^{3+}, and ReO_2^{+}). Incorporation of the metal within the HSA linker or HSA linker conjugate can be achieved by various methods common in the art of coordination chemistry. When the metal is technetium-99 m, the following general procedure may be used to form a technetium complex. An HSA linker-chelator conjugate solution is formed initially by dissolving the HSA linker or HSA linker conjugate in aqueous alcohol such as ethanol. The solution is then degassed to remove oxygen then thiol protecting groups are removed with a suitable reagent, for example, with sodium hydroxide, and then neutralized with an organic acid, such as acetic acid (pH 6.0-6.5). In the labeling step, a stoichiometric excess of sodium pertechnetate, obtained from a molybdenum generator, is added to a solution of the conjugate with an amount of a reducing agent such as stannous chloride sufficient to reduce technetium and heated. The labeled HSA linker or HSA linker conjugate may be separated from contaminants $^{99m}TcO_4^{-}$ and colloidal $^{99m}TcO_2$ chromatographically, for example, with a C-18 Sep Pak cartridge.

In an alternative method, labeling of an HSA linker of the invention can be accomplished by a transchelation reaction. The technetium source is a solution of technetium complexed with labile ligands facilitating ligand exchange with the selected chelator. Suitable ligands for transchelation include tartarate, citrate, and heptagluconate. In this instance the preferred reducing reagent is sodium dithionite. It will be appreciated that the HSA linker or HSA linker conjugate may be labeled using the techniques described above, or alternatively the chelator itself may be labeled and subsequently coupled to an HSA linker protein of the invention to form an HSA linker-chelator conjugate; a process referred to as the "prelabeled ligand" method.

Another approach for labeling an HSA linker of the invention, or any agent conjugated thereto, involves immobilizing the HSA linker-chelator conjugate on a solid-phase support through a

linkage that is cleaved upon metal chelation. This is achieved when the chelator is coupled to a functional group of the support by one of the complexing atoms. Preferably, a complexing sulfur atom is coupled to the support which is functionalized with a sulfur protecting group such as maleimide.

When labeled with a diagnostically useful metal, an agent of the invention that includes an HSA linker-chelator conjugate can be used to detect tissue at risk of developing cancer (e.g., lung cancer, breast cancer, colon cancer, and prostate cancer), age-related diseases (e.g., cardiovascular disease, cerebrovascular disease, or Alzheimer's disease), tobacco-related diseases (e.g., emphysema, aortic aneurysms, esophageal cancer, or squamous cell cancer of the head and neck) by procedures established in the art of diagnostic imaging. An agent of the invention that incorporates an HSA linker labeled with a radionuclide metal, such as technetium-99 m, may be administered to a mammal (e.g., a human) by intravenous injection in a pharmaceutically acceptable solution, such as isotonic saline, or by other methods described herein. The amount of a labeled agent of the invention appropriate for administration is dependent upon the distribution profile of the chosen HSA linker or HSA linker conjugate in the sense that an agent of the invention that incorporates a rapidly cleared HSA linker or HSA linker conjugate may be administered at higher doses than an agent that incorporates an HSA linker or HSA linker conjugate that clears less rapidly. Unit doses acceptable for imaging tissues are in the range of about 5-40 mCi for a 70 kg individual. The *in vivo* distribution and localization of an agent of the invention that incorporates a labeled HSA linker or HSA linker conjugate can be tracked by standard techniques described herein at an appropriate time subsequent to administration, typically between 30 minutes and 180 minutes and up to about 5 days depending upon the rate of accumulation at the target site with respect to the rate of clearance at non-target tissue.

An HSA linker of the invention, or any molecule or moiety conjugated thereto, can also be modified or labeled to facilitate diagnostic or therapeutic uses. Detectable labels such as a radioactive, fluorescent, heavy metal, or other molecules may be bound to any of the agents of the invention. Single, dual, or multiple labeling of an agent may be advantageous. For example, dual labeling with radioactive iodination of one or more residues combined with the additional coupling of, for example, ^{90}Y via a chelating group to amine-containing side or reactive groups, would allow combination labeling. This may be useful for specialized diagnostic needs such as identification of widely dispersed small neoplastic cell masses.

An HSA linker of the invention, or any molecule or moiety conjugated thereto, can also be modified, for example, by halogenation of the tyrosine residues of the peptide component. Halogens include fluorine, chlorine, bromine, iodine, and astatine. Such halogenated agents may be detectably labeled, e.g., if the halogen is a radioisotope, such as, for example, ^{18}F, ^{75}Br, ^{77}Br, ^{122}I, ^{123}I, ^{124}I, ^{125}I, ^{129}I, ^{131}I, or ^{211}At. Halogenated agents of the invention contain a halogen covalently bound to at least one amino acid, and preferably to D-Tyr residues in each agent molecule. Other suitable detectable modifications include binding of other compounds (e.g., a fluorochrome such as fluorescein) to a lysine residue of the agent of the invention, or analog, particularly an agent or analog having a linker including lysines.

Radioisotopes for radiolabeling an HSA linker of the invention, or any molecule or moiety conjugated thereto, include any radioisotope that can be covalently bound to a residue of the peptide component of the agent of the invention or analog thereof. The radioisotopes can also be selected from radioisotopes that emit either beta or gamma radiation, or alternatively, any of the agents of the invention can be modified to contain chelating groups that, for example, can be covalently bonded to lysine residue(s) of the HSA linker or any peptidic agent conjugated thereto. The chelating groups can then be modified to contain any of a variety of radioisotopes, such as gallium, indium, technetium, ytterbium, rhenium, or thallium (e.g., ^{125}I, ^{67}Ga, ^{111}In, ^{99}mTc, ^{169}Yb, ^{186}Re).

An HSA linker of the invention, or any molecule or moiety conjugated thereto, can be modified by attachment of a radioisotope. Preferable radioisotopes are those having a radioactive half-life corresponding to, or longer than, the biological half-life of the HSA conjugate used. More preferably, the radioisotope is a radioisotope of a halogen atom (e.g. a radioisotope of fluorine, chlorine, bromine, iodine, and astatine), even more preferably ^{75}Br, ^{77}Br, ^{76}Br, ^{122}I, ^{123}I, ^{124}I, ^{125}I, ^{129}I, ^{131}I, or ^{211}At.

An agent of the invention that incorporates an HSA linker, or any molecule or moiety conjugated thereto, can be coupled to radioactive metals and used in radiographic imaging or radiotherapy. Preferred radioisotopes also include ^{99m}Tc, ^{51}Cr, ^{67}Ga, ^{68}Ga, ^{111}In, ^{168}Yb, ^{140}La, ^{90}Y, ^{88}Y, ^{153}Sm, ^{156}Ho, ^{165}Dy, ^{64}Cu, ^{97}Ru, ^{103}Ru, ^{186}Re, ^{188}Re, ^{203}Pb, ^{211}Bi, ^{212}Bi, ^{213}Bi, and ^{214}Bi. The choice of metal is determined based on the desired therapeutic or diagnostic application.

An HSA linker of the invention, or any molecule or moiety conjugated thereto, can be coupled to a metal component, to produce agent of the invention that can be used as a diagnostic or therapeutic agent. A detectable label may be a metal ion from heavy elements or rare earth ions,

such as Gd^{3+}, Fe^{3+}, Mn^{3+}, or Cr^{2+}. An agent of the invention that incorporates an HSA linker having paramagnetic or superparamagnetic metals conjoined thereto are useful as diagnostic agents in MRI imaging applications. Paramagnetic metals that can be coupled to the agents of the invention include, but are not limited to, chromium (III), manganese (II), iron (II), iron (III), cobalt (II), nickel (II), copper (II), praseodymium (III), neodymium (III), samarium (III), gadolinium (III), terbium (III), dysprosium (III), holmium (III), erbium (III), and ytterbium (III).

Chelating groups may be used to indirectly couple detectable labels or other molecules to an HSA linker of the invention or to an agent conjugated thereto. Chelating groups can link agents of the invention with radiolabels, such as a bifunctional stable chelator, or can be linked to one or more terminal or internal amino acid reactive groups. An HSA linker of the invention, or any molecule or moeity conjugated thereto, can be linked via an isothiocyanate β-Ala or appropriate non-α-amino acid linker which prevents Edman degradation. Examples of chelators known in the art include, for example, the ininocarboxylic and polyaminopolycarboxylic reactive groups, ininocarboxylic and polyaminopolycarboxylic reactive groups, diethylenetriaminepentaacetic acid (DTPA), and 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA).

An HSA linker of the invention, when expressed recombinantly, can be joined to a peptidic detectable label or diagnostic agent. Peptides and proteins that can be used as a detectable label with an HSA linker include, but are not limited to, fluorescent proteins, bioluminescent proteins, and epitope tags, each of which is discussed in detail below. One or more of these detectable labels can also be incorporated into an HSA linker of the invention that also includes a therapeutic, cytotoxic, or cytostatic agent.

Fluorescent proteins or fluorochromes, such as green fluorescent protein (GFP; SEQ ID NO:47), enhanced GFP (eGFP), yellow fluorescent protein (SEQ ID NO: 48; YFP), cyan fluorescent protein (SEQ ID NO: 49; CFP), and red fluorescent protein (SEQ ID NO: 50; RFP or DsRed), can be used as detectable label joined to an HSA linker of the invention. Fluorescent proteins can be recombinantly expressed in a cell (e.g., a blood cell, such as a lymphocyte) following transfection or transduction of the cell with an expression vector that encodes the nucleotide sequence of the fluorescent protein. Upon exposure of the fluorescent protein to a stimulating frequency of light, the fluorescent protein will emit light at a low, medium, or high intensity that can be observed by eye under a microscope or by an optical imaging device. Exemplary fluorescent proteins suitable for use as the diagnostic sequence in agents of the

invention are described in, e.g., U.S. Patent Nos. 7,417,131 and 7,413,874, each of which is herein incorporated by reference.

Bioluminescent proteins can also be used as a detectable label incorporated into an HSA linker of the invention. Bioluminescent proteins, such as luciferase (e.g., firefly (SEQ ID NO:51), *Renilla* (SEQ ID NO:52), and *Omphalotus* luciferase) and aequorin, emit light as part of a chemical reaction with a substrate (e.g., luciferin and coelenterazine). In one embodiment of the invention, a vector encoding a luciferase gene provides for the *in vivo, in vitro*, or *ex vivo* detection of cells (e.g., blood cells, such as lymphocytes) that have been transduced or transfected according to the methods of the invention. Exemplary bioluminescent proteins suitable for use as diagnostic sequence of the invention and methods for their use are described in, e.g., U.S. Patent Nos. 5,292,658, 5,670,356, 6,171,809, and 7,183,092, each of which is herein incorporated by reference.

Epitope tags are short amino acid sequences, e.g., 5-20 amino acid residues in length, that can be incorporated into an HSA linker of the invention as a detectable label to facilitate detection once expressed in a cell, secreted from the cell, or bound to a target cell. An agent of the invention that incorporates an epitope tag as a diagnostic sequence can be detected by virtue of its interaction with an antibody, antibody fragment, or other binding molecule specific for the epitope tag. Nucleotide sequences encoding the epitope tag are produced either by cloning appropriate portions of natural genes or by synthesizing a polynucleotide that encodes the epitope tag. An antibody, antibody fragment, or other binding molecule that binds an epitope tag can directly incorporate a detectable label (e.g., a fluorochrome, radiolabel, heavy metal, or enzyme such as horseradish peroxidase) or serve itself as a target for a secondary antibody, antibody fragment, or other binding molecule that incorporates such a label. Exemplary epitope tags that can be used as a diagnostic sequence include c-myc (SEQ ID NO:33), hemagglutinin (HA; SEQ ID NO:34), and histidine tag (His_6; SEQ ID NO:35). Furthermore, fluorescent (e.g., GFP) and bioluminescent proteins can also serve as epitope tags, as antibodies, antibody fragments, and other binding molecules are commercially available for the detection of these proteins.

The *in vivo*, *in vitro*, or *ex vivo* detection, imaging, or tracking of a an HSA linker of the invention that incorporates a diagnostic sequence (e.g., a fluorescent protein, bioluminescent protein, or epitope tag) or any cell expressing or bound thereto can be accomplished using a

microscope, flow cytometer, luminometer, or other state of the art optical imaging device, such as an IVIS® Imaging System (Caliper LifeSciences, Hopkinton, MA).

Therapeutic or Cytotoxic Agents Coupled to the HSA Linker of the Invention

An HSA linker protein of the invention, or any molecule or moiety conjugated thereto, can be coupled to any known cytotoxic or therapeutic moiety to form an agent of the invention that can be administered to treat, inhibit, reduce, or ameliorate disease (e.g., a cancer, autoimmune disease, or cardiovascular disease) or one or more symptoms of disease. Examples include but are not limited to antineoplastic agents such as: Acivicin; Aclarubicin; Acodazole Hydrochloride; Acronine; Adozelesin; Adriamycin; Aldesleukin; Altretamine; Ambomycin; A. metantrone Acetate; Aminoglutethimide; Amsacrine; Anastrozole; Anthramycin; Asparaginase; Asperlin; Azacitidine; Azetepa; Azotomycin; Batimastat; Benzodepa; Bicalutamide; Bisantrene Hydrochloride; Bisnafide Dimesylate; Bizelesin; Bleomycin Sulfate; Brequinar Sodium; Bropirimine; Busulfan; Cactinomycin; Calusterone; Camptothecin; Caracemide; Carbetimer; Carboplatin; Carmustine; Carubicin Hydrochloride; Carzelesin; Cedefingol; Chlorambucil; Cirolemycin; Cisplatin; Cladribine; Combretestatin A-4; Crisnatol Mesylate; Cyclophosphamide; Cytarabine; Dacarbazine; DACA (N-[2- (Dimethyl-amino) ethyl] acridine-4-carboxamide); Dactinomycin; Daunorubicin Hydrochloride; Daunomycin; Decitabine; Dexormaplatin; Dezaguanine; Dezaguanine Mesylate; Diaziquone; Docetaxel; Dolasatins; Doxorubicin; Doxorubicin Hydrochloride; Droloxifene; Droloxifene Citrate; Dromostanolone Propionate; Duazomycin; Edatrexate; Eflornithine Hydrochloride; Ellipticine; Elsamitrucin; Enloplatin; Enpromate; Epipropidine; Epirubicin Hydrochloride; Erbulozole; Esorubicin Hydrochloride; Estramustine; Estramustine Phosphate Sodium; Etanidazole; Ethiodized Oil I 131; Etoposide; Etoposide Phosphate; Etoprine; Fadrozole Hydrochloride; Fazarabine; Fenretinide; Floxuridine; Fludarabine Phosphate; Fluorouracil; 5-FdUMP; Flurocitabine; Fosquidone; Fostriecin Sodium; Gemcitabine; Gemcitabine Hydrochloride; Gold Au 198; Homocamptothecin; Hydroxyurea; Idarubicin Hydrochloride; Ifosfamide; Ilmofosine; Interferon Alfa-2a; Interferon Alfa-2b; Interferon Alfa-nl; Interferon Alfa-n3; Interferon Beta-I a; Interferon Gamma-I b; Iproplatin; Irinotecan Hydrochloride; Lanreotide Acetate; Letrozole; Leuprolide Acetate; Liarozole Hydrochloride; Lometrexol Sodium; Lomustine; Losoxantrone Hydrochloride; Masoprocol; Maytansine; Mechlorethamine Hydrochloride; Megestrol Acetate; Melengestrol Acetate; Melphalan; Menogaril; Mercaptopurine; Methotrexate; Methotrexate Sodium; Metoprine;

Meturedepa; Mitindomide; Mitocarcin; Mitocromin; Mitogillin; Mitomalcin; Mitomycin; Mitosper; Mitotane; Mitoxantrone Hydrochloride; Mycophenolic Acid; Nocodazole; Nogalamycin;Ormaplatin; Oxisuran; Paclitaxel; Pegaspargase; Peliomycin; Pentamustine; PeploycinSulfate; Perfosfamide; Pipobroman; Piposulfan; Piroxantrone Hydrochloride; Plicamycin; Plomestane; Porfimer Sodium; Porfiromycin; Prednimustine; Procarbazine Hydrochloride; Puromycin; Puromycin Hydrochloride; Pyrazofurin; Rhizoxin; Rhizoxin D; Riboprine; Rogletimide; Safingol; Safingol Hydrochloride; Semustine; Simtrazene; Sparfosate Sodium; Sparsomycin; Spirogermanium Hydrochloride; Spiromustine; Spiroplatin; Streptonigrin; Streptozocin; Strontium Chloride Sr 89; Sulofenur; Talisomycin; Taxane; Taxoid; Tecogalan Sodium; Tegafur; Teloxantrone Hydrochloride; Temoporfin; Teniposide; Teroxirone; Testolactone; Thiamiprine; Thioguanine; Thiotepa; Thymitaq; Tiazofurin; Tirapazamine; Tomudex; TOP53; Topotecan Hydrochloride; Toremifene Citrate; Trestolone Acetate; Triciribine Phosphate; Trimetrexate; Trimetrexate Glucuronate; Triptorelin; Tubulozole Hydrochloride; Uracil Mustard; Uredepa; Vapreotide; Verteporfin; Vinblastine; Vinblastine Sulfate; Vincristine; Vincristine Sulfate; Vindesine; Vindesine Sulfate; Vinepidine Sulfate; Vinglycinate Sulfate; Vinleurosine Sulfate; Vinorelbine Tartrate; Vinrosidine Sulfate; Vinzolidine Sulfate; Vorozole; Zeniplatin; Zinostatin; Zorubicin Hydrochloride; 2-Chlorodeoxyadenosine; 2' Deoxyformycin; 9-aminocamptothecin; raltitrexed; N-propargyl-5,8-dideazafolic acid; 2chloro-2'-arabino-fluoro-2'-deoxyadenosine; 2-chloro-2'-deoxyadenosine; anisomycin; trichostatin A; hPRL-G129R; CEP-751; linomide; sulfur mustard; nitrogen mustard (mechlor ethamine); cyclophosphamide; melphalan; chlorambucil; ifosfamide; busulfan; N-methyl-Nnitrosourea (MNU); N, N'-Bis (2-chloroethyl)-N-nitrosourea (BCNU); N- (2-chloroethyl)-N' cyclohexyl-N-nitrosourea (CCNU); N- (2-chloroethyl)-N'- (trans-4-methylcyclohexyl-N-nitrosourea (MeCCNU); N- (2-chloroethyl)-N'- (diethyl) ethylphosphonate-N-nitrosourea (fotemustine); streptozotocin; diacarbazine (DTIC); mitozolomide; temozolomide; thiotepa; mitomycin C; AZQ; adozelesin; Cisplatin; Carboplatin; Ormaplatin; Oxaliplatin;C1-973; DWA 2114R; JM216; JM335; Bis (platinum); tomudex; azacitidine; cytarabine; gemcitabine; 6-Mercaptopurine; 6-Thioguanine; Hypoxanthine; teniposide 9-amino camptothecin; Topotecan; CPT-11; Doxorubicin; Daunomycin; Epirubicin; darubicin; mitoxantrone; losoxantrone; Dactinomycin (Actinomycin D); amsacrine; pyrazoloacridine; all-trans retinol; 14-hydroxy-retro-retinol; all-trans retinoic acid; N- (4-Hydroxyphenyl) retinamide; 13-cis retinoic acid; 3-Methyl TTNEB; 9-cis retinoic acid; fludarabine (2-F-ara-AMP); or 2-chlorodeoxyadenosine (2-Cda).

Other therapeutic compounds include, but are not limited to, 20-pi-1,25 dihydroxyvitamin D3; 5-ethynyluracil; abiraterone; aclarubicin; acylfulvene; adecypenol; adozelesin; aldesleukin; ALL-TK antagonists; altretamine; ambamustine; amidox; amifostine; aminolevulinic acid; amrubicin; amsacrine; anagrelide; anastrozole; andrographolide; angiogenesis inhibitors; antagonist D; antagonist G; antarelix; anti-dorsalizing morphogenetic protein-1; antiandrogen, prostatic carcinoma; antiestrogen; antineoplaston; antisense oligonucleotides; aphidicolin glycinate; apoptosis gene modulators; apoptosis regulators; apurinic acid; ara-CDP-DL-PTBA; arginine deaminase; asulacrine; atamestane; atrimustine; axinastatin 1; axinastatin 2; axinastatin 3; azasetron; azatoxin; azatyrosine; baccatin III derivatives; balanol; batimastat; BCR/ABL antagonists; benzochlorins; benzoylstaurosporine; beta lactam derivatives; beta-alethine; betaclamycin B; betulinic acid; bFGF inhibitor; bicalutamide; bisantrene; bisaziridinylspermine; bisnafide; bistratene A; bizelesin; breflate; bleomycin A2; bleomycin B2; bropirimine; budotitane; buthionine sulfoximine; calcipotriol; calphostin C; camptothecin derivatives (e.g., 10-hydroxy-camptothecin); canarypox IL-2; capecitabine; carboxamide-amino-triazole; carboxyamidotriazole; CaRest M3; CARN 700; cartilage derived inhibitor; carzelesin; casein kinase inhibitors (ICOS); castanospermine; cecropin B; cetrorelix; chlorins; chloroquinoxaline sulfonamide; cicaprost; cis-porphyrin; cladribine; clomifene analogues; clotrimazole; collismycin A ; collismycin B; combretastatin A4; combretastatin analogue; conagenin; crambescidin 816 ; crisnatol; cryptophycin 8; cryptophycin A derivatives; curacin A; cyclopentanthraquinones; cycloplatam; cypemycin; cytarabine ocfosfate; cytolytic factor; cytostatin; dacliximab; decitabine; dehydrodidemnin B; 2'deoxycoformycin (DCF); deslorelin; dexifosfamide; dexrazoxane; dexverapamil; diaziquone; didemnin B; didox; diethylnorspermine; dihydro-5-azacytidine; dihydrotaxol, 9- ; dioxamycin; diphenyl spiromustine; discodermolide; docosanol; dolasetron; doxifluridine; droloxifene; dronabinol; duocarmycin SA; ebselen; ecomustine; edelfosine; edrecolomab; eflornithine; elemene; emitefur; epirubicin; epothilones (A, R = H; B, R = Me); epithilones; epristeride; estramustine analogue; estrogen agonists; estrogen antagonists; etanidazole; etoposide; etoposide 4'-phosphate (etopofos); exemestane; fadrozole; fazarabine; fenretinide; filgrastim; finasteride; flavopiridol; flezelastine; fluasterone; fludarabine; fluorodaunorunicin hydrochloride; forfenimex; formestane; fostriecin; fotemustine; gadolinium texaphyrin; gallium nitrate; galocitabine; ganirelix; gelatinase inhibitors; gemcitabine; glutathione inhibitors; hepsulfam; heregulin; hexamethylene bisacetamide; homoharringtonine (HHT); hypericin; ibandronic acid; idarubicin; idoxifene; idramantone; ilmofosine; ilomastat;

imidazoacridones; imiquimod; immunostimulant peptides; insulin-like growth factor-1 receptor inhibitor; interferon agonists; interferons; interleukins; iobenguane; iododoxorubicin; ipomeanol, 4- ; irinotecan; iroplact; irsogladine; isobengazole; isohomohalicondrin B; itasetron; jasplakinolide; kahalalide F; lamellarin-N triacetate; lanreotide; leinamycin; lenograstim; lentinan sulfate; leptolstatin; letrozole; leukemia inhibiting factor; leukocyte alpha interferon; leuprolide + estrogen + progesterone; leuprorelin; levamisole; liarozole; linear polyamine analogue; lipophilic disaccharide peptide; lipophilic platinum compounds; lissoclinamide 7; lobaplatin; lombricine; lometrexol; lonidamine; losoxantrone; lovastatin; loxoribine; lurtotecan; lutetium texaphyrin; lysofylline; lytic peptides; maytansine; mannostatin A; marimastat; masoprocol; maspin; matrilysin inhibitors; matrix metalloproteinase inhibitors; menogaril; merbarone; meterelin; methioninase; metoclopramide; MIF inhibitor; mifepristone; miltefosine; mirimostim; mismatched double stranded RNA; mithracin; mitoguazone; mitolactol; mitomycin analogues; mitonafide; mitotoxin fibroblast growth factor-saporin; mitoxantrone; mofarotene; molgramostim; monoclonal antibody, human chorionic gonadotrophin; monophosphoryl lipid A + myobacterium cell wall sk; mopidamol; multiple drug resistance gene inhibitor; multiple tumor suppressor 1-based therapy; mustard anticancer agent; mycaperoxide B; mycobacterial cell wall extract; myriaporone; N-acetyldinaline; N-substituted benzamides; nafarelin; nagrestip; naloxone + pentazocine; napavin; naphterpin; nartograstim; nedaplatin; nemorubicin; neridronic acid; neutral endopeptidase; nilutamide; nisamycin; nitric oxide modulators; nitroxide antioxidant; nitrullyn; 06-benzylguanine; octreotide; okicenone; oligonucleotides; onapristone; ondansetron; ondansetron; oracin; oral cytokine inducer; ormaplatin; osaterone; oxaliplatin; oxaunomycin; paclitaxel analogues; paclitaxel derivatives; palauamine; palmitoylrhizoxin; pamidronic acid; panaxytriol; panomifene; parabactin; pazelliptine; pegaspargase; peldesine; pentosan polysulfate sodium; pentostatin; pentrozole; perflubron; perfosfamide; perillyl alcohol; phenazinomycin; phenylacetate; phosphatase inhibitors; picibanil; pilocarpine hydrochloride; pirarubicin; piritrexim; placetin A; placetin B; plasminogen activator inhibitor; platinum complex; platinum compounds; platinum-triamine complex; podophyllotoxin; porfimer sodium; porfiromycin; propyl bis-acridone; prostaglandin J2; proteasome inhibitors; protein A-based immune modulator; protein kinase C inhibitor; protein kinase C inhibitors, microalgal; protein tyrosine phosphatase inhibitors; purine nucleoside phosphorylase inhibitors; purpurins; pyrazoloacridine; pyridoxylated hemoglobin polyoxyethylene conjugate; raf antagonists; raltitrexed; ramosetron; ras farnesyl protein transferase inhibitors; ras inhibitors; ras-GAP inhibitor; retelliptine

demethylated; rhenium Re 186 etidronate; rhizoxin; ribozymes; RII retinamide; rogletimide; rohitukine; romurtide; roquinimex; rubiginone B 1; ruboxyl; safingol; saintopin; SarCNU; sarcophytol A; sargramostim; Sdi 1 mimetics; semustine; senescence derived inhibitor 1; sense oligonucleotides; signal transduction inhibitors; signal transduction modulators; single chain antigen binding protein; sizofiran; sobuzoxane; sodium borocaptate; sodium phenylacetate; solverol; somatomedin binding protein; sonermin; sparfosic acid; spicamycin D; spiromustine; splenopentin; spongistatin 1; squalamine; stem cell inhibitor; stem-cell division inhibitors; stipiamide; stromelysin inhibitors; sulfinosine; superactive vasoactive intestinal peptide antagonist; suradista; suramin; swainsonine; synthetic glycosaminoglycans; tallimustine; tamoxifen methiodide; tauromustine; tazarotene; tecogalan sodium; tegafur; tellurapyrylium; telomerase inhibitors; temoporfin; temozolomide; teniposide; tetrachlorodecaoxide; tetrazomine; thaliblastine; thalidomide; thiocoraline; thrombopoietin; thrombopoietin mimetic; thymalfasin; thymopoietin receptor agonist; thymotrinan; thyroid stimulating hormone; tin ethyl etiopurpurin; tirapazamine; titanocene dichloride; topotecan; topsentin; toremifene; totipotent stem cell factor; translation inhibitors; tretinoin; triacetyluridine; triciribine; trimetrexate; triptorelin; tropisetron; turosteride; tyrosine kinase inhibitors; tyrphostins; UBC inhibitors; ubenimex; urogenital sinus-derived growth inhibitory factor; urokinase receptor antagonists; vapreotide; variolin B; vector system, erythrocyte gene therapy; velaresol; veramine; verdins; verteporfin; vinorelbine; vinxaltine; vitaxin; vorozole; zanoterone; zeniplatin; zilascorb; and zinostatin stimalamer.

The HSA linker agents of the invention can also include site-specifically conjugated molecules and moieties. Site-specific conjugation allows for the controlled stoichiometric attachment to specific residues in the HSA linker of cytotoxic, immunomodulatory, or cytostatic agents including, e.g., anti tubulin agents, DNA minor groove binders, DNA replication inhibitors, alkylating agents, anthracyclines, antibiotics, antifolates, antimetabolites, chemotherapy or radiation sensitizer, duocarmycins, etoposides, fluorinated pyrimidines, ionophores, lexitropsins, nitrosoureas, platinols, purine antimetabolites, puromycins, steroids, taxanes, topoisomerase inhibitors, and vinca alkaloids or any other molecules or moieties described herein.

Techniques for conjugating therapeutic agents to proteins, and in particular to antibodies, are well-known (e.g., Arnon *et al.*, "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy," in Monoclonal Antibodies And Cancer Therapy (Reisfeld *et al.*, eds., Alan R. Liss, Inc., 1985); Hellstrom *et al.*, "Antibodies For Drug Delivery," in Controlled Drug Delivery (Robinson *et*

al., eds., Marcel Dekker, Inc., 2nd ed. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review," in Monoclonal Antibodies '84: Biological And Clinical Applications (Pinchera *et al.*, eds., 1985); "Analysis, Results, and Future Prospective of the Therapeutic Use of Radiolabeled Antibody In Cancer Therapy," in Monoclonal Antibodies For Cancer Detection And Therapy (Baldwin *et al.*, eds., Academic Press, 1985); Thorpe *et al., Immunol. Rev.* 62:119-58 (1982); and Doronina *et al.*, "Development of potent monoclonal antibody auristatin conjugates for cancer therapy," *Nature Biotech.* 21:(7)778-784 (2003)). See also, e.g., PCT publication WO 89/12624.

An HSA linker of the invention, or any molecule or moiety conjugated thereto, can also be coupled to a lytic peptide to form an agent of the invention. Such lytic peptides induce cell death and include, but are not limited to, streptolysin O; stoichactis toxin; phallolysin; staphylococcus alpha toxin; holothurin A; digitonin; melittin; lysolecithin; cardiotoxin; and cerebratulus A toxin (Kem *et al., J. Biol. Chem.* 253(16):5752-5757, 1978). An agent of the invention can also be formed by conjugating an HSA linker of the invention, or any molecule or moiety conjugated thereto (e.g., antibody or antibody fragment conjugates), to a synthetic peptide that shares some sequence homology or chemical characteristics with any of the naturally occurring peptide lysins; such characteristics include, but are not limited to, linearity, positive charge, amphipathicity, and formation of alpha-helical structures in a hydrophobic environment (Leuschner *et al., Biology of Reproduction* 73:860-865, 2005). An HSA linker of the invention, or any molecule or moiety conjugated thereto, can also be coupled to an agent that induces complement-mediated cell lysis such as, for example, the immunoglobulin F_c subunit. An HSA linker of the invention, or any molecule or moiety conjugated thereto, can also coupled to any member of the phospholipase family of enzymes (including phospholipase A, phospholipase B, phospholipase C, or phospholipase D) or to a catalytically-active subunit thereof.

An HSA linker of the invention, or any molecule or moiety conjugated thereto, can also be coupled to a radioactive agent to form an agent that can be used for detection or therapeutic applications. Radioactive agents that can be used include but are not limited to Fibrinogen ^{125}I; Fludeoxyglucose ^{18}F; Fluorodopa ^{18}F; Insulin ^{125}I; Insulin ^{131}I; lobenguane ^{123}I; Iodipamide Sodium ^{131}I; Iodoantipyrine ^{131}I; Iodocholesterol ^{131}I; Iodohippurate Sodium ^{123}I; Iodohippurate Sodium ^{125}I; Iodohippurate Sodium ^{131}I; Iodopyracet ^{125}I; Iodopyracet ^{131}I; Iofetamine Hydrochloride ^{123}I; Iomethin ^{125}I; Iomethin ^{131}I; Iothalamate Sodium ^{125}I; Iothalamate Sodium ^{131}I; tyrosine ^{131}I;

Liothyronine ^{125}I; Liothyronine ^{131}I; Merisoprol Acetate ^{197}Hg; Merisoprol Acetate ^{203}Hg; Merisoprol ^{197}Hg; Selenomethionine ^{75}Se; Technetium ^{99m}Tc Antimony Trisulfide Colloid; Technetium ^{99m}Tc Bicisate; Technetium ^{99m}Tc Disofenin; Technetium ^{99m}Tc Etidronate; Technetium ^{99m}Tc Exametazime; Technetium ^{99m}Tc Furifosmin; Technetium ^{99m}Tc Gluceptate; Technetium ^{99m}Tc Lidofenin; Technetium ^{99m}Tc Mebrofenin; Technetium ^{99m}Tc Medronate; Technetium ^{99m}Tc Medronate Disodium; Technetium ^{99m}Tc Mertiatide; Technetium ^{99m}Tc Oxidronate; Technetium ^{99m}Tc Pentetate; Technetium ^{99m}Tc Pentetate Calcium Trisodium; Technetium ^{99m}Tc Sestamibi; Technetium ^{99m}Tc Siboroxime; Technetium ^{99m}Tc; Succimer; Technetium ^{99m}Tc Sulfur Colloid; Technetium ^{99m}Tc Teboroxime; Technetium ^{99m}Tc Tetrofosmin; Technetium ^{99m}Tc Tiatide; Thyroxine ^{125}I; Thyroxine ^{131}I; Tolpovidone ^{131}I; Triolein ^{125}I; or Triolein ^{131}I.

Additionally, a radioisotope could be site-specifically conjoined to an HSA linker or HSA linker conjugate. The available reactive groups could be used to conjugate site-specific bifunctional chelating agents for labeling of radioisotopes, including ^{123}I, ^{124}I, ^{125}I, ^{131}I, ^{99m}Tc, ^{111}In, ^{64}Cu, ^{67}Cu, ^{186}Re, ^{188}Re, ^{177}Lu, ^{90}Y, ^{77}As, ^{72}As, ^{86}Y, ^{89}Zr, ^{211}At, ^{212}Bi, ^{213}Bi, or ^{225}Ac.

Therapeutic or cytotoxic agents incorporated into or coupled with an HSA linker or an HSA linker conjugate may further include, for example, anti-cancer Supplementary Potentiating Agents, including, but not limited to: tricyclic anti-depressant drugs (e.g., imipramine, desipramine, amitryptyline, clomipramine, trimipramine, doxepin, nortriptyline, protriptyline, amoxapine, and maprotiline); non-tricyclic anti-depressant drugs (e.g., sertraline, trazodone, and citalopram); Ca^{2+} antagonists (e.g., verapamil, nifedipine, nitrendipine, and caroverine); Calmodulin inhibitors (e.g., prenylamine, trifluoroperazine, and clomipramine); Amphotericin B; Triparanol analogs (e.g., tamoxifen); antiarrhythmic drugs (e.g., quinidine); antihypertensive drugs (e.g., reserpine); Thiol depleters (e.g., buthionine and sulfoximine) and Multiple Drug Resistance reducing agents such as Cremaphor EL.

An agent of the invention that includes an HSA linker, or any molecule or moiety conjugated thereto, can also be coupled to or administered with cytokines (e.g., granulocyte colony stimulating factor, interferon-alpha, and tumor necrosis factor-alpha). An HSA linker of the invention, or any molecule or moiety conjugated thereto, can also be coupled to an antimetabolic agent. Antimetabolic agents include, but are not limited to, the following compounds and their derivatives: azathioprine, cladribine, cytarabine, dacarbazine, fludarabine phosphate, fluorouracil, gencitabine chlorhydrate, mercaptopurine, methotrexate, mitobronitol, mitotane, proguanil chlorohydrate, pyrimethamine,

raltitrexed, trimetrexate glucuronate, urethane, vinblastine sulfate, vincristine sulfate, etc. More preferably, an HSA linker or conjugate of the invention can be coupled to a folic acid-type antimetabolite, a class of agents that includes, for example, methotrexate, proguanil chlorhydrate, pyrimethanime, trimethoprime, or trimetrexate glucuronate, or derivatives of these compounds.

An HSA linker of the invention, or any molecule or moiety conjugated thereto, can also be coupled to a member of the anthracycline family of neoplastic agents, including but not limited to aclarubicine chlorhydrate, daunorubicine chlorhydrate, doxorubicine chlorhydrate, epirubicine chlorhydrate, idarubicine chlorhydrate, pirarubicine, or zorubicine chlorhydrate; a camptothecin, or its derivatives or related compounds, such as 10, 11 methylenedioxycamptothecin; or a member of the maytansinoid family of compounds, which includes a variety of structurally-related compounds, e.g., ansamitocin P3, maytansine, 2'-N-demethylmaytanbutine, and maytanbicyclinol.

An HSA linker of the invention, or any molecule or moiety conjugated thereto, can also be coupled directly to a cytotoxic or therapeutic agent using known chemical methods, or coupled indirectly to a cytotoxic or therapeutic agent via an indirect linkage. For example, an HSA linker can be attached to a chelating group that is attached to the cytotoxic or therapeutic agent. Chelating groups include, but are not limited to, ininocarboxylic and polyaminopolycarboxylic reactive groups, diethylenetriaminepentaacetic acid (DTPA), and 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA). For general methods, see, e.g., Liu *et al., Bioconjugate Chem.* 12(4):653, 2001; Cheng *et al.,* WO 89/12631; Kieffer *et al.,* WO 93/12112; Albert *et al.,* U.S.P.N. 5,753,627; and WO 91/01144 (each of which are hereby incorporated by reference).

An agent of the invention that includes, e.g., an HSA linker, one or more binding moieties (with or without intervening polypeptide connectors, as defined herein), and a therapeutic or cytotoxic agent, can be specifically targeted by the binding moiety (e.g., antibody, antibody fragment, or receptor/ligand) to a cell or tissue, thereby allowing selective destruction of the target cell or tissue to which the binding moiety is directed. For example, an agent of the invention can be used to target and destroy cancer cells of the lung, breast, prostate, and colon in order to prevent, stabilize, inhibit the progression of, or treat cancers originating in these organs when the agent includes a binding moiety that specifically binds to the cancer cells in these organs. Also, for example, the agents of the invention can be used to target and destroy cells of the vasculature, brain, liver, kidney, heart, lung, prostate, colon, nasopharynx, oropharynx, larynx, bronchus, and skin in order to prevent, stabilize, inhibit the progression of, or treat age-related, tobacco-related, or

autoimmune diseases or conditions relating to these organs by targeting, in the case of autoimmune disease for example, autoreactive T cells (e.g., by binding to and agonizing tumor necrosis factor receptor 2 (TNFR2) present on the autoreactive T cells).

An HSA linker of the invention, when expressed recombinantly, can be joined to a cytotoxic polypeptide. Cytotoxic polypeptides, when brought into contact with a target cell (e.g., a cancer cell), exerts cytotoxic or cytostatic effects on the cell. For example, a cytotoxic polypeptide, when joined with an HSA linker of the invention, can induce events in a target cell upon binding of the target cell that leads to cell death through, for example, apoptosis, necrosis, or senescence. Alternatively, a cytotoxic polypeptide joined with an HSA linker of the invention can interfere or inhibit normal cellular biological activities, such as division, metabolism, and growth, or abnormal cellular biological activities, such as metastasis.

For example, an HSA linker of the invention joined to caspase 3 will bind a target cell (e.g., a cancer cell) and undergoe endocytosis. Once internalized by the target cell, the caspase portion of the HSA linker conjugate can initiate the pro-apoptotic caspase cascade, ultimately resulting in the apoptosis of the target cell.

In a preferred embodiment, an HSA linker of the invention includes a cytotoxic polypeptide capable of killing a cancer cell. In another embodiment of the invention, the cytotoxic polypeptide inhibits the growth or metastasis of a cancer cell. The cytotoxic polypeptide joined with an HSA linker of the invention can also be used to kill or inhibit the growth of cells associated with, necessary for, or beneficial to cancer growth, such as endothelial cells that form blood vessels that perfuse solid tumors.

In an embodiment, an HSA linker of the invention can include two or more cytotoxic polypeptides so as to modulate (e.g., increase) the specificity, intensity, or duration of the cytotoxic or cytostatic effect on a target cell (e.g., a cancer cell).

In another embodiment, the HSA linker of the invention is joined to an activatable form of cytotoxic polypeptide (e.g., a biologically-inactive pro-agent that is capable of activation upon cleavage by an enzyme or drug). In this embodiment, texposure (e.g., *in vivo*) of the cytotoxic polypeptide pro-agent to an enzyme or drug capable of cleaving the cytotoxic polypeptide, renders the cytotoxic polypeptide biologically-active (e.g., cytotoxic or cytostatic). An example of a biologically-inactive cytotoxic polypeptide that can be converted to a biologically-active form for use with an HSA linker of the invention is a procaspase (e.g., procaspase 8 or 3). For

example, the procaspase 8 domain of an HSA linker of the invention can be cleaved by TRAIL or FasL upon internalization by a target cell (e.g., a cancer cell). Once cleaved, the biologically active caspase 8 can promote apoptosis of the target cell.

In one embodiment of the invention, the cytotoxic polypeptide joined to an HSA linker of the invention can include a full-length peptide, polypeptide, or protein, or biologically-active fragment thereof (e.g., a "death domain"), known to have cytotoxic or cytostatic properties. Peptides, polypeptides, or proteins with cytotoxic or cytostatic properties can be altered (e.g., by making amino acid substitutions, mutations, truncations, or additions) to facilitate incorporation of the cytotoxic sequence into an agent of the invention. Desirable alterations include, for example, changes to the amino acid sequence that facilitate protein expression, longevity, cell secretion, and target cell toxicity.

The invention also features a nucleic acid molecule encoding a cytotoxic polypeptide as a fusion protein with an HSA linker of the invention, optionally including binding moieities and polypeptide connectors. The nucleic acid molecule can be incorporated into a vector (e.g., an expression vector), such that, upon expression of the HSA linker of the invention in a cell transfected or transduced with the vector, the cytotoxic polypeptide, HSA linker, and binding moieties, if present, are operably linked (e.g., fused, contiguously-joined, or tethered together). Examples of peptides, polypeptides, and proteins that can be used as a cytotoxic polypeptide of the present invention include, but are not limited to, apoptosis-inducing proteins such as cytochrome c (SEQ ID NO:39); caspases (e.g., caspase 3 (SEQ ID NO:36) and caspase 8 (SEQ ID NO:37)); procaspases, granzymes (e.g., granzymes A and B (SEQ ID NO: 38)); tumor necrosis factor (TNF) and TNF receptor family members, including TNF-alpha (TNFα; SEQ ID NO:40)), TNF-beta, Fas (SEQ ID NO:41) and Fas ligand; Fas-associated death domain-like IL-1β converting enzyme (FLICE); TRAIL/APO2L (SEQ ID NO:45) and TWEAK/APO3L (see, e.g., U.S. Patent Application Publication No. 2005/0187177, herein incorporated by reference); pro-apoptotic members of the Bcl-2 family, including Bax (SEQ ID NO:46), Bid, Bik, Bad (SEQ ID NO:42), Bak, and RICK (see, e.g., U.S. Patent Application Publication No. 2004/0224389, herein incorporate by reference); vascular apoptosis inducing proteins 1 and 2 (VAP1 and VAP2; Masuda et al., *Biochem. Biophys. Res. Commun.* 278:197-204 (2000)); pierisin (SEQ ID NO:44; Watanabe et al., *Biochemistry* 96:10608-10613 (1999)); apoptosis-inducing protein (SEQ ID NO:43; AIP; Murawaka et al., *Nature* 8:298-307 (2001)); IL-1α propiece polypeptide (see, e.g., U.S. Patent 6,191,269, herein incorporated by

reference); apoptin and apoptin-associated proteins such as AAP-1 (see, e.g., European Patent Application Publication No. EP 1083224, herein incorporated by reference); anti-angiogenic factors such as endostatin and angiostatin; and other apoptosis-inducing proteins, including those described in the following International and U.S. Patent Application Publications, each herein incorporated by reference: U.S. 2003/0054994, U.S. 2003/0086919, U.S. 2007/0031423, WO 2004/078112, WO 2007/012430, and WO 2006/0125001 (intracellular domain of delta 1 and jagged 1).

Wild-type HSA Linker Conjugates

The present invention also encompasses a naturally-occurring wild-type HSA linker, the amino acid and nucleotide sequences of which are provided in SEQ ID NOS:3 and 4, respectively, in the formation of binding, diagnostic, or therapeutic agents of the invention. In all embodiments of the invention utilizing an HSA linker with the amino acid sequence listed in SEQ ID NO:3, one or more polypeptide connectors, as described above, are covalently attached to the amino and/or carboxy termini of the HSA linker, or to an amino acid residue within the HSA linker sequence, to facilitate conjugation of one or more binding moieties.

Truncations

The invention further features a conjugate that is formed using a truncated wild-type HSA polypeptide, which can be combined with one or more polypeptide connectors or binding moieties. A wild-type HSA polypeptide lacking 1, 2, 3, 4, 5, 10, 15, 20, 50, 100, 200 or more amino acids of the full-length wild-type HSA amino acid sequence (i.e., SEQ ID NO:3) can be conjoined to any of the binding moieties or diagnostic or therapeutic agents described herein. Truncations can occur at one or both ends of the HSA linker, or can include a deletion of internal residues. Truncation of more than one amino acid residue need not be linear. Examples of wild-type HSA linkers of the invention include those having, in combination with one or more polypeptide connectors or binding moieties, one or more of domain I (SEQ ID NO:56; residues 1-197 of SEQ ID NO:3), domain II (SEQ ID NO:54; residues 189-385 of SEQ ID NO:3), or domain III (SEQ ID NO:57; residues 381-585 of SEQ ID NO:3), or combinations thereof, e.g., domains I and II, I and III, and II and III.

Serum clearance rates of a conjugate of the invention (e.g., a bispecific HSA-drug or radioisotope-containing agent), can be optimized by testing conjugates containing a truncated wild-type HSA linker, as describe above.

Additional HSA Linker Modifications

The invention further provides site-specific chemical modification of amino acid residues in the HSA linker polypeptide. The correctly-folded tertiary structure of HSA displays certain amino acid residues on the external face of the protein. Chemically-reactive amino acid residues (e.g., cysteine) can be substituted for these surface-exposed residues to allow site-specific conjugation of a diagnostic or therapeutic agent.

The invention also provides for the addition or removal of asparagine, serine, or threonine residues from an HSA linker polypeptide sequence to alter glycosylation of these amino acid residues. Glycosylation sites added to an HSA linker are preferably surface-exposed, as discussed herein. Glycosyl or other carbohydrate moieties introduced to an HSA linker can be directly conjugated to diagnostic, therapeutic, or cytotoic agents.

Cysteine (Thiol) Conjugation

The invention features the substitution of surface-exposed amino acid residues of the HSA linker polypeptide with cysteine residues to allow for chemical conjugation of diagnostic, therapeutic, or cytotoxic agents. Cysteine residues exposed on the surface of the HSA linker polypeptide (when folded into its native tertiary structure) allow the specific conjugation of a diagnostic, therapeutic, or cytotoxic agent to a thiol reactive group such as maleimide or haloacetyl. The nucleophilic reactivity of the thiol functionality of a cysteine residue to a maleimide group is about 1000 times higher compared to any other amino acid functionality in a protein, such as the amino group of a lysine residue or the N-terminal amino group. Thiol specific functionality in iodoacetyl and maleimide reagents may react with amine groups, but higher pH (>9.0) and longer reaction times are required (Garman, 1997, Non-Radioactive Labelling: A Practical Approach, Academic Press, London). The amount of free thiol in a protein may be estimated using the standard Ellman's assay. In some instances, reduction of the disulfide bonds with a reagent such as dithiothreitol (DTT) or selenol (Singh *et al., Anal. Biochem.* 304:147-156 (2002)) is required to generate the reactive free thiol.

Sites for cysteine substitution can be identified by analysis of surface accessibility of the HSA linker (e.g., the identification of serine and threonine residues as suitable for substitution are described in Example 1 below). The surface accessibility can be expressed as the surface area (e.g., square angstroms) that can be contacted by a solvent molecule, e.g., water. The occupied space of

water is approximated as a sphere with a 1.4 angstrom radius. Software for calculating the surface accessibility of each amino acid of a protein is freely available or licensable. For example, the CCP4 Suite of crystallography programs which employ algorithms to calculate the surface accessibility of each amino acid of a protein with known x-ray crystallography derived coordinates ("The CCP4 Suite: Programs for Protein Crystallography" *Acta. Cryst.* D50:760-763 (1994); www.ccp4.ac.uk/dist/html/INDEX.html). Solvent accessibility may also be assessed using the free software DeepView Swiss PDB Viewer downloaded from the Swiss Institute of Bioinformatics. The substitution of cysteines at surface-exposed sites allows for conjugation of the reactive cysteine to a thiol reactive group linked to the diagnostic or therapeutic agent.

Glycosylation

In addition, altered serum clearance rates can be achieved by engineering glycosylation sites into the HSA linker. In certain embodiments, an HSA linker of the invention is glycosylated. Glycosylation of polypeptides is typically either N-linked or O-linked. N-linked refers to the attachment of a carbohydrate moiety to the side chain of an asparagine residue. The tripeptide sequences asparagine-X-serine and asparagine-X-threonine, where X represents any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tripeptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-aceylgalactosamine, galactose, or xylose to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used.

Addition or deletion of glycosylation sites to the HSA linker is conveniently accomplished by altering the amino acid sequence such that one or more of the above-described tripeptide sequences (for N-linked glycosylation sites) is created. The alteration may also be made by the addition, deletion, or substitution of one or more serine or threonine residues to the sequence of the original HSA linker (for O-linked glycosylation sites). The resulting carbohydrate structures on HSA can also be used for site-specific conjugation of cytotoxic, immunomodulatory or cytostatic agents as described above.

HSA Linker Agents in Combination with Therapeutic Agents

The invention further provides for the administration of the HSA linker agents described herein with one or more of the therapeutic, cytotoxic, or cytostatic agents described herein. For example, a patient suffering from breast cancer can be administered an HSA linker containing ErbB2 and ErbB3 scFvs (e.g., B2B3-1) can be co-administered with, e.g., doxorubicin, cyclophosphamide, and paclitaxel, a common chemotherapeutic regimen for the treatment of breast cancer. Additional biological and chemical agents useful for the treatment of cancer are listed in Appendix C.

HSA Linker Agents in Combination with Radiotherapy or Surgery

The invention provides for the administration of an HSA linker agent of the invention prior to, concurrent with, or following radiotherapy or surgery. For example, a patient suffering from a proliferative disorder (e.g., breast cancer) can receive an HSA linker agent, alone or in combination with other therapeutic, cytotoxic, or cytotoxic agents as described herein concurrent with targeted radiotherapy or surgical intervention (e.g., lumpectomy or mastectomy) at the site of the cancerous tissue. Radiotherapies suitable for use in combination with HSA linker agents of the invention include brachytherapy and targeted intraoperative radiotherapy (TARGIT).

Pharmaceutical Compositions

Pharmaceutical compositions of the invention contain a therapeutically or diagnostically effective amount of an HSA linker conjugate of the invention that includes one or more of a binding moiety (e.g., antibodies or antibody fragments), diagnostic agent (e.g., radionuclide or chelating agents), or a therapeutic agent (e.g., cytotoxic or immunomodulatory agents) agent. The active ingredients, an HSA linker conjugate of the invention (prepared with one or more of a binding moiety, diagnostic agent, or therapeutic agent) can be formulated for use in a variety of drug delivery systems. One or more physiologically acceptable excipients or carriers can also be included in the compositions for proper formulation. Suitable formulations for use in the present invention are found in *Remington's Pharmaceutical Sciences*, Mack Publishing Company, Philadelphia, PA, 17th ed. (1985). For a brief review of methods for drug delivery, see, Langer *Science* 249:1527-1533 (1990).

The pharmaceutical compositions are intended for parenteral, intranasal, topical, oral, or local administration, such as by a transdermal means, for prophylactic and/or therapeutic treatment.

Commonly, the pharmaceutical compositions are administered parenterally (e.g., by intravenous, intramuscular, or subcutaneous injection), or by oral ingestion, or by topical application. Thus, the invention provides compositions for parenteral administration that include an HSA linker of the invention, with or without one or more binding, diagnostic, and/or therapeutic agent conjugated thereto, dissolved or suspended in an acceptable carrier, preferably an aqueous carrier, e.g., water, buffered water, saline, PBS, and the like. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions, such as pH adjusting and buffering agents, tonicity adjusting agents, wetting agents, detergents and the like. The invention also provides compositions for oral delivery, which may contain inert ingredients such as binders or fillers for the formulation of a tablet, a capsule, and the like. Furthermore, this invention provides compositions for local administration, which may contain inert ingredients such as solvents or emulsifiers for the formulation of a cream, an ointment, and the like.

These compositions may be sterilized by conventional sterilization techniques, or may be sterile filtered. The resulting aqueous solutions may be packaged for use as is, or lyophilized, the lyophilized preparation being combined with a sterile aqueous carrier prior to administration. The pH of the preparations typically will be between 3 and 11, more preferably between 5 and 9 or between 6 and 8, and most preferably between 7 and 8, such as 7 to 7.5. The resulting compositions in solid form may be packaged in multiple single dose units, each containing a fixed amount of an HSA linker conjugate (prepared with one or more of a binding, diagnostic, and/or therapeutic agent) in a sealed package of tablets or capsules, for example. The composition in solid form can also be packaged in a container for a flexible quantity, such as in a squeezable tube designed for a topically applicable cream or ointment.

The compositions containing an effective amount of an HSA linker conjugate (prepared with one or more of a binding, diagnostic, and/or therapeutic agent) can be administered to a mammal (e.g., a human) for prophylactic and/or therapeutic treatments. In prophylactic applications, compositions of the invention containing an HSA linker conjugate (prepared with one or more of a binding, diagnostic, and/or therapeutic agent) are administered to a patient susceptible to or otherwise at risk of developing a disease or condition (e.g., a cancer, autoimmune disease, or cardiovascular disease). Such an amount is defined to be a "prophylactically effective dose." In this use, the precise amounts again depend on the patient's state of health, but generally range from about 0.5 mg to about 400 mg of an HSA linker conjugate (prepared with one or more of a binding,

diagnostic, and/or therapeutic agent) per dose (e.g., 10 mg, 50 mg, 100 mg, 200 mg, 300 mg, or 400 mg per dose) and from about 0.1 μg to about 300 mg of one or more immunomodulatory agents per dose (e.g., 10 μg, 30 μg, 50 μg, 0.1 mg, 10 mg, 50 mg, 100 mg, or 200 mg per dose). A dose of an HSA linker conjugate (prepared with one or more of a binding, diagnostic, and/or therapeutic agent) can be administered prophylactically to a patient one or more times per hour, day, week, month, or year (e.g., 2, 4, 5, 6, 7, 8, 9, 10, 11, or 12 times per hour, day, week, month, or year). More commonly, a single dose per week of an HSA linker conjugate (prepared with one or more of a binding, diagnostic, and/or therapeutic agent) is administered.

In therapeutic applications, a dose of an HSA linker conjugate (prepared with one or more of a binding, diagnostic, and/or therapeutic agent) is administered to a mammal (e.g., a human) already suffering from a disease or condition (e.g., a cancer, autoimmune disease, or cardiovascular disease) in an amount sufficient to cure or at least partially arrest or alleviate one or more of the symptoms of the disease or condition and its complications. An amount adequate to accomplish this purpose is defined as a "therapeutically effective dose." Amounts effective for this use may depend on the severity of the disease or condition and general state of the patient, but generally range from about 0.5 mg to about 400 mg of an HSA linker conjugate (prepared with one or more of a binding, diagnostic, and/or therapeutic agent) per dose (e.g., 10 mg, 50 mg, 100 mg, 200 mg, 300 mg, or 400 mg per dose). A dose of an HSA linker conjugate (prepared with one or more of a binding, diagnostic, and/or therapeutic agent) can be administered therapeutically to a patient one or more times per hour, day, week, month, or year (e.g., 2, 4, 5, 6, 7, 8, 9, 10, 11, or 12 times per hour, day, week, month, or year). More commonly, a single dose per week of an HSA linker conjugate (prepared with one or more of a binding, diagnostic, and/or therapeutic agent) is administered.

In several embodiments, the patient may receive an HSA linker conjugate (prepared with one or more of a binding, diagnostic, and/or therapeutic agent) in the range of about 0.5 to about 400 mg per dose one or more times per week (e.g., 2, 3, 4, 5, 6, or 7 or more per week), preferably about 5 mg to about 300 mg per dose one or more times per week, and even more preferably about 5 mg to about 200 mg per dose one or more times per week. The patient may also receive a biweekly dose of an HSA linker conjugate (prepared with one or more of a binding, diagnostic, and/or therapeutic agent) in the range of about 50 mg to about 800 mg or a monthly dose of an HSA linker of the invention, or any binding, diagnostic, and/or therapeutic agent conjugated thereto, in the range of about 50 mg to about 1,200 mg.

In other embodiments, an HSA linker conjugate (prepared with one or more of a binding, diagnostic, and/or therapeutic agent) may be administered to a patient in a typical dosage range of about 0.5 mg per week to about 400 mg per week, about 1.0 mg per week to about 300 mg per week, about 5 mg per week to about 200 mg per week, about 10 mg per week to about 100 mg per week, about 20 mg per week to about 80 mg per week, about 100 mg per week to about 300 mg per week, or about 100 mg per week to about 200 mg per week. An HSA linker conjugate (prepared with one or more of a binding, diagnostic, and/or therapeutic agent) may be administered in the range of about 0.5 mg every other day to about 100 mg every other day, preferably about 5 mg every other day to about 75 mg every other day, more preferably about 10 mg every other day to about 50 mg every other day, and even more preferably 20 mg every other day to about 40 mg every other day. An HSA linker conjugate (prepared with one or more of a binding, diagnostic, and/or therapeutic agent) may also be administered in the range of about 0.5 mg three times per week to about 100 mg three times per week, preferably about 5 mg three times per week to about 75 mg three times per week, more preferably about 10 mg three times per week to about 50 mg three times per week, and even more preferably about 20 mg three times per week to about 40 mg three times per week.

In non-limiting embodiments of the methods of the present invention, an HSA linker conjugate (prepared with one or more of a binding, diagnostic, and/or therapeutic agent) is administered to a mammal (e.g., a human) continuously for 1, 2, 3, or 4 hours; 1, 2, 3, or 4 times a day; every other day or every third, fourth, fifth, or sixth day; 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 times a week; biweekly; 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 times a month; bimonthly; 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 times every six months; 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 times a year; or biannually. An HSA linker conjugate (prepared with one or more of a binding, diagnostic, and/or therapeutic agent) may be administered at different frequencies during a therapeutic regime. In additional embodiments, an HSA linker conjugate (prepared with one or more of a binding, diagnostic, and/or therapeutic agent) may be administered to a patient at the same frequency or at a different frequency.

The amount of one or more diagnostic or therapeutic agents and an HSA linker, or any agent conjugated thereto, required to achieve the desired therapeutic effect depends on a number of factors, such as the specific diagnostic or therapeutic agent(s) chosen, the mode of administration, and clinical condition of the recipient. A skilled artisan will be able to determine the appropriate dosages

of one or more diagnostic or therapeutic agents and an HSA linker, or any agent conjugated thereto, to achieve the desired results.

Single or multiple administrations of the compositions comprising an effective amount of an HSA linker conjugate (prepared with one or more of a binding, diagnostic, and/or therapeutic agent) can be carried out with dose levels and pattern being selected by the treating physician. The dose and administration schedule can be determined and adjusted based on the severity of the disease or condition in a mammal (e.g., a human), which may be monitored throughout the course of treatment according to the methods commonly practiced by clinicians or those described herein.

An HSA linker conjugate (prepared with one or more of a binding, diagnostic, and/or therapeutic agent) can be administered to a mammalian subject, such as a human, directly or in combination with any pharmaceutically acceptable carrier or salt known in the art. Pharmaceutically acceptable salts may include non-toxic acid addition salts or metal complexes that are commonly used in the pharmaceutical industry. Examples of acid addition salts include organic acids such as acetic, lactic, pamoic, maleic, citric, malic, ascorbic, succinic, benzoic, palmitic, suberic, salicylic, tartaric, methanesulfonic, toluenesulfonic, or trifluoroacetic acids or the like; polymeric acids such as tannic acid, carboxymethyl cellulose, or the like; and inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid phosphoric acid, or the like. Metal complexes include zinc, iron, and the like. One exemplary pharmaceutically acceptable carrier is physiological saline. Other physiologically acceptable carriers and their formulations are known to one skilled in the art and described, for example, in Remington's Pharmaceutical Sciences, (18^{th} edition), ed. A. Gennaro, 1990, Mack Publishing Company, Easton, PA.

Diagnostic and Therapeutic Applications

The HSA linker conjugates of the invention, as described herein, can be used for diagnostic and therapeutic applications in a human, including, for example, the diagnosis or treatment of proliferative diseases (e.g., cancers, such as melanoma, clear cell sarcoma, and renal cancer) and autoimmune diseases (e.g., multiple sclerosis, rheumatoid arthritis, and uveitis). The following discussion of human proliferative and autoimmune diseases is meant to provide the skilled practitioner with a general understanding of how HSA linker conjugates of the invention can be applied in diagnostic and therapeutic applications and is not meant to limit the scope of the present invention.

Proliferative Diseases (Cancer)

An HSA linker conjugate of the invention can be used to diagnose, treat, prevent, or eliminate proliferative diseases such as, but not limited to, breast cancer, melanoma, clear cell sarcoma, renal cancer (e.g., renal cell carcinoma), prostate cancer, lung cancer, gastric cancer, and ovarian cancer. Binding moieties to be conjoined with an HSA linker of the invention for diagnostic or therapeutic application in a patient suspected of having or suffering from a proliferative disease may be chosen based on its ability to specifically bind, agonize, activate, antagonize, or inhibit target molecules (e.g., cell surface receptors such as tyrosine kinase receptors) associated with a proliferative disease. Binding moieties that target, for example, insulin-like growth factor receptor (IGFR, e.g., IGF1R and IGF2R), fibroblast growth factor receptor (FGFR), platelet-derived growth factor receptor (PDGFR), vascular endothelial growth factor receptor (VEGFR), tumor necrosis factor receptor (TNFR), epidermal growth factor receptor (EGFR, e.g., ErbB2 (HER2/neu)), Fc receptor, c-kit receptor, or mesenchymal epithelial transition factor receptor (c-met; also known as hepatocyte growth factor receptor (HGFR)) can be conjoined to an HSA linker of the invention to diagnose or treat a proliferative disease. Specific binding of a cancer cell by an HSA linker conjugate can allow for detection (e.g., an HSA linker conjoined to a detectable label, as defined herein) or destruction (e.g., an HSA linker conjoined to a cytotoxic agent) of the bound cancer cell. Specific application of HSA linker conjugates of the invention for the treatment of breast and renal cancer is described below.

Breast Cancer

Common forms of breast cancer include invasive ductal carcinoma, a malignant cancer in the breast's ducts, and invasive lobular carcinoma, a malignant cancer in the breast's lobules. Some types of breast cancer cells are known to highly express high levels of epidermal growth factor receptors, especially ErbB2 (i.e., HER2/neu). Aberrant signaling or unregulated activation of EGFRs has been linked to the development and progression of many cancers, including breast cancer. Uncontrolled cellular proliferation mediated via dysfunctional EGFR pathways can be found in a wide variety of solid tumors of epithelial origin and data have linked tumor EGFR expression, overexpression, and dysregulation to advanced disease, metastatic phenotype, resistance to chemotherapy, and an overall poorer prognosis.

An HSA linker of the invention conjoined to one or more binding moieties specific for an EGFR (e.g., anti-ErbB2; trastuzumab) can be used with a diagnostic (e.g., a detectable label) or

cytotoxic, cytostatic, or therapeutic agent, as described herein, to diagnose or treat breast cancer. Alternatively, a bispecific HSA linker conjugate that consists of binding moieties specific for ErbB2 and ErbB3, such as "B2B3-1," described further herein, can be employed to diagnose or treat cancers, e.g., breast, kidney, ovarian, and lung cancers.

As described above, an HSA linker conjugate of the invention used to treat breast cancer can be administered prior to (e.g., neoadjuvant chemotherapy), concurrent with, or following (e.g., adjuvant chemotherapy) radiotherapy or surgical intervention. An HSA linker conjugate can also be co-administered with other compounds (e.g., antineoplastic agents, such as biological or chemical therapeutics) useful for the treatment of breast cancer. For example, the antineoplastic agents listed in Table 1, including mitotic inhibitors (e.g., taxanes), topoisomerase inhibitors, alkylating agents (including, e.g., platinum-based agents), selective estrogen modulators (SERM), aromatase inhibitors, antimetabolites, antitumor antibiotics (e.g., anthracycline antibiotics), anti-VEGF agents, anti-ErbB2 (HER2/neu) agents, and anti-ErbB3 agents, are known to be particularly useful for the treatment of breast cancer. An HSA linker conjugate of the invention can be administered by a clinician in combination with any compound, including those listed in Appendix C, known or thought to be beneficial for the treatment of breast cancer.

Table 1: Exemplary antineoplastic agents for treatment of breast cancer in combination with HSA linker conjugates of the invention.

Therapeutic Class	**Exemplary Agent (Generic/Tradename)**	**Exemplary Dose**
Mitotic Inhibitors	paclitaxel (TAXOL®; ABRAXANE®)	175 mg/m^2
	docctaxcl (TAXOTERE®)	60-100 mg/m^2
Topoisomerase Inhibitors	camptothecin	
	topotecan hydrochloride (HYCAMTIN®)	
	etoposide (EPOSIN®)	
Alkylating Agents	cyclophosphamide (CYTOXAN®)	600 mg/m^2
Platinum-Based Agents	cisplatin	20-100 mg/m^2
	carboplatin (PARAPLATIN®)	300 mg/m^2
	nedaplatin (AQUPLA®)	
	oxaliplatin (ELOXATIN®)	65-85 mg/m^2
	satraplatin (SPERA®)	
	triplatin tetranitrate	
Selective Estrogen Modulators (SERM)	tamoxifen (NOLVADEX®)	20-40 mg/day
	raloxifene (EVISTA®)	60 mg/day

	toremifene (FARESTON®)	
Antimetabolites	methotrexate	40 mg/m^2
	fluorouracil (5-FU)	500 mg/m^2
	raltitrexed	
Antitumor Antibiotics	doxorubicin (ADRIAMYCIN®)	40-75 mg/m^2
	epirubicin (ELLENCE®)	60-120 mg/m^2
Aromatase Inhibitors	aminoglutethimide (CYTADREN®)	250-2000 mg/day
	anastrozole (ARIMIDEX®)	1 mg/day
	letrozole (FEMARA®)	2.5 mg/day
	vorozole	
	exemestane (AROMASIN®)	25-50 mg/day
	testolactone	
	fadrozole (AFEMA®)	
Anti-VEGF Agents	bevacizumab (AVASTIN®)	10 mg/kg
Anti-ErbB2 (HER2/neu) Agents	trastuzumab (HERCEPTIN®)	2-8 mg/kg
	pertuzumab (OMNITARG®)	
Anti-ErbB3 (HER3) Agents	U3-1287 (AMG 888)	

Renal Cancer

Kidney cancers, such as renal cell carcinoma, are particularly resistant to traditional radiological and chemical therapies. As such, the application of biological therapeutics, conjoined with an HSA linker of the invention represents an attractive option for patients suffering from these cancers. For example, an HSA linker conjoined with binding moieties that agonize type I interferon or interleukin 2 receptors can be used to treat a renal cancer. As a solid tumor, binding moieties that target and inhibit tumor vascularization (e.g., anti-vascular endothelial growth factor (VEGF) antibodies such as bevacizumab) can also be used for therapeutic effect.

Autoimmune Diseases

An HSA linker conjugate of the invention can be used to diagnose, treat, prevent, or stabilize autoimmune diseases and disorders in e.g., a human patient, such as, e.g., multiple sclerosis (MS), insulin-dependent diabetes mellitus (IDDM), rheumatoid arthritis (RA), uveitis, Sjögren's syndrome, Grave's disease, psoriasis, and myasthenia gravis. Autoimmune diseases and disorders are caused by self-reactive elements of the immune system (e.g., T cells, B cells, and self-reactive antibodies). As such, binding moieties that inhibit, block, antagonize, or deplete (e.g., anti-lymphocyte or anti-thymocyte globulins; basiliximab, daclizumab, or muromonab-CD3 monoclonal antibodies) self-

reactive immune cells and antibodies can be conjoined with an HSA linker of the invention for therapeutic use. Binding moieties that function as inflammatory signaling inhibitors (ISI), as defined herein, can be conjoined to an HSA linker of the invention for the treatment of autoimmunity. In addition, binding moieties that inhibit or antagonize integrin function (e.g., an integrin antagonist, as defined herein) can ameliorate or halt disease progression.

In other embodiments of the invention, the binding moiety is a soluble TNF receptor, such as etanercept or lenercept; an antibody directed against a pro-inflammatory cytokine or a pro-inflammatory cell surface signaling molecule, such as adalimumab, certolizumab, inflixamab, golimumab, and rituxan; a dominant-negative pro-inflammatory cytokine variant, such as XENP345, XPROTM1595, anakinra, and variants disclosed in U.S. Patent Application Publication Nos. 20030166559 and 20050265962; an inhibitor of the signaling pathways downstream of pro-inflammatory cytokine or pro-inflammatory cell surface signaling molecules, such as DE 096, 5-amino-2-carbonylthiopene derivatives (as described in WO2004089929), ARRY-797, BIRB 796 BS, (1-5-tert-butyl-2-p-tolyl-2H-pyrazol-3-yl)-3-[4-2(morpholin-4-yl-ethoxy)-naphtalen-1-yl]-urea, CHR-3620, CNI-1493, FR-167653 (Fujisawa Pharmaceutical, Osaka, Japan), ISIS 101757 (Isis Pharmaceuticals), ML3404, NPC31145, PD169316, PHZ1112, RJW67657, 4-(4-(4-fluorophenyl)-1-(3-phenylpropyl)-5-(4-pyridinyl)-1H-imidazol-2-yl)-3-butyn-1-ol, SCIO-469, SB202190, SB203580, (4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)1H-imidazole), SB239063, trans-1-(4-hydroxycyclohexyl)-4-(4-fluorophenyl-methoxypyridimidin-4-yl)imidazole, SB242235, SD-282, SKF-86002, TAK 715, VX702, and VX745; or an inhibitor of TNF-alpha converting enzyme (TACE), such as BB-1101, BB-3103, BMS-561392, butynyloxyphenyl β-sulfone piperidine hydroxomates, CH4474, DPC333, DPH-067517, GM6001, GW3333, Ro 32-7315, TAPI-1, TAPI-2, and TMI 005); or an anti-idiotypic agent, such as monoclonal antibodies, LJP 394 (abetimus, RIQUENT®, La Jolla Pharmaceuticals).

In other embodiments, the binding moiety is an interferon, as described herein. Binding moieties that can be conjoined to an IISA linker of the invention include, e.g., interferon-beta (REBIF® (IFN-β-1a), AVONEX® (IFN- β-1a), and BETASERON® (IFN- β-1b)), interferon-t (TAUFERONTM), interferon-alpha (e.g., ROFERON-A® (IFN-α-2a), INTRON-A® (IFN-α-2b), REBETRON® (IFN- α-2b), ALFERON-N® (IFN-α-n3), PEG-INTRON® (IFN-α-2b covalently conjugated with monomethoxy polyethylene glycol), INFERGEN® (a non-naturally occurring type 1

interferon with 88% homology to IFN-α-2b), or PEGASYS® (pegylated IFN-α-1a)), and ACTIMMUNE® (IFN-g-1b).

The present invention further provides HSA linker conjugates with binding moieties that antagonize these pro-inflammatory molecules or their specific receptors to treat autoimmunity. Specific application of HSA linker conjugates of the invention for the diagnosis and treatment of MS and RA are described below.

Multiple Sclerosis

Multiple sclerosis (MS) is a neurological disease characterized by irreversible degeneration of the nerves of the central nervous system (CNS). Although the underlying cause is unclear, the neurodegeneration in MS is the direct result of demyelination, or the stripping of myelin, a protein that normally lines the outer layer and insulates the nerves. T cells play a key role in the development of MS. Inflamed MS lesions, but not normal white matter, can have infiltrating $CD4^+$ T cells that respond to self antigens presented by MHC class II-linked molecules such as human HLA-DR2. The infiltrating CD4 T cells (T_H1 cells) produce the pro-inflammatory cytokines IL-2, IFN-γ, and TNF-α that activate antigen-presenting cells (APCs) such as macrophages to produce additional pro-inflammatory cytokines (e.g., IL-1β, IL-6, IL-8, and IL-12. IL-12 induces further IFN-γ synthesis. The result is progressive demyelination of neuronal sheaths, leading to human disease.

HSA linker conjugates can be used to aid in the diagnosis of MS. Diagnostic HSA linker conjugates that include binding moieties, as defined herein, that specifically target one or more (e.g., a bispecific HSA linker conjugate) immune cell activation markers (e.g., CD69, CD28, HLA-DR, and CD45). An imbalance of one or more of these pro-inflammatory or immune cell activation mediators relative to other factors or cells may be using measured by an HSA linker conjugate conjoined with a diagnostic agent (e.g., a radioisotope or fluorochrome).

An HSA linker conjugate of the invention can be used to treat a person at risk of developing or suffering from MS or to prevent, ameliorate, or cure the symptoms of the disease. For example, binding moieties, as defined herein, that specifically target and antagonize α4 integrin (e.g., natalizumab), CD52 (e.g., alemtuzumab), CD80, P-selectin, sphingosine-1-phosphate receptor-1 (S1PR1), hyaluronate receptor, leukocyte function antigen-1 (LFA-1), CD11 (e.g., efalizumab), CD18, CD20 (e.g., rituximab), CD85, ICOS ligand, CCR2, CXCR3, or CCR5 can be useful when conjoined to an HSA linker of the invention for therapeutic use in a patient suffering from MS.

Similary, binding moieties that neutralize type I interferons (e.g., interferons -alpha and –beta) or that antagonize type I interferon receptors (e.g., IFNαR1) can also be conjoined to an HSA linker of the invention for therapeutic application.

Rheumatoid arthritis

Rheumatoid arthritis (RA) is a chronic, inflammatory autoimmune disorder that causes the immune system to attack the joints. It is a disabling and painful inflammatory condition, which can lead to substantial loss of mobility due to pain and joint destruction. RA is a systemic disease, often affecting extra-articular tissues throughout the body including the skin, blood vessels, heart, lungs, and muscles.

Patients suffering from RA frequently have an increase in cellular expression of the HLA-DR4/DR1 cluster. HSA linker conjugates specific for one or both of these cell surface molecules are useful for the diagnosis of RA.

An HSA linker conjugate of the invention can be used to treat a person at risk of developing of suffering from RA to prevent, ameliorate, or cure the symptoms of the disease. For example, binding moieties, as defined herein, that specifically target and antagonize TNF-α (e.g., etanercept, infliximab, and adalimumab), IL-1 (e.g., anakinra), or CTLA-4 (e.g., abatacept). Binding moieties that specifically target and deplete B cells (e.g., an anti-CD20 antibody, such as rituximab) can also be conjoined to the HSA linker described herein to treat or prevent RA.

Uveitis

Uveitis specifically refers to inflammation of the middle layer of the eye, but may refer to any inflammatory process involving the interior of the eye. Uveitis may be autoimmune or idiopathic in origin

An HSA linker conjugate of the invention can be used to treat a person at risk of developing of suffering from autoimmune uveitis to prevent, ameliorate, or cure the symptoms of the disease. For example, alpha-fetoprotein (e.g., human AFP; NCBI Accession No. NM_001134), or biologically-active fragments thereof, can be conjoined to an HSA linker of the invention to reduce or eliminate inflammation associated with autoimmune or idiopathic uveitis.

Kits

The invention provides kits that include a pharmaceutical composition containing an HSA linker of the invention, and one or more of a binding moiety (e.g., antibodies or antibody fragments), a diagnostic agent (e.g., radionuclide or chelating agents), and a therapeutic agent (e.g., cytotoxic or immunomodulatory agents) with reagents that can be used to conjugate them to the HSA linker, if necessary, and including a pharmaceutically-acceptable carrier, in a therapeutically effective amount for treating a disease or condition (e.g., a cancer, autoimmune disease, or cardiovascular disease).. The kits include instructions to allow a practitioner (e.g., a physician, nurse, or patient) to administer the composition contained therein.

Preferably, the kits include multiple packages of the single-dose pharmaceutical composition(s) containing an effective amount of an HSA linker of the invention, or any binding (e.g., antibodies or antibody fragments (e.g., scFv)), diagnostic (e.g., radionuclide or chelating agents), and/or therapeutic (e.g., cytotoxic or immunomodulatory agents) conjugate thereof. Optionally, instruments or devices necessary for administering the pharmaceutical composition(s) may be included in the kits. For instance, a kit of this invention may provide one or more pre-filled syringes containing an effective amount of an HSA linker of the invention, or any binding, diagnostic, and/or therapeutic agent conjugated thereto. Furthermore, the kits may also include additional components such as instructions or administration schedules for a patient suffering from a disease or condition (e.g., a cancer, autoimmune disease, or cardiovascular disease) to use the pharmaceutical composition(s) containing an HSA linker of the invention, or any binding, diagnostic, and/or therapeutic agent conjugated thereto.

It will be apparent to those skilled in the art that various modifications and variations can be made in the compositions, methods, and kits of the present invention without departing from the spirit or scope of the invention. Thus, it is intended that the present invention cover the modifications and variations of this invention provided they come within the scope of the appended claims and their equivalents.

EXAMPLES

The following examples are provided by way of illustration only and not by way of limitation. Those of skill in the art will readily recognize a variety of non-critical parameters that could be changed or modified to yield essentially the same or similar results.

Example 1: Methods to identify residues in the HSA linker for site-specific conjugation of cytotoxic or cytostatic drugs.

To identify sites for specific conjugation of drug to HSA the crystal structure was studied and surface exposed serine and threonine residues were identified. These particular surface-exposed amino acids can then be mutated to cysteine allowing drug conjugation to the substituted cysteine using a thiol-specific conjugating agent such as maleimide. Mild reduction may be required prior to drug conjugation. The number of drugs conjugated is controlled by the number of surface exposed cysteine residues introduced into HSA. Serine and threonine are selected as the most suitable residues for mutation as they share the most structural identity with cysteine, however, other surface exposed residues may also be mutated to cysteine and successfully conjugated to cytostatic or cytotoxic drugs.

The crystal structure of HSA is deposited in the RSCB Protein Data Bank (1bm0 – Sugio *et al.*, "Crystal structure of human serum albumin at 2.5 A resolution," *Protein Eng.* 12:439-446 (1999)). This structure was analyzed using the DeepView Swiss PDB Viewer downloaded from the Swiss Institute of Bioinformatics. Serine and threonine residues with 50%, 40%, and 30% surface exposure were identified as the most suitable for mutation to cysteine (Table 2). Mutations can be introduced using standard molecular biology procedures. Conjugation of a thiol reactive drug or chelating agent to introduced cysteines can be tested using standard protein chemistry techniques.

Table 2

% Surface Exposure	Residue
50	T496
40	S58
30	T76, T79, T83, T125, T236, S270, S273, S304, S435, T478, T506, T508

Example 2: Methods to identify residues in the HSA linker for introduction of asparagine-linked glycosylation sites.

To identify regions for introduction of asparagine-linked glycosylation sites in HSA, the crystal structure was studied to identify surface exposed (>30%) asparagine, serine and threonine residues that would be suitable for mutation. Glycosylation occurs on asparagine residues when the

consensus sequence asparagine – x – serine/threonine is present, where x cannot be a proline. Table 2 lists possible mutation sites in HSA for the introduction of asparagine-linked glycosylation.

Table 3

Residue	Proposed Mutation
Gln32	Asn
Val46	Ser/Thr
Asp56	Asn
Asp63	Ser/Thr*
Glu231	Asn
Asp237	Asn
Gln268	Asn
Asp269	Ser/Thr
Glu285	Asn
Ala320	Ser/Thr*
Asp340	Asn
Glu354	Asn
Gln437	Asn
Glu425	Asn
Glu465	Asn
Asp494	Asn*

*These mutations have also been reported to occur very rarely in HSA (Carlson *et al.*, "Alloalbuminemia in Sweden: Structural study and phenotypic distribution of nine albumin variants," *Proc.Nat.Acad.Sci.USA* 89:8225- 8229 (1992); Madison *et al.*, "Genetic variants of human serum albumin in Italy: point mutants and a carboxyl-terminal variant," *Proc.Nat.Acad.Sci.USA* 91:6476-6480 (1994); Hutchinson *et al.*, "The N-terminal sequence of albumin Redhill, a variant of human serum albumin," *FEBS Lett.* 193:211-212 (1985); Brennan *et al.*, "Albumin Redhill (-1 Arg, 320 Ala-Thr): a glycoprotein variant of human serum albumin whose precursor has an aberrant signal peptidase cleavage site," *Proc.Nat.Acad.Sci.USA* 87:26-30 (1990); Minchiotti *et al.*, "Structural characterization of four genetic variants of human serum albumin associated with alloalbuminemia in Italy," *Eur.J.Biochem.* 247:476-482 (1997); Peach *et al.*, "Structural characterization of a glycoprotein variant of human serum albumin: albumin Casebrook (494 Asp-Asn)," *Biochim.Biophys.Acta* 1097:49-54 (1991)).

Example 3

B2B3-1 is a bispecific scFv antibody fusion molecule consisting of B1D2, a human anti-ErbB2 scFv antibody (SEQ ID NO:27) and H3, a human anti-ErbB3 scFv (SEQ ID NO:26). The two scFvs are joined by a modified human serum albumin (HSA) linker. The anti-ErbB3 scFv, H3, is recombinantly fused to the amino terminus of the HSA linker incorporating a short connector polypeptide and the anti-ErbB2 scFv, B1D2, is recombinantly fused to the carboxy terminus of the

modified HSA linker incorporating an additional short connector polypeptide. Each connector polypeptide was selected based on protease resistance properties. The modified HSA linker contains two amino acid substitutions. A cysteine residue at position 34 of native HSA was mutated to serine in order to reduce potential protein heterogeneity due to oxidation at this site. An asparagine residue at amino acid 503 of native HSA, which in native HSA is sensitive to deamidation and can result in decreased pharmacologic half-life, was mutated to glutamine. B2B3-1 selectively binds ErbB2 over-expressing tumors by virtue of its high affinity anti-ErbB2 scFv binding moiety, which has a kD in the range of 10.0 nM to 0.01 nM and more preferably a kD of about 0.3 nM. Subsequent binding of ErbB3 by the anti-ErbB3 scFv, which has a kD in the range of 50 to 1 nM and more preferably about 16 nM, prevents HRG induced phosphorylation of ErbB3. The modified HSA linker confers an extended circulating half-life on the bispecific molecule. B2B3-1 has a molecular weight of 119.6 kDa and is not glycosylated.

B2B3-1 inhibits ligand-induced phosphorylation of ErbB3 with sub-nanomolar potency by exploiting the abundant expression of its dimerization partner, ErbB2, to specifically target cancer cells that express both receptors.

Example 4

As shown in Figure 2, B2B3-1 variants inhibit HRG-induced pErbB3 in ZR75-1 breast cancer cells. ZR75-1 breast cancer cells were treated with a dose range of B2B3-1 variants for 24 hours followed by HRG stimulation. pErbB3 levels were measured in cell lysates and IC_{50} values were calculated together with the percent of inhibition. Shown are the mean IC_{50} values (Y axis) with error bars representing replicate experiments. Percent inhibition values are shown above the corresponding bar.

Example 5

Inhibition of phosphorylated ErbB3 (Figures 3A-D), AKT (Figures 4A-D), and ERK (Figures 5A-D) following 24 hour pre-treatment with B2B3-1 variants A5-HSA-B1D2 (panel A of Figures 3-5), H3-HSA-B1D2 (panel B of Figures 3-5), H3-HSA-F5B6H2 (panel C of Figures 3-5), and F4-HSA-F5B6H2 (panel D of Figures 3-5). BT474 breast cancer cells were treated with a dose range of B2B3-1 variants for 24 hours followed by HRG stimulation. pErbB3, pAKT, and pERK levels were measured in cell lysates and IC_{50} values were calculated together with the percent of inhibition.

Example 6

As shown in Figure 6, treatment of BT474 breast tumor cells with B2B3-1 variants causes G1 cell cycle arrest and a decrease in the population of cells in S phase. BT474 cells were treated with 1 μM of B2B3-1 variants and controls for 72 hours. After the end of treatment, cells were trypsinized, gently resuspended in hypotonic solution containing propidium iodide and single cells were analyzed by flow cytometry. Cell cycle distribution in G1 and S phases was measured using curve-fitting algorithms designed for cell cycle analysis (FlowJo software cell cycle platform).

Example 7

B2B3-1 (SEQ ID NO:16) inhibits ErbB3 activation, utilizing the abundant expression of its dimerization partner, ErbB2, to target tumor cells. A high affinity anti-ErbB2 scFv antibody, B1D2, facilitates targeting of B2B3-1 to tumor cells over-expressing ErbB2. B1D2 is connected by a modified HSA linker to a lower affinity anti-ErbB3 scFv antibody, H3, which blocks binding of ErbB3's ligand, HRG, thereby inhibiting ErbB3 phosphorylation and downstream AKT signaling. The ErbB2 binding scFv, B1D2, is derived from parent scFv C6.5, which possesses neither agonistic nor antagonistic activity. B1D2, therefore, likely functions solely as a targeting agent. The lower affinity binding of the ErbB3 binding scFv prevents strong binding of B2B3-1 to normal, non-cancerous tissues which express ErbB3 but little or no ErbB2, thereby reducing the potential for non-specific toxicity. In tumor cells expressing both ErbB2 and ErbB3, the avidity effect of bispecific B2B3-1 binding to both receptors overcomes the low affinity of the ErbB3 scFv allowing strong inhibition of HRG interaction.

The ability of B2B3-1 to inhibit HRG binding to ErbB3 was investigated using flow cytometry (FACS). Cells of the breast cancer cell line BT-474-M3 (a variant of BT-474 that over-express ErbB2), were pretreated with 1 μM B2B3-1 then incubated with 10 nM biotinylated HRG 1β EGF domain. After extensive washing, binding was assessed using streptavidin-AlexaFluor 647 conjugate. All incubations were performed at 4°C. Figure 7 shows that B2B3-1 is capable of blocking the binding of HRG to ErbB3.

Example 8

After demonstrating B2B3-1's ability to block HRG binding to ErbB3, we investigated the effect of B2B3-1 on ErbB3 signaling *in vitro* on two cell lines that express ErbB3 and over-express ErbB2. Breast cancer cell lines BT-474-M3 and ZR75-3 0 were serum starved overnight, pre-treated with a dose titration of B2B3-1 for 24 hours and then stimulated for 10 minutes with 5 nM of HRG 1β EGF domain. The phosphorylation status of ErbB3 and AKT was then examined using an ELISA assay. The results show that B2B3-1 inhibits HRG induced activation of both ErbB3 and AKT phosphorylation in a dose-dependent manner and with potent IC_{50}s in both cell lines (Figures 8A-D).

Example 9

Figure 9 shows the effect of B2B3-1 treatment on signaling proteins in BT474 breast cancer cells. Cells were treated with a dose range of B2B3-1 for 24 hours, followed by heregulin stimulation. Levels of pErbB2, pErbB3, pErk and pAKT and their corresponding total protein levels were determined on cell lysates by Western blot analysis.

Example 10

Figure 10 shows the immunoprecipitation-Western blot analysis of B2B3-1 treated BT474 breast cancer cells. Cells were treated with a dose range of B2B3-1 for 24 hours, followed by heregulin stimulation. ErbB2-associated complexes were isolated from cell lysates using an anti-ErbB2 antibody followed by Western blot analysis to detect pErbB2 and pErbB3 and their corresponding total protein levels.

Example 11

The anti-tumor activity of B2B3-1 was investigated *in vitro* using a number of assays. In the first assay, the effect of B2B3-1 on the accumulation of BT-474 or SKBR3 cells in G1 phase and the concomitant decrease in S phase of the cell cycle was examined. Briefly, cells were treated with 1 μM B2B3-1 or PBS vehicle for 72 hours. After the end of treatment, cells were trypsinized, gently resuspended in hypotonic solution containing propidium iodide and single cells were analyzed by flow cytometry. Cell cycle distribution in G1 and S phases was measured using curve –fitting algorithms designed for cell cycle analysis (FlowJo software cell cycle platform). B2B3-1 was found to modestly decrease the percentage of cells in S phase and increase the population of cells in

G1 phase (Figure 11A). In a second experiment, the number of cell colonies formed following treatment with B2B3-1 was studied. BT-474 and SKBR3 breast cancer cells were plated in the presence of 1 μM B2B3-1 and compared to cells plated in media only. Media only or media including treatment was replenished every 3 days. After 14 days the number of colonies were counted and compared to untreated cells. Figure 11B illustrates the 40-45% decrease in the number of colonies formed when cells are treated with B2B3-1 compared to control cells. Finally, the ability of B2B3-1 to inhibit cell proliferation was assessed in a cell impedance assay using a Real-Time Cell Electronic Sensing System (RT-CES: ACEA Biosciences). BT- 474 cells were seeded on plates integrated with microelectronic sensor arrays and treated with a dose titration of B2B3-1 or media only for 72 hours. Data reflecting the generation of cell-electrode impedance response was collected every hour for 72 hours and IC_{50} values were calculated 68 hours after treatment. Figure 11C illustrates that B2B3-1 was able to inhibit impedance of BT-474 cells with an IC_{50} of 33 nM.

Example 12

We also investigated whether B2B3-1 exhibits agonistic activity based on its ability to simultaneously bind and cross-link ErbB2 and ErbB3 receptors. Serum starved ZR75-1 breast cancer cells were incubated with 1 μM B2B3-1 or PBS vehicle for 24 hours. Cells were also treated with B2B3-1 or PBS vehicle for 24 hours followed by a 10-minute stimulation with 5 nM HRG 1β EGF domain. Cells were lysed and the pErbB3 content of the lysates was assessed by ELISA. Figure 12 shows that cells treated with B2B3-1 alone contained comparable levels of phosphorylated ErbB3 as untreated cells, indicating that B2B3-1 is non-agonistic.

Example 13

The ability of B2B3-1 to bind with specificity to ErbB2 and ErbB3 and not to related ErbB family members, EGFR and ErbB4, was investigated by ELISA. Plates were coated with the recombinant extracellular domain of either ErbB2 or ErbB3. Plates were blocked and incubated with a half-maximal binding concentration of B2B3-1 in the presence of a dilution series of competing recombinant extracellular domains of EGFR, ErbB2, ErbB3 or ErbB4. The results show only soluble ErbB2 extracellular domain blocked B2B3-1 binding to ErbB2-coated plates (Figure 13A). Likewise, only soluble ErbB3 extracellular domain blocked B2B3-1 binding to ErbB3-coated plates (Figure 13B). These results demonstrate the specificity of the anti-ErbB2 arm B1D2 for ErbB2, and

of the anti-ErbB3 arm H3 for ErbB3. The increased signal observed when soluble ErbB2 extracellular domain was incubated with B2B3-1 on the ErbB3 coated plate is assumed to be due to formation of an ErbB2, ErbB3, B2B3-1 complex on the plate.

Example 14

The ability of B2B3-1 to bind avidly to tumor cells expressing both ErbB2 and ErbB3 was studied. SKO2 and SKO3 are variants of B2B3-1 that lack the ability to interact with ErbB2 or ErbB3, respectively. MALME-3M melanoma cells, which express approximately equal numbers of ErbB2 and ErbB3 receptors, were incubated with a dilution series of B2B3-1, SKO2, or SKO3 in the presence of saturating concentrations of a goat anti-HSA Alexafluor-647 conjugated antibody. Cell binding was assessed by flow cytometry and apparent binding affinities were determined for each molecule. Control cells were incubated with secondary antibody alone. No measurable cell binding was observed for SKO2, which retains only low affinity binding to ErbB3 and lacks ErbB2 binding activity. SKO3, which retains a functional, high affinity ErbB2 binding scFv but lacks the ability to bind ErbB3 had a K_D of 6.7 nM. B2B3-1 bound cells with a K_D of 0.2 nM, demonstrating avidity binding of this bispecific molecule (Figure 14).

Example 15

The stability of B2B3-1 under physiological conditions was assessed by incubating 100 nM B2B3-1 in human, Cynomolgus monkey, or mouse serum at 37°C for a period of 120 hours. Samples were removed at 0, 24, 48, 72, 96 and 120 hours and the ability of B2B3-1 to bind both ErbB2 and ErbB3 was measured by ELISA. This ELISA involves coating a 96-well plate with recombinant ErbB2 extracellular domain overnight followed by a blocking step and then incubation with a dilution series of B2B3-1. Plates are then incubated with an Fc-ErbB3 extracellular domain fusion protein followed by a goat antihuman-Fc-HRP conjugate. Plates are developed by addition of supersignal chemiluminescense substrate. Figures 15A-C show that B2B3-1 remains stable in serum from all three species under physiological conditions, retaining comparable ability to bind both ErbB2 and ErbB3 at all time points measured.

Example 16: B2B3-1 dose response in BT-474-M3 breast cancer xenograft model.

The efficacy of B2B3-1 *in vivo* was assessed using nude mice bearing BT-474-M3 xenografts. Ten mice per group were treated with 12 doses of 0.3, 1, 3, 10, 30 or 90 mg/kg of B2B3-1 every 3 days. Control groups were administered PBS vehicle or HSA at an equimolar dose to the 90 mg/kg B2B3-1 dose. All doses were administered interperitoneally (i.p.). Tumor size was measured twice a week and the corresponding tumor volume was calculated. The results show that B2B3-1 treatment leads to significant reduction in BT-474-M3 tumor size as compared to the control group (Figure 16). Complete regressions were observed in each of the B2B3-1 treatment groups except mice treated with the lowest dose of B2B3-1 (0.1 mg/kg).

Example 17

As shown in Figures 17A-E, B2B3-1 is effective in multiple xenograft models in an ErbB2 dependent manner. B2B3-1 was efficacious in the Calu-3 (Figure 17A), SKOV-3 (Figure 17B), NCI-N87 (Figure 17C), and MDA-MB-361 (Figure 17E) xenograft models expressing ErbB2 at > 1 x 10^5 receptors/cell but worked less well in the ACHN (Figure 17D) xenograft model which expresses 4.5 x 10^4 ErbB2 receptors/cell. Mice were treated with 30 mg/kg of B2B3-1 every 3 days.

Example 18

Over-expression of ErbB2 converts B2B3-1 non-responder ADRr breast cancer xenograft model into a responder to B2B3-1 (Figures 18A B). ErbB2 was over-expressed in ADRr breast cancer cells using a retroviral expression system. Transfected cells expressing high levels of ErbB2 (ADRr –E2) were selected using FACS and subsequently injected subcutaneously into nude mice to establish xenograft tumors. Mice were treated with 30 mg/kg of B2B3-1 every 3 days. While no response to B2B3-1 was observed in wild type ADRr xenografts (Figure 18A), ADRr-E2 xenografts (Figure 18B) responded to B2B3-1.

Example 19

As shown in Figures 19A-B, B2B3-1 activity correlates positively with ErbB2 expression levels *in vitro* (Figure 19A) and *in vivo* (Figure 19B). B2B3-1 inhibition of ErbB3 phosphorylation was determined in 9 tumor cell lines with expression levels of ErbB2 ranging from 5 x 10^4 receptors/cell to 2.4 x 10^6 receptors/cell using an ELISA assay. The extent of B2B3-1's ability to

inhibit ErbB3 phosphorylation to basal levels (% pErbB3 inhibition) was found to correlate positively with ErbB2 expression levels. Similarly, B2B3-1 activity was assessed in 10 tumor xenograft models expressing low to high levels of ErbB2. Xenograft response also correlated positively with ErbB2 expression levels.

Example 20

B2B3-1 treatment of BT474-M3 breast tumor cells results in translocation of $p27^{kip1}$ to the nucleus (Figure 20A). BT474-M3 cells were treated with 1 μM of B2B3-1 for 6 hours. The sub-cellular location of $p27^{kip1}$ was assessed using immunofluorescence techniques. In cells treated with B2B3-1, $p27^{kip1}$ translocated to the nucleus, which has been shown to result in inhibition of cell proliferation. $p27^{kip1}$ remained in the cytoplasm of untreated cells.

To further study the effect of B2B3-1 on the cell cycle, BT-474-M3 cells treated with B2B3-1 for 72 hours were probed for the cell cycle regulator Cyclin D1 using Western blot analysis (Figure 20B). The cytoskeleton protein vinculin was used as a protein loading control in this experiment. B2B3-1 treatment resulted in a decrease in the levels of Cyclin D1 compared to untreated cells.

Example 21

As shown in Figures 21A-B, B2B3-1 treatment of BT474-M3 breast tumor xenografts results in translocation of $p27^{kip1}$ to the nucleus. BT474 breast tumor xenografts were treated with B2B3-1 (Figures 21A) at a dose of 30 mg/kg or an equimolar dose of HSA (Figures 21B) every 3 days for a total of 4 doses. Increased nuclear staining for $p27^{kip1}$ was observed in B2B3-1 treated tumors compared to HSA control tumors indicating an anti-proliferative effect of B2B3-1 *in vivo*.

Example 22

B2B3-1 treatment results in a reduction of the proliferation marker Ki67 in BT474 breast cancer xenograft tumors. BT474-M3 breast tumor xenografts were treated with B2B3-1 (Figure 22A) at a dose of 30 mg/kg or an equimolar dose of HSA (Figure 22B) every 3 days for a total of 4 doses. Subsequent staining of tumor sections for Ki67 demonstrated a reduced expression pattern for B2B3-1 treated tumors compared to HSA treated tumors.

Example 23

B2B3-1 treatment results in a reduction of vessel density in BT474-M3 breast cancer xenograft tumors, as determined by assaying for CD31 expression (Figures 23A-B). BT474 breast tumor xenografts were treated with B2B3-1 (Figure 23A) at a dose of 30 mg/kg or an equimolar dose of HSA (Figure 23B) every 3 days for a total of 4 doses. Tumors were stained for the presence of vascular marker CD31. Tumors treated with B2B3-1 show a marked decrease in vessel density compared to control tumors treated with HSA.

Example 24: B2B3-1 inhibits phosphorylation of ErbB3 in vivo.

BT-474-M3 xenograft tumors were treated with 30 mg/kg B2B3-1 or 17.5 mg/kg HSA every 3 days for a total of 4 doses and tumors were harvested 24 hours after the final dose. Tumors were lysed and subjected to SDS-PAGE followed by Western analysis to assess relative levels of phosphorylation of B2B3-1's target ErbB3. Equal quantities of protein were loaded in each lane and total protein levels were controlled by probing for beta tubulin. Western blot analysis using antibodies specific for phosphorylated ErbB3 show that B2B3-1 treated tumors contain less pErbB3 than HSA treated tumors (Figure 24A). Densitometry of the western blot analysis followed by normalization of the mean pErbB3 integral band intensity to the mean beta tubulin integral band intensity demonstrated that B2B3-1 treated tumors contained significantly less pErbB3 than control HSA treated tumors (Figure 24B). These data confirmed that B2B3-1 inhibits phosphorylation of its target ErbB3 *in vivo*.

Example 25: In vivo activity of B2B3-1 in BT-474-M3 xenografts which have reduced PTEN activity.

ShRNA technology was applied to knock out the activity of the tumor suppressor gene phosphatase and tensin homolog (PTEN) in BT-474-M3 breast cancer cells. Briefly, cultured BT-474-M3 cells were transfected with shPTEN or shControlRNA by retroviral transfection. Transfected cells with reduced PTEN were selected using FACS and subsequently injected subcutaneously into the right flank of nude mice to establish xenograft tumors. Cells transfected with a control vector were injected into the left flank of the same mouse. Mice were treated with 30mg/kg B2B3-1 every 3 days or 10mg/kg Herceptin every week. HSA was injected as a control at an equimolar dose to B2B3-1. All injections were done i.p.

B2B3-1 and Herceptin promoted a reduction in the size of tumors formed by control BT-474-M3 breast cancer cells (Figure 25A), whereas only B2B3-1 (and not Herceptin) promoted a reduction in the size of tumors formed by BT-474-M3 breast cancer cells lacking expression of PTEN (Figure 25B).

Example 26: B2B3-1 inhibits ErbB3 signaling in BT-474-M3 breast cancer cells having reduced PTEN activity.

The ability of B2B3-1 to inhibit phosphorylation of ErbB3 signaling in tumor xenografts was studied using the PTEN deficient BT-474-M3 model. Xenograft tumors of the engineered cell line or control cell line were treated with 30 mg/kg B2B3-1, 17.5 mg/kg HSA every 3 days or 10 mg/kg Herceptin weekly and tumors were harvested 24 hours after the final dose. Tumors were lysed and subjected to SDS-PAGE followed by Western analysis to assess relative levels of phosphorylation of B2B3-1's target ErbB3, AKT and total PTEN levels. Equal quantities of protein were loaded in each lane and total protein levels were controlled by probing for PCNA. Western blot analysis using antibodies specific for phosphorylated ErbB3 show that B2B3-1 treated tumors contain less pAKT than HSA treated or Herceptin treated tumors (Figure 26A). Densitometry of the western blot analysis followed by normalization of the mean pAKT integral band intensity to the mean PCNA integral band intensity demonstrated that B2B3-1 treated tumors contained significantly less pAKT than control HSA treated and Herceptin-treated tumors (Figure 26B).

Example 27

The pharmacokinetic parameters for B2B3-1 were investigated in nu/nu mice. Animals were randomized into groups and administered intravenous (IV) with a single dose of 5, 15, 30, or 45 mg/kg B2B3-1 (Figures 27A-D, respectively). Blood was collected pre-dose and at 0.5, 4, 8, 24, 48, 72, and 120 hours post dose. Three mice were used for each time point. Serum levels of B2B3-1 were measured using two ELISA methods. The first method requires functional binding of B2B3-1 to both ErbB2 and ErbB3 while the second method measures only the HSA component of B2B3-1 in the serum. The HSA ELISA utilizes a polyclonal-anti HSA capture antibody and a HRP-conjugated polyclonal anti-HSA detection antibody. A reduction in B2B3-1 serum concentration measured using the ErbB2/ErbB3 binding method versus the HSA method would indicate a loss in functional

B2B3-1. Figures 27A-D show that the pharmacokinetic properties of B2B3-1 are comparable when assessed using either ELISA method, indicating that B2B3-1 is stable in circulation in mice.

Example 28

B2B3-1 serum concentrations were fit using a two-compartment, biexponential model and showed biphasic disposition. Terminal half-lives were calculated to be 17, 16, 23, and 18 hrs for the 5, 15, 30, or 45 mg/kg doses, respectively, and are shown in Table 4. Increases in B2B3-1 dose resulted in a linear increase in exposure (Figure 28).

Table 4: Pharmacokinetic properties of B2B3-1 in mice and Cynomolgus monkeys.

Dose (mg/kg)	Species	N	T½β (hrs)	AUC (hr-μg/ml)	Clearance (ml/hr/kg)
5	Mouse (single dose)	3	16.9	1.58E+03	3.19
15	Mouse (single dose)	3	16.2	6.10E+03	2.47
30	Mouse (single dose)	3	22.6	1.18E+04	2.54
45	Mouse (single dose)	3	17.5	1.84E+04	2.46
4	Cynomolgus monkey (1st dose)	2	39.1	3.44E+03	1.17
4	Cynomolgus monkey (4th dose)	2	44.9	7.20E+03	0.60
20	Cynomolgus monkey (1st dose)	2	33.1	2.29E+04	0.88
20	Cynomolgus monkey (4th dose)	2	122.5	8.20E+04	0.25
200	Cynomolgus monkey (1st dose)	4	68.8	3.18E+05	0.64
200	Cynomolgus monkey (4th dose)	2	69.7	5.72E+05	0.35
200	Cynomolgus monkey (4th dose)*	2	66.6	5.99E+05	0.34

*recovery animals

Example 29

Blood samples for pharmacokinetic analysis were also obtained from a dose range-finding toxicology study in female Cynomolgus monkeys. In this study, animals were infused with 4, 20 or 200 mg/kg of B2B3-1 administered every 3 days for 4 doses. Sampling occurred prior to and 5 minutes after dosing on each dosing day (study days 1, 4, 7 and 10) to provide pre-dose and

peak/trough concentrations, and at 1, 2, 4, 8, 24 and 48 hours after the end of the first infusion on day 1 and at 1, 2, 4, 8, 24, 48, 72 and 120 hours after the last infusion on day 10. For recovery animals dosed at 200 mg/kg serum samples were also collected at 168, 336 and 456 hours after the last infusion.

Cynomolgus monkey serum samples were assayed using the ErbB2/ErbB3 ELISA method described previously. Serum concentrations for each dose over the time course are shown in Figure 29. The analysis showed that mean concentration-time profiles for serum B2B3-1 after dosing on days 1 and 10 were qualitatively similar with concentrations generally declining with time from Cmax. Mean half-life estimates ranged from 38.3-67.2 hours on day 1 and 45.0 to 121.0 hours on day 10 (Table 4).

Example 30

The plasmid encoding the B2B3-1 bispecific scFv antibody fusion protein was created combining gene sequences of a unique human anti-ErbB3 scFv (designated "H3"), a human anti-ErbB2 scFv (designated "B1D2"), and a modified human serum albumin (HSA) linker. The anti-ErbB3 scFv, H3, is recombinantly linked to the amino terminus of the HSA linker via a connecting peptide (Ala-Ala-Ser) and the anti-ErbB2 scFv, B1D2, is genetically linked the carboxy terminus of the HSA linker via a connecting peptide (Ala-Ala- Ala-Leu). The peptide connectors were formed through the introduction of restriction sites during construction of the mammalian expression vector and were synthesized with optimized codon usage for mammalian expression together with the single chain antibody fragments and HSA linker.

The B1D2 scFv was selected from a combinatorial phage display library created by mutagenesis of the ErbB2-binding scFv C6.5, which was selected from a non-immune phage display library. The H3 scFv was selected from a non-immune phage display library originally made by Sheets et al. The gene sequences encoding the B1D2 and H3 single chain antibody fragments were optimized for CHO cell codon preferences and synthesized for subsequent construction of the B2B3-1 encoding plasmid.

The modified HSA linker contains two amino acid substitutions. A cysteine residue at position 34 was mutated to serine in order to reduce potential protein heterogeneity due to oxidation at this site. An asparagine residue at amino acid 503 was mutated to glutamine, which in wild type HSA is sensitive to deamination and can result in decreased pharmacologic half-life.

The gene sequence encoding the modified HSA linker was synthesized with optimized codon usage for mammalian expression for subsequent construction of the B2B3-1 encoding plasmid.

Example 31

The B2B3-1 coding sequence was cloned into pMP10k base vector using standard molecular biology techniques to create plasmid pMP10k4H3-mHSA-B1D2, shown in Figure 30. For the most part this construct employs commonly used genetic elements. B2B3-1 expression is driven by the human GAPD promoter. This vector utilizes genetic elements referred to as Matrix Attachment Regions or MAR elements. The MAR genetic elements control the dynamic organization of chromatin, and insulate nearby genes from the effect of surrounding chromatin thereby increasing copy number dependent, position-independent, expression of genes. MAR elements have been shown to improve the probability of isolating a clone exhibiting the desired level of expression for the production of a recombinant protein and to increase the stability of production. The MAR elements used in the B2B3-1 constructs are non-coding human MAR elements. In addition to the B2B3-1 plasmid, a neomycin antibiotic resistance plasmid (Figure 31) and a hygromycin resistance plasmid (Figure 32) were also used to select for stable transformants.

Example 32: First round of Gene Transfection

Chinese Hamster Ovary CHO-K1 cells were purchased from ATCC (ATCC # CCL-61). The CHO-K1 cell line is a serum and proline dependent adherent sub-clone of the parental CHO cell line created by T.T. Puck. The CHO-K1 cells used for B2B3-1 transfection were pre-adapted for suspension growth in serum free media prior to transfection. An iterative transfection procedure was used to develop the B2B3-1 cell line. Twenty-four hours before transfection, CHO-K1 cells were sub passaged to $1.0x10^6$ cells/mL in SFM4CHO (Serum Free) medium (HyClone, Logan, UT) supplemented with 8mM L-glutamine, 0.1 mM sodium hypoxanthine, and 0.016 mM thymidine. On the day of transfection, cells were resuspended in OptiMEM medium (Invitrogen Corp, Carlsbad, CA) and 40,000 cells were placed in each well of a twenty-four well plate. In the first transfection, the B2B3-1 expression plasmid (pMP10k4H3-mHSA-B1D2) and the neomycin resistance plasmid (Figure 30; pSV2-neo (Selexis, Inc., Marlborough, MA) were mixed together using a molar plasmid ratio of 75:1 (B2B3-1:neomycin resistance). The plasmid mixture was subsequently mixed with a cationic lipid transfection reagent (Lipofectamine LTX, Invitrogen Corp, Carlsbad, CA) and

lipid/DNA complexes were allowed to form for thirty minutes. The DNA/Lipid complex was than added to the CHO-K1 cells and the 24-well plates were placed in a 37°C, 5% CO_2 incubator.

Example 33: Selection and Screening for High Producers

The contents of each transfection well were washed with PBS, trypsinized and distributed across two, ninety-six well plates. The growth media used consisted of DMEM/F12 (Invitrogen Corp, Carlsbad, CA) with 10% FBS (Invitrogen Corp, Carlsbad, CA) and 500 mg/L of geneticin (G418; Invitrogen Corp, Carlsbad, CA). Media in the 96-well plates was changed on day 4 to SFM4CHO medium supplemented with 8mM L-glutamine, 0.1 mM sodium hypoxanthine, 0.016 mM thymidine, and 500mg/L geneticin. Following an additional two weeks of culture in selection medium, surviving cells had formed well-defined colonies. The clones were evaluated using quantitative spot blot techniques. The top producing colonies were trypsinized, and expanded to a single well of a 24-well plate.

A seven day productivity assay was used to screen for high B2B3-1 producing colonies. Upon expansion the cells in 24-well plates were allowed to proliferate for seven days in SFM4CHO medium supplemented with 8 mM L-glutamine, 0.1 mM sodium hypoxanthine, and 0.016 mM thymidine. The B2B3-1 concentration in the spent media was determined. Top clones from the 24-well scale were expanded into 125 mL baffled shake flasks. A seven day study in the shake flask in SFM4CHO medium supplemented with 8mM L-glutamine, 0.1 mM sodium hypoxanthine, and 0.016 mM thymidine was used to screen the cell pools for growth and B2B3-1 production.

Example 34: Second Round of Gene Transfection

The highest producing cell pool determined from the first round of transfection (*supra*) was transfected a second time to increase production. Twenty-four hours before transfection, the cell pool was sub passaged to $1.0x10^6$ cells/mL in SFM4CHO (Serum Free) medium supplemented with 8mM L-glutamine, 0.1 mM sodium hypoxanthine, and 0.016 mM thymidine. On the day of transfection, cells were resuspended in OptiMEM medium (Invitrogen Corp, Carlsbad, CA) and 40,000 cells were placed in each well of a twenty-four well plate. In the first transfection, the B2B3-1 and hygromycin resistance plasmid (Figure 32; pTK-Hyg (Clontech, Mountain View, CA)) were mixed together using a molar plasmid ratio of 50:1 (B2B3-1:hygromycin resistance). The plasmid mixture was subsequently mixed with a cationic lipid transfection reagent (Lipofectamine LTX,

Invitrogen Corp) and lipid/DNA complexes were allowed to form for thirty minutes. The DNA/Lipid complex was than added to the cell pool and the 24-well plates were placed in a 37°C, 5% CO_2 incubator.

Example 35: Selection and Screening for High Producers From Second Transfection

The contents of each transfection well were washed with PBS, trypsinized and distributed across two, 96-well plates. The growth media used consisted of DMEM/F12 supplemented with 10% FBS and 400 mg/L of hygromycin B (Invitrogen Corp). Media in the 96-well plates was changed on day 4 to Hyclone SFM4CHO medium supplemented with 8mM L-glutamine, 0.1 mM sodium hypoxanthine, 0.016 mM thymidine , and 400 mg/L of hygromycin B. After an additional two weeks of selection, surviving cells had formed well-defined colonies. The clones were evaluated using quantitative spot blot techniques. The top producing colonies were trypsinized, and expanded to a single well of a 24-well plate.

A seven day productivity assay was used to screen for high B2B3-1 producing colonies. Upon expansion the cells were allowed to proliferate for seven days, and the B2B3-1 concentration in the spent media was determined.

Top clones from the 24-well plates were expanded into 125 mL baffled shaker flasks in the Hyclone SFM4CHO medium supplemented with 8mM L-glutamine, 0.1 mM sodium hypoxanthine, and 0.016 mM thymidine. A seven day study in shake flask was used to screen the cell pools for growth and B2B3-1 production. The spent media was quantitated using Protein Aresin and an HPLC instrument.

Example 36: Limiting Dilution Cloning

The best growing and highest B2B3-1-producing colony identified by the productivity assay was transferred from the 125 mL shaker flask and plated in five 96-well plates at a cell concentration calculated to give one cell/well. The 96-well plates were placed in an incubator at 37°C and 5% CO_2. The wells were examined bi-weekly to track formation of colonies. Colonies arising from a single cell were identified based on the symmetrical shape of the colony. Wells containing such colonies were marked for further screening by 24-well 7-day assessment, and 125mL shaker flask 7-day assessment.

The second round of limiting dilution cloning was performed in a similar manner to the first round. An additional 100 mL fed batch evaluation was performed to confirm clone choice. A pre-seed bank was cryopreserved.

Example 37: Formulation of B2B3-1

B2B3-1 can be administered once a week via intravenous infusion over a period of 60 or 90 minutes, depending on patient tolerability. B2B3-1 can be formulated in a sterile 20 mM L-histidine hydrochloride, 150 mM sodium chloride, pH 6.5 solution at a concentration of 25mg/mL for administration to a patient (e.g., a human).

Example 38: Treatment of Breast Cancer

An HSA linker of the invention (e.g., B2B3-1; SEQ ID NO:16) can be administered to a patient diagnosed with breast cancer. For example, a patient whose cancer is diagnosed as expressing high levels of epidermal growth factor receptors, such as ErbB2 (HER2/neu) can be treated with an HSA linker conjoined to an ErbB2 biding moiety. A physician providing care to a patient diagnosed with breast cancer can administer an HSA linker of the invention suitable and capable of providing the best therapeutic benefit to the patient. Selection of, for example, the binding moieties, conjoined to an HSA linker of the invention can be based on clinical determinants of the patient and specific cancer variant. For example, genotypic or histologic screens of cancer biopsies can reveal increased expression of ErbB2, indicating that an HSA linker of the invention that incorporates and anti-ErbB2 binding moiety (e.g., B2B3-1) can be used therapeutically.

In one embodiment of the invention, B2B3-1 can be administered to a patient diagnosed with breast cancer once a week via intravenous infusion over a period of, e.g., 60 or 90 minutes, depending on patient tolerability. B2B3-1 can be formulated in a sterile 20 mM L-histidine hydrochloride, 150 mM sodium chloride, pH 6.5 solution at a concentration of 25mg/mL for administration to a patient (e.g., a human). A clinician supervising the administration of B2B3-1 or any other HSA linker of the invention can follow common formulation and dosing practices to determine the proper course of therapy for any given patient.

The invention further provides for the co-administration of one or more therapeutic drugs or compounds in combination with the HSA linker of the invention (e.g., B2B3-1; SEQ ID

NO:16), such as the common chemotherapeutic regimen for the treatment of breast cancer includes doxorubicin, cyclophosphamide, and paclitaxel. Alternatively, a clinician can administer the HSA linker of invention (e.g., B2B3-1) in combination with surgical or radiation therapy to treat breast cancer in a patient in need thereof.

Example 39: Treatment of Ovarian Cancer

An HSA linker of the invention can be administered to a patient diagnosed with ovarian cancer (ovarian carcinoma), for example, a patient whose cancer is diagnosed as expressing high levels of epidermal growth factor receptors, such as ErbB2 (HER2/neu) can be treated with an HSA linker conjoined to an ErbB2 biding moiety. A physician providing care to a patient diagnosed with ovarian cancer can administer an HSA linker of the invention suitable and capable of providing the best therapeutic benefit to the patient. Selection of, for example, the binding moieties, conjoined to an HSA linker of the invention can be based on clinical determinants of the patient and specific cancer variant. For example, genotypic or histologic screens of cancer biopsies can reveal increased expression of ErbB2 or other targeting molecules (e.g., overexpressed receptors or ligands specific to the cancer), which can be targeted by an HSA linker of the invention that incorporates an anti-ErbB2 binding moiety (e.g., B2B3-1) or other target specific binding moiety.

In one embodiment of the invention, B2B3-1 can be administered to a patient diagnosed with ovarian cancer once a week via intravenous infusion over a period of, e.g., 60 or 90 minutes, depending on patient tolerability. B2B3-1 can be formulated in a sterile 20 mM L-histidine hydrochloride, 150 mM sodium chloride, pH 6.5 solution at a concentration of 25mg/mL for administration to a patient (e.g., a human). A clinician supervising the administration of B2B3-1 or any other HSA linker of the invention for the treatment of ovarian cancer can follow common formulation and dosing practices (e.g., intraperitoneal injection) to determine the proper course of therapy for any given patient.

The invention further provides for the co-administration of one or more therapeutic drugs or compounds in combination with the HSA linker of the invention (e.g., B2B3-1; SEQ ID NO:16). Alternatively, a clinician can administer an HSA linker of invention in combination with surgical or radiation therapy to treat ovarian cancer in a patient in need thereof.

Example 40

HSA linker conjugates of the invention can be constructed using one or more of the elements (groups A-E) listed in Table 5 below. In particular, an HSA linker of the invention, which is shown as Group C in Table 5 below, can incorporate one or more binding moieties selected from groups A and E shown in Table 5. In addition, the HSA linker conjugates can also include one or more peptide connectors, which are selected from groups B and D in Table 5, at each of the amino and carboxy terminal ends of the HSA linker. Peptide connectors can be repeated or truncated to increase or decrease the length of the connector sequence.

The invention provides specific embodiments of the HSA linkers, polypeptide connectors, and binding moieties discussed above. Table 6 lists ten HSA linker conjugates with varying ErbB2-specific or ErbB3-specific binding moieties, as well as polypeptide connectors, at the amino and carboxy termini of an HSA linker of the invention.

Table 6

Agent of the Invention	Amino Terminal Binding Moiety	N-Terminal Connector	HSA	C-Terminal Connector	Carboxy Terminal Binding Moiety
B2B3-1 (SEQ ID NO:16)	H3 (SEQ ID NO:26)	AAS	mHSA (SEQ ID NO:1)	AAAL (SEQ ID NO:5)	B1D2 (SEQ ID NO:27)
B2B3-2 (SEQ ID NO:17)	A5 (SEQ ID NO:28)	AAS	mHSA (SEQ ID NO:1)	AAAL (SEQ ID NO:5)	B1D2 (SEQ ID NO:27)
B2B3-3 (SEQ ID NO:18)	A5 (SEQ ID NO:28)	AAS	mHSA (SEQ ID NO:1)	AAAL (SEQ ID NO:5)	F5B6H2 (SEQ ID NO:32)
B2B3-4 (SEQ ID NO:19)	A5 (SEQ ID NO:28)	AAS	mHSA (SEQ ID NO:1)	AAAL (SEQ ID NO:5)	ML3.9 (SEQ ID NO:29)
B2B3-5 (SEQ ID NO:20)	B12 (SEQ ID NO:30)	AAS	mHSA (SEQ ID NO:1)	AAAL (SEQ ID NO:5)	B1D2 (SEQ ID NO:27)
B2B3-6 (SEQ ID NO:21)	B12 (SEQ ID NO:30)	AAS	mHSA (SEQ ID NO:1)	AAAL (SEQ ID NO:5)	F5B6H2 (SEQ ID NO:32)
B2B3-7 (SEQ ID NO:22)	F4 (SEQ ID NO:31)	AAS	mHSA (SEQ ID NO:1)	AAAL (SEQ ID NO:5)	B1D2 (SEQ ID NO:27)
B2B3-8 (SEQ ID NO:23)	F4 (SEQ ID NO:31)	AAS	mHSA (SEQ ID NO:1)	AAAL (SEQ ID NO:5)	F5B6H2 (SEQ ID NO:32)
B2B3-9 (SEQ ID NO:24)	H3 (SEQ ID NO:26)	AAS	HSA (SEQ ID NO:3)	AAAL (SEQ ID NO:5)	B1D2 (SEQ ID NO:27)
B2B3-10 (SEQ ID NO:25)	H3 (SEQ ID NO:26)	AAS	mHSA (SEQ ID NO:1)	AAAL (SEQ ID NO:5)	F5B6H2 (SEQ ID NO:32)

APPENDIX A

SEQUENCE LISTINGS

SEQ ID NO:1
Human Serum Albumin (HSA) Linker with C34S and N503Q substitutions ("mHSA") Amino Acid Sequence

DAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQSPFEDHVKLVNEVTEFAKTCVADESAE
NCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECFLQHKDDNPNLPRLVRPEV
DVMCTAFHDNEETFLKKYLYEIARRHPYFYAPELLFFAKRYKAAFTECCQAADKAACLLP
KLDELRDEGKASSAKQRLKCASLQKFGERAFKAWAVARLSQRFPKAEFAEVSKLVTDLTK
VHTECCHGDLLECADDRADLAKYICENQDSISSKLKECCEKPLLEKSHCIAEVENDEMPAD
LPSLAADFVESKDVCKNYAEAKDVFLGMFLYEYARRHPDYSVVLLLRLAKTYETTLEKCC
AAADPHECYAKVFDEFKPLVEEPQNLIKQNCELFEQLGEYKFQNALLVRYTKKVPQVSTPT
LVEVSRNLGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLCVLHEKTPVSDRVTKCCTESLV
NRRPCFSALEVDETYVPKEFQAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQL
KAVMDDFAAFVEKCCKADDKETCFAEEGKKLVAASQAALGL

SEQ ID NO:2
Human Serum Albumin (HSA) Linker w/C34S and N503Q substitutions ("mHSA") Nucleotide Sequence

GACGCTCACAAGAGCGAAGTGGCACATAGGTTCAAAGATCTGGGCGAAGAGAACTTTA
AGGCCCTCGTCCTGATCGCTTTCGCACAGTACCTCCAGCAGTCTCCCTTTGAAGATCAC
GTGAAACTGGTCAATGAGGTGACCGAATTTGCCAAGACATGCGTGGCTGATGAGAGTG
CAGAAAACTGTGACAAATCACTGCATACTCTCTTTGGAGATAAGCTGTGCACCGTCGCC
ACACTCAGAGAGACTTATGGGGAAATGGCTGACTGTTGCGCAAAACAGGAGCCTGAAC
GGAATGAGTGTTTCCTCCAGCACAAGGATGACAACCCAAATCTGCCCCGCCTCGTGCGA
CCTGAGGTCGATGTGATGTGCACCGCCTTTCATGACAACGAAGAGACATTCCTGAAGA
AATACCTGTATGAAATTGCTCGTAGGCACCCATACTTTTATGCCCCCGAGCTCCTGTTCT
TTGCAAAGAGATACAAAGCTGCCTTCACTGAATGTTGCCAGGCAGCTGATAAGGCCGC
ATGTCTCCTGCCTAAACTGGACGAGCTCCGGGATGAAGGTAAGGCTTCCAGCGCCAAA
CAGCGCCTGAAGTGCGCTTCTCTCCAGAAGTTTGGCGAGCGAGCATTCAAAGCCTGGGC
TGTGGCCCGTCTCAGTCAGAGGTTTCCAAAGGCAGAATTTGCTGAGGTCTCAAAACTGG
TGACCGACCTCACAAAGGTCCATACTGAGTGTTGCCACGGAGATCTGCTGGAATGTGCC
GACGATAGAGCAGACCTCGCTAAATATATCTGCGAGAATCAGGATTCCATTAGCTCTAA
GCTGAAAGAATGTTGCGAGAAGCCCCTCCTGGAAAAGAGTCATTGTATCGCCGAGGTG
GAAAACGACGAGATGCCAGCAGATCTGCCATCACTCGCTGCCGACTTTGTGGAATCCA
AAGATGTCTGCAAGAATTACGCAGAGGCTAAAGACGTGTTCCTGGGGATGTTTCTGTAT
GAGTACGCCCGGCGTCACCCCGATTATAGCGTCGTGCTCCTGCTCCGACTGGCAAAGAC
CTACGAAACAACTCTGGAGAAATGTTGCGCTGCCGCAGACCCTCATGAATGTTATGCTA
AGGTGTTCGATGAGTTTAAGCCACTCGTCGAAGAGCCCCAGAACCTGATTAAACAGAA
TTGCGAACTGTTCGAGCAGCTCGGTGAATACAAGTTTCAGAACGCCCTGCTCGTGCGTT
ATACCAAAAAGGTCCCTCAGGTGTCTACACCAACTCTGGTGGAGGTCAGTAGGAATCT
GGGCAAAGTGGGATCAAAGTGTTGCAAACACCCCGAGGCAAAGAGAATGCCTTGTGCT

GAAGATTACCTCTCCGTCGTGCTGAACCAGCTCTGCGTGCTGCATGAAAAGACCCCAGT
CAGCGATCGGGTGACAAAATGTTGCACCGAATCTCTGGTCAATCGCCGACCCTGTTTCA
GTGCCCTCGAAGTGGACGAAACTTATGTGCCTAAGGAGTTTCAGGCTGAAACATTCACC
TTTCACGCCGATATCTGCACTCTGTCCGAGAAAGAAAGGCAGATTAAGAAACAGACAG
CACTGGTCGAGCTCGTGAAGCATAAACCAAAGGCTACCAAGGAGCAGCTGAAAGCCGT
CATGGACGATTTCGCAGCTTTTGTGGAAAAGTGTTGCAAAGCCGACGATAAGGAGACT
TGTTTCGCAGAAGAGGGGAAAAAGCTCGTGGCTGCCAGCCAGGCAGCTCTGGGTCTG

SEQ ID NO:3
Human Serum Albumin (HSA)
Amino Acid Sequence

DAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQCPFEDHVKLVNEVTEFAKTCVADESAE
NCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECFLQHKDDNPNLPRLVRPEV
DVMCTAFHDNEETFLKKYLYEIARRHPYFYAPELLFFAKRYKAAFTECCQAADKAACLLP
KLDELRDEGKASSAKQRLKCASLQKFGERAFKAWAVARLSQRFPKAEFAEVSKLVTDLTK
VHTECCHGDLLECADDRADLAKYICENQDSISSKLKECCEKPLLEKSHCIAEVENDEMPAD
LPSLAADFVESKDVCKNYAEAKDVFLGMFLYEYARRHPDYSVVLLLRLAKTYETTLEKCC
AAADPHECYAKVFDEFKPLVEEPQNLIKQNCELFEQLGEYKFQNALLVRYTKKVPQVSTPT
LVEVSRNLGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLCVLHEKTPVSDRVTKCCTESLV
NRRPCFSALEVDETYVPKEFNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQL
KAVMDDFAAFVEKCCKADDKETCFAEEGKKLVAASQAALGL

SEQ ID NO:4
Human Serum Albumin (HSA)
Nucleotide Sequence

GATGCACACAAGAGTGAGGTTGCTCATCGGTTTAAAGATTTGGGAGAAGAAAATTTCA
AAGCCTTGGTGTTGATTGCCTTTGCTCAGTATCTTCAGCAGTGTCCATTTGAAGATCATG
TAAAATTAGTGAATGAAGTAACTGAATTTGCAAAAACATGTGTAGCTGATGAGTCAGC
TGAAAATTGTGACAAATCACTTCATACCCTTTTTGGAGACAAATTATGCACAGTTGCAA
CTCTTCGTGAAACCTATGGTGAAATGGCTGACTGCTGTGCAAAACAAGAACCTGAGAG
AAATGAATGCTTCTTGCAACACAAAGATGACAACCCAAACCTCCCCCGATTGGTGAGA
CCAGAGGTTGATGTGATGTGCACTGCTTTTCATGACAATGAAGAGACATTTTTGAAAAA
ATACTTATATGAAATTGCCAGAAGACATCCTTACTTTTATGCCCCGGAACTCCTTTTCTT
TGCTAAAAGGTATAAAGCTGCTTTTACAGAATGTTGCCAAGCTGCTGATAAAGCTGCCT
GCCTGTTGCCAAAGCTCGATGAACTTCGGGATGAAGGGAAGGCTTCGTCTGCCAAACA
GAGACTCAAATGTGCCAGTCTCCAAAAATTTGGAGAAAGAGCTTTCAAAGCATGGGCA
GTGGCTCGCCTGAGCCAGAGATTTCCCAAAGCTGAGTTTGCAGAAGTTTCCAAGTTAGT
GACAGATCTTACCAAAGTCCACACGGAATGCTGCCATGGAGATCTGCTTGAATGTGCTG
ATGACAGGGCGGACCTTGCCAAGTATATCTGTGAAAATCAGGATTCGATCTCCAGTAA
ACTGAAGGAATGCTGTGAAAAACCTCTGTTGGAAAAATCCCACTGCATTGCCGAAGTG
GAAAATGATGAGATGCCTGCTGACTTGCCTTCATTAGCTGCTGATTTTGTTGAAAGTAA

GGATGTTTGCAAAAACTATGCTGAGGCAAAGGATGTCTTCCTGGGCATGTTTTTGTATG
AATATGCAAGAAGGCATCCTGATTACTCTGTCGTGCTGCTGCTGAGACTTGCCAAGACA
TATGAAACCACTCTAGAGAAGTGCTGTGCCGCTGCAGATCCTCATGAATGCTATGCCAA
AGTGTTCGATGAATTTAAACCTCTTGTGGAAGAGCCTCAGAATTTAATCAAACAAAACT
GTGAGCTTTTTAAGCAGCTTGGAGAGTACAAATTCCAGAATGCGCTATTAGTTCGTTAC
ACCAAGAAAGTACCCCAAGTGTCAACTCCAACTCTTGTAGAGGTCTCAAGAAACCTAG
GAAAAGTGGGCAGCAAATGTTGTAAACATCCTGAAGCAAAAAGAATGCCCTGTGCAGA
AGACTATCTATCCGTGGTCCTGAACCAGTTATGTGTGTTGCATGAGAAAACGCCAGTAA
GTGACAGAGTCACAAAATGCTGCACAGAGTCCTTGGTGAACAGGCGACCATGCTTTTC
AGCTCTGGAAGTCGATGAAACATACGTTCCCAAAGAGTTTAATGCTGAAACATTCACCT
TCCATGCAGATATATGCACACTTTCTGAGAAGGAGAGACAAATCAAGAAACAAACTGC
ACTTGTTGAGCTTGTGAAACACAAGCCCAAGGCAACAAAAGAGCAACTGAAAGCTGTT
ATGGATGATTTCGCAGCTTTTGTAGAGAAGTGCTGCAAGGCTGACGATAAGGAGACCT
GCTTTGCCGAGGAGGGTAAAAAACTTGTTGCTGCAAGTCAAGCTGCCTTAGGCTTA

SEQ ID NO:5
Polypeptide Connector
Amino Acid Sequence

AAAL

SEQ ID NO:6
Human Serum Albumin (HSA) Linker with C34S and N503Q substitutions ("mHSA") and Polypeptide Connector
Amino Acid Sequence

AASDAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQSPFEDHVKLVNEVTEFAKTCVA
DESAENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECFLQHKDDNPNLP
RLVRPEVDVMCTAFHDNEETFLKKYLYEIARRHPYFYAPELLFFAKRYKAAFTECCQAA
DKAACLLPKLDELRDEGKASSAKQRLKCASLQKFGERAFKAWAVARLSQRFPKAEFAE
VSKLVTDLTKVHTECCHGDLLECADDRADLAKYICENQDSISSKLKECCEKPLLEKSHCI
AEVENDEMPADLPSLAADFVESKDVCKNYAEAKDVFLGMFLYEYARRHPDYSVVLLL
RLAKTYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNCELFEQLGEYKFQNA
LLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLCVLH
EKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFQAETFTFHADICTLSEKERQIK
KQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCCKADDKETCFAEEGKKLVAASQA
ALGL

SEQ ID NO:7
Human Serum Albumin (HSA) Linker with C34S and N503Q substitutions ("mHSA") and Polypeptide Connector
Amino Acid Sequence

AAQDAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQSPFEDHVKLVNEVTEFAKTCVA
DESAENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECFLQHKDDNPNLP

RLVRPEVDVMCTAFHDNEETFLKKYLYEIARRHPYFYAPELLFFAKRYKAAFTECCQAA
DKAACLLPKLDELRDEGKASSAKQRLKCASLQKFGERAFKAWAVARLSQRFPKAEFAE
VSKLVTDLTKVHTECCHGDLLECADDRADLAKYICENQDSISSKLKECCEKPLLEKSHCI
AEVENDEMPADLPSLAADFVESKDVCKNYAEAKDVFLGMFLYEYARRHPDYSVVLLL
RLAKTYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNCELFEQLGEYKFQNA
LLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLCVLH
EKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFQAETFTFHADICTLSEKERQIK
KQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCCKADDKETCFAEEGKKLVAASQA
ALGL

SEQ ID NO:8
Human Serum Albumin (HSA) Linker with C34S and N503Qsubstitutions ("mHSA") and Polypeptide Connector
Amino Acid Sequence

DAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQSPFEDHVKLVNEVTEFAKTCVADES
AENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECFLQHKDDNPNLPRLV
RPEVDVMCTAFHDNEETFLKKYLYEIARRHPYFYAPELLFFAKRYKAAFTECCQAADK
AACLLPKLDELRDEGKASSAKQRLKCASLQKFGERAFKAWAVARLSQRFPKAEFAEVS
KLVTDLTKVHTECCHGDLLECADDRADLAKYICENQDSISSKLKECCEKPLLEKSHCIAE
VENDEMPADLPSLAADFVESKDVCKNYAEAKDVFLGMFLYEYARRHPDYSVVLLLRL
AKTYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNCELFEQLGEYKFQNALL
VRYTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLCVLHEK
TPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFQAETFTFHADICTLSEKERQIKKQ
TALVELVKHKPKATKEQLKAVMDDFAAFVEKCCKADDKETCFAEEGKKLVAASQAAL
GLAAAL

SEQ ID NO:9
Human Serum Albumin (HSA) Linker with C34S and N503Q substitutions ("mHSA") and Polypeptide Connector
Amino Acid Sequence

AASDAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQSPFEDHVKLVNEVTEFAKTCVA
DESAENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECFLQHKDDNPNLP
RLVRPEVDVMCTAFHDNEETFLKKYLYEIARRHPYFYAPELLFFAKRYKAAFTECCQAA
DKAACLLPKLDELRDEGKASSAKQRLKCASLQKFGERAFKAWAVARLSQRFPKAEFAE
VSKLVTDLTKVHTECCHGDLLECADDRADLAKYICENQDSISSKLKECCEKPLLEKSHCI
AEVENDEMPADLPSLAADFVESKDVCKNYAEAKDVFLGMFLYEYARRHPDYSVVLLL
RLAKTYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNCELFEQLGEYKFQNA
LLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLCVLH
EKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFQAETFTFHADICTLSEKERQIK
KQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCCKADDKETCFAEEGKKLVAASQA
ALGLAAAL

SEQ ID NO:10
Human Serum Albumin (HSA) Linker with C34S and N503Q substitutions ("mHSA") and Polypeptide Connector
Amino Acid Sequence

AAQDAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQSPFEDHVKLVNEVTEFAKTCVA
DESAENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECFLQHKDDNPNLP
RLVRPEVDVMCTAFHDNEETFLKKYLYEIARRHPYFYAPELLFFAKRYKAAFTECCQAA
DKAACLLPKLDELRDEGKASSAKQRLKCASLQKFGERAFKAWAVARLSQRFPKAEFAE
VSKLVTDLTKVHTECCHGDLLECADDRADLAKYICENQDSISSKLKECCEKPLLEKSHCI
AEVENDEMPADLPSLAADFVESKDVCKNYAEAKDVFLGMFLYEYARRHPDYSVVLLL
RLAKTYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNCELFEQLGEYKFQNA
LLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLCVLH
EKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFQAETFTFHADICTLSEKERQIK
KQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCCKADDKETCFAEEGKKLVAASQA
ALGLAAAL

SEQ ID NO:11
Human Serum Albumin (HSA) Linker and Polypeptide Connector
Amino Acid Sequence

AASDAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQCPFEDHVKLVNEVTEFAKTCVA
DESAENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECFLQHKDDNPNLP
RLVRPEVDVMCTAFHDNEETFLKKYLYEIARRHPYFYAPELLFFAKRYKAAFTECCQAA
DKAACLLPKLDELRDEGKASSAKQRLKCASLQKFGERAFKAWAVARLSQRFPKAEFAE
VSKLVTDLTKVHTECCHGDLLECADDRADLAKYICENQDSISSKLKECCEKPLLEKSHCI
AEVENDEMPADLPSLAADFVESKDVCKNYAEAKDVFLGMFLYEYARRHPDYSVVLLL
RLAKTYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNCELFEQLGEYKFQNA
LLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLCVLH
EKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFNAETFTFHADICTLSEKERQIK
KQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCCKADDKETCFAEEGKKLVAASQA
ALGL

SEQ ID NO:12
Human Serum Albumin (HSA) Linker and Polypeptide Connector
Amino Acid Sequence

AAQDAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQCPFEDHVKLVNEVTEFAKTCVA
DESAENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECFLQHKDDNPNLP
RLVRPEVDVMCTAFHDNEETFLKKYLYEIARRHPYFYAPELLFFAKRYKAAFTECCQAA
DKAACLLPKLDELRDEGKASSAKQRLKCASLQKFGERAFKAWAVARLSQRFPKAEFAE
VSKLVTDLTKVHTECCHGDLLECADDRADLAKYICENQDSISSKLKECCEKPLLEKSHCI
AEVENDEMPADLPSLAADFVESKDVCKNYAEAKDVFLGMFLYEYARRHPDYSVVLLL
RLAKTYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNCELFEQLGEYKFQNA
LLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLCVLH

EKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFNAETFTFHADICTLSEKERQIK
KQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCCKADDKETCFAEEGKKLVAASQA
ALGL

SEQ ID NO:13
Human Serum Albumin (HSA) Linker and Polypeptide Connector Amino Acid Sequence

DAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQCPFEDHVKLVNEVTEFAKTCVADES
AENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECFLQHKDDNPNLPRLV
RPEVDVMCTAFHDNEETFLKKYLYEIARRHPYFYAPELLFFAKRYKAAFTECCQAADK
AACLLPKLDELRDEGKASSAKQRLKCASLQKFGERAFKAWAVARLSQRFPKAEFAEVS
KLVTDLTKVHTECCHGDLLECADDRADLAKYICENQDSISSKLKECCEKPLLEKSHCIAE
VENDEMPADLPSLAADFVESKDVCKNYAEAKDVFLGMFLYEYARRHPDYSVVLLLRL
AKTYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNCELFEQLGEYKFQNALL
VRYTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLCVLHEK
TPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFNAETFTFHADICTLSEKERQIKKQ
TALVELVKHKPKATKEQLKAVMDDFAAFVEKCCKADDKETCFAEEGKKLVAASQAAL
GLAAAL

SEQ ID NO:14
Human Serum Albumin (HSA) Linker and Polypeptide Connector Amino Acid Sequence

AASDAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQCPFEDHVKLVNEVTEFAKTCVA
DESAENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECFLQHKDDNPNLP
RLVRPEVDVMCTAFHDNEETFLKKYLYEIARRHPYFYAPELLFFAKRYKAAFTECCQAA
DKAACLLPKLDELRDEGKASSAKQRLKCASLQKFGERAFKAWAVARLSQRFPKAEFAE
VSKLVTDLTKVHTECCHGDLLECADDRADLAKYICENQDSISSKLKECCEKPLLEKSHCI
AEVENDEMPADLPSLAADFVESKDVCKNYAEAKDVFLGMFLYEYARRHPDYSVVLLL
RLAKTYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNCELFEQLGEYKFQNA
LLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLCVLH
EKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFNAETFTFHADICTLSEKERQIK
KQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCCKADDKETCFAEEGKKLVAASQA
ALGLAAAL

SEQ ID NO:15
Human Serum Albumin (HSA) Linker and Polypeptide Connector Amino Acid Sequence

AAQDAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQCPFEDHVKLVNEVTEFAKTCVA
DESAENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECFLQHKDDNPNLP
RLVRPEVDVMCTAFHDNEETFLKKYLYEIARRHPYFYAPELLFFAKRYKAAFTECCQAA
DKAACLLPKLDELRDEGKASSAKQRLKCASLQKFGERAFKAWAVARLSQRFPKAEFAE
VSKLVTDLTKVHTECCHGDLLECADDRADLAKYICENQDSISSKLKECCEKPLLEKSHCI
AEVENDEMPADLPSLAADFVESKDVCKNYAEAKDVFLGMFLYEYARRHPDYSVVLLL

RLAKTYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNCELFEQLGEYKFQNA
LLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLCVLH
EKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFNAETFTFHADICTLSEKERQIK
KQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCCKADDKETCFAEEGKKLVAASQA
ALGLAAAL

SEQ ID NO:16

mHSA Linker, Polypeptide Connectors, H3 (anti-ErbB3) scFv, and B1D2 (anti-ErbB2) scFv ("B2B3-1")
Amino Acid Sequence

QVQLQESGGGLVKPGGSLRLSCAASGFTFSSYWMSWVRQAPGKGLEWVANINRDGSA
SYYVDSVKGRFTISRDDAKNSLYLQMNSLRAEDTAVYYCARDRGVGYFDLWGRGTLV
TVSSASTGGGGSGGGGSGGGGSQSALTQPASVSGSPGQSITISCTGTSSDVGGYNFVSWY
QQHPGKAPKLMIYDVSDRPSGVSDRFSGSKSGNTASLIISGLQADDEADYYCSSYGSSST
HVIFGGGTKVTVLGAASDAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQSPFEDHVKL
VNEVTEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNE
CFLQHKDDNPNLPRLVRPEVDVMCTAFHDNEETFLKKYLYEIARRHPYFYAPELLFFAK
RYKAAFTECCQAADKAACLLPKLDELRDEGKASSAKQRLKCASLQKFGERAFKAWAV
ARLSQRFPKAEFAEVSKLVTDLTKVHTECCHGDLLECADDRADLAKYICENQDSISSKL
KECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNYAEAKDVFLGMFLYE
YARRHPDYSVVLLLRLAKTYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNC
ELFEQLGEYKFQNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCAE
DYLSVVLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFQAETFTF
HADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCCKADDKETC
FAEEGKKLVAASQAALGLAAALQVQLVQSGAEVKKPGESLKISCKGSGYSFTSYWIAW
VRQMPGKGLEYMGLIYPGDSDTKYSPSFQGQVTISVDKSVSTAYLQWSSLKPSDSAVYF
CARHDVGYCTDRTCAKWPEWLGVWGQGTLVTVSSGGGGSSGGGSGGGGSQSVLTQPP
SVSAAPGQKVTISCSGSSSNIGNNYVSWYQQLPGTAPKLLIYDHTNRPAGVPDRFSGSKS
GTSASLAISGFRSEDEADYYCASWDYTLSGWVFGGGTKLTVLG

SEQ ID NO:17
B2B3-2 (A5-mHSA-B1D2)

QVQLVQSGGGLVKPGGSLRLSCAASGFSFNTYDMNWVRQAPGKGLEWVSSISSSSSYIY
YADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARDGVATTPFDYWGQGTLVT
VSSGGGGSGGGGSGGGGSQSVLTQPPSVSGAPGQRVTISCTGSSSNIGAGYDVHWYQQL
PGTAPKLLIYGNSNRPSGVPDRFSGSKSGTSASLAITGLQAEDEADYYCQSYDSSLSALF
GGGTKLTVLGAASDAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQSPFEDHVKLVNE
VTEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECFL
QHKDDNPNLPRLVRPEVDVMCTAFHDNEETFLKKYLYEIARRHPYFYAPELLFFAKRYK
AAFTECCQAADKAACLLPKLDELRDEGKASSAKQRLKCASLQKFGERAFKAWAVARL
SQRFPKAEFAEVSKLVTDLTKVHTECCHGDLLECADDRADLAKYICENQDSISSKLKEC
CEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNYAEAKDVFLGMFLYEYAR
RHPDYSVVLLLRLAKTYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNCELF
EQLGEYKFQNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCAEDYL

SVVLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFQAETFTFHADI
CTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCCKADDKETCFAEE
GKKLVAASQAALGLAAALQVQLVQSGAEVKKPGESLKISCKGSGYSFTSYWIAWVRQ
MPGKGLEYMGLIYPGDSDTKYSPSFQGQVTISVDKSVSTAYLQWSSLKPSDSAVYFCAR
HDVGYCTDRTCAKWPEWLGVWGQGTLVTVSSGGGGSSGGGSGGGGSQSVLTQPPSVS
AAPGQKVTISCSGSSSNIGNNYVSWYQQLPGTAPKLLIYDHTNRPAGVPDRFSGSKSGTS
ASLAISGFRSEDEADYYCASWDYTLSGWVFGGGTKLTVLG

SEQ ID NO:18
B2B3-3 (A5-mHSA-F5B6H2)

QVQLVQSGGGLVKPGGSLRLSCAASGFSFNTYDMNWVRQAPGKGLEWVSSISSSSSYIY
YADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARDGVATTPFDYWGQGTLVT
VSSGGGGSGGGGSGGGGSQSVLTQPPSVSGAPGQRVTISCTGSSSNIGAGYDVHWYQQL
PGTAPKLLIYGNSNRPSGVPDRFSGSKSGTSASLAITGLQAEDEADYYCQSYDSSLSALF
GGGTKLTVLGAASDAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQSPFEDHVKLVNE
VTEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECFL
QHKDDNPNLPRLVRPEVDVMCTAFHDNEETFLKKYLYEIARRHPYFYAPELLFFAKRYK
AAFTECCQAADKAACLLPKLDELRDEGKASSAKQRLKCASLQKFGERAFKAWAVARL
SQRFPKAEFAEVSKLVTDLTKVHTECCHGDLLECADDRADLAKYICENQDSISSKLKEC
CEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNYAEAKDVFLGMFLYEYAR
RHPDYSVVLLLRLAKTYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNCELF
EQLGEYKFQNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCAEDYL
SVVLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFQAETFTFHADI
CTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCCKADDKETCFAEE
GKKLVAASQAALGLAAALQVQLVESGGGLVQPGGSLRLSCAASGFTFRSYAMSWVRQ
APGKGLEWVSAISGRGDNTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCA
KMTSNAVGFDYWGQGTLVTVSSGGGGSGGGGSGGGGSGQSVLTQPPSVSGAPGQRVTIS
CTGRHSNIGLGYGVHWYQQLPGTAPKLLIYGNTNRPSGVPDRFSGFKSGTSASLAITGLQ
AEDEADYYCQSYDRRTPGWVFGGGTKLTVLG

SEQ ID NO:19
B2B3-4 (A5-mHSA-ML3.9)

QVQLVQSGGGLVKPGGSLRLSCAASGFSFNTYDMNWVRQAPGKGLEWVSSISSSSSYIY
YADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARDGVATTPFDYWGQGTLVT
VSSGGGGSGGGGSGGGGSQSVLTQPPSVSGAPGQRVTISCTGSSSNIGAGYDVHWYQQL
PGTAPKLLIYGNSNRPSGVPDRFSGSKSGTSASLAITGLQAEDEADYYCQSYDSSLSALF
GGGTKLTVLGAASDAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQSPFEDHVKLVNE
VTEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECFL
QHKDDNPNLPRLVRPEVDVMCTAFHDNEETFLKKYLYEIARRHPYFYAPELLFFAKRYK
AAFTECCQAADKAACLLPKLDELRDEGKASSAKQRLKCASLQKFGERAFKAWAVARL
SQRFPKAEFAEVSKLVTDLTKVHTECCHGDLLECADDRADLAKYICENQDSISSKLKEC
CEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNYAEAKDVFLGMFLYEYAR
RHPDYSVVLLLRLAKTYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNCELF
EQLGEYKFQNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCAEDYL

SVVLNQLCVLIIEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFQAETFTFHADI
CTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCCKADDKETCFAEE
GKKLVAASQAALGLAAALQVQLVQSGAEVKKPGESLKISCKGSGYSFTSYWIAWVRQ
MPGKGLEYMGLIYPGDSDTKYSPSFQGQVTISVDKSVSTAYLQWSSLKPSDSAVYFCAR
HDVGYCSSSNCAKWPEYFQHWGQGTLVTVSSGGGGSSGGGSGGGGSQSVLTQPPSVSA
APGQKVTISCSGSSSNIGNNYVSWYQQLPGTAPKLLIYDHTNRPAGVPDRFSGSKSGTSA
SLAISGFRSEDEADYYCASWDYTLSGWVFGGGTKLTVLG

SEQ ID NO:20
B2B3-5 (B12-mHSA-B1D2)

QVQLVQSGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSGISWNSGSI
GYADSVKGRFTISRDNAKNSLYLQMNSLRPEDTAVYYCARDLGAKQWLEGFDYWGQG
TLVTVSSASTGGGGSGGGGSGGGGSSYELTQDPAVSVALGQTVRITCQGDSLRSYYAS
WYQQKPGQAPVLVIYGKNNRPSGIPDRFSGSTSGNSASLTITGAQAEDEADYYCNSRDS
SGNHWVFGGGTKVTVLGAASDAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQSPFED
HVKLVNEVTEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEP
ERNECFLQHKDDNPNLPRLVRPEVDVMCTAFHDNEETFLKKYLYEIARRHPYFYAPELL
FFAKRYKAAFTECCQAADKAACLLPKLDELRDEGKASSAKQRLKCASLQKFGERAFKA
WAVARLSQRFPKAEFAEVSKLVTDLTKVHTECCHGDLLECADDRADLAKYICENQDSIS
SKLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNYAEAKDVFLGMF
LYEYARRHPDYSVVLLLRLAKTYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIK
QNCELFEQLGEYKFQNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMP
CAEDYLSVVLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFQAET
FTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCCKADDK
ETCFAEEGKKLVAASQAALGLAAALQVQLVQSGAEVKKPGESLKISCKGSGYSFTSYWI
AWVRQMPGKGLEYMGLIYPGDSDTKYSPSFQGQVTISVDKSVSTAYLQWSSLKPSDSA
VYFCARHDVGYCTDRTCAKWPEWLGVWGQGTLVTVSSGGGGSSGGGSGGGGSQSVL
TQPPSVSAAPGQKVTISCSGSSSNIGNNYVSWYQQLPGTAPKLLIYDHTNRPAGVPDRFS
GSKSGTSASLAISGFRSEDEADYYCASWDYTLSGWVFGGGTKLTVLG

SEQ ID NO:21
B2B3-6 (B12-mHSA-F5B6H2)

QVQLVQSGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSGISWNSGSI
GYADSVKGRFTISRDNAKNSLYLQMNSLRPEDTAVYYCARDLGAKQWLEGFDYWGQG
TLVTVSSASTGGGGSGGGGSGGGGSSYELTQDPAVSVALGQTVRITCQGDSLRSYYAS
WYQQKPGQAPVLVIYGKNNRPSGIPDRFSGSTSGNSASLTITGAQAEDEADYYCNSRDS
SGNHWVFGGGTKVTVLGAASDAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQSPFED
HVKLVNEVTEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEP
ERNECFLQHKDDNPNLPRLVRPEVDVMCTAFHDNEETFKKYLYEIARRHPYFYAPELLF
FAKRYKAAFTECCQAADKAACLLPKLDELRDEGKASSAKQRLKCASLQKFGERAFKA
WAVARLSQRFPKAEFAEVSKLVTDLTKVHTECCHGDLLECADDRADLAKYICENQDSIS
SKLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNYAEAKDVFLGMF
LYEYARRHPDYSVVLLLRLAKTYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIK
QNCELFEQLGEYKFQNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMP

CAEDYLSVVLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFQAET
FTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCCKADDK
ETCFAEEGKKLVAASQAALGLAAALQVQLVESGGGLVQPGGSLRLSCAASGFTFRSYA
MSWVRQAPGKGLEWVSAISGRGDNTYYADSVKGRFTISRDNSKNTLYLQMNSLRAED
TAVYYCAKMTSNAVGFDYWGQGTLVTVSSGGGGSGGGSGGGGSGQSVLTQPPSVSGA
PGQRVTISCTGRHSNIGLGYGVHWYQQLPGTAPKLLIYGNTNRPSGVPDRFSGFKSGTSA
SLAITGLQAEDEADYYCQSYDRRTPGWVFGGGTKLTVLG

SEQ ID NO:22
B2B3-7 (F4-mHSA-B1D2)

QVQLQESGGGLVKPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSTISGSGGST
YYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKGYSSSWSEVASGYWGQG
TLVTVSSASTGGGGSGGGGSGGGGSAIVMTQSPSSLSASVGDRVTITCRASQGIRNDLG
WYQQKAGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPDDFATYFCQQAHSF
PPTFGGGTKVEIKRGAASDAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQSPFEDHVK
LVNEVTEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERN
ECFLQHKDDNPNLPRLVRPEVDVMCTAFHDNEETFLKKYLYEIARRHPYFYAPELLFFA
KRYKAAFTECCQAADKAACLLPKLDELRDEGKASSAKQRLKCASLQKFGERAFKAWA
VARLSQRFPKAEFAEVSKLVTDLTKVHTECCHGDLLECADDRADLAKYICENQDSISSK
LKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNYAEAKDVFLGMFLY
EYARRHPDYSVVLLLRLAKTYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQN
CELFEQLGEYKFQNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCA
EDYLSVVLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFQAETFT
FHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCCKADDKET
CFAEEGKKLVAASQAALGLAAALQVQLVQSGAEVKKPGESLKISCKGSGYSFTSYWIA
WVRQMPGKGLEYMGLIYPGDSDTKYSPSFQGQVTISVDKSVSTAYLQWSSLKPSDSAV
YFCARHDVGYCTDRTCAKWPEWLGVWGQGTLVTVSSGGGGSSGGGSGGGGSQSVLT
QPPSVSAAPGQKVTISCSGSSSNIGNNYVSWYQQLPGTAPKLLIYDHTNRPAGVPDRFSG
SKSGTSASLAISGFRSEDEADYYCASWDYTLSGWVFGGGTKLTVLG

SEQ ID NO:23
B2B3-8 (F4-mHSA-F5B6H2)

QVQLQESGGGLVKPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSTISGSGGST
YYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKGYSSSWSEVASGYWGQG
TLVTVSSASTGGGGSGGGGSGGGGSAIVMTQSPSSLSASVGDRVTITCRASQGIRNDLG
WYQQKAGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPDDFATYFCQQAHSF
PPTFGGGTKVEIKRGAASDAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQSPFEDHVK
LVNEVTEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERN
ECFLQHKDDNPNLPRLVRPEVDVMCTAFHDNEETFLKKYLYEIARRHPYFYAPELLFFA
KRYKAAFTECCQAADKAACLLPKLDELRDEGKASSAKQRLKCASLQKFGERAFKAWA
VARLSQRFPKAEFAEVSKLVTDLTKVHTECCHGDLLECADDRADLAKYICENQDSISSK
LKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNYAEAKDVFLGMFLY
EYARRHPDYSVVLLLRLAKTYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQN
CELFEQLGEYKFQNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCA

EDYLSVVLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFQAETFT
FHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCCKADDKET
CFAEEGKKLVAASQAALGLAAALQVQLVESGGGLVQPGGSLRLSCAASGFTFRSYAMS
WVRQAPGKGLEWVSAISGRGDNTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTA
VYYCAKMTSNAVGFDYWGQGTLVTVSSGGGGSGGGSGGGGSGQSVLTQPPSVSGAPG
QRVTISCTGRHSNIGLGYGVHWYQQLPGTAPKLLIYGNTNRPSGVPDRFSGFKSGTSASL
AITGLQAEDEADYYCQSYDRRTPGWVFGGGTKLTVLG

SEQ ID NO:24
B2B3-9 (H3-HSA-B1D2)

QVQLQESGGGLVKPGGSLRLSCAASGFTFSSYWMSWVRQAPGKGLEWVANINRDGSA
SYYVDSVKGRFTISRDDAKNSLYLQMNSLRAEDTAVYYCARDRGVGYFDLWGRGTLV
TVSSASTGGGGSGGGGSGGGGSQSALTQPASVSGSPGQSITISCTGTSSDVGGYNFVSWY
QQHPGKAPKLMIYDVSDRPSGVSDRFSGSKSGNTASLIISGLQADDEADYYCSSYGSSST
HVIFGGGTKVTVLGAASDAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQCPFEDHVK
LVNEVTEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERN
ECFLQHKDDNPNLPRLVRPEVDVMCTAFHDNEETFLKKYLYEIARRHPYFYAPELLFFA
KRYKAAFTECCQAADKAACLLPKLDELRDEGKASSAKQRLKCASLQKFGERAFKAWA
VARLSQRFPKAEFAEVSKLVTDLTKVHTECCHGDLLECADDRADLAKYICENQDSISSK
LKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNYAEAKDVFLGMFLY
EYARRHPDYSVVLLLRLAKTYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQN
CELFEQLGEYKFQNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCA
EDYLSVVLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFNAETFT
FHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCCKADDKET
CFAEEGKKLVAASQAALGLAAALQVQLVQSGAEVKKPGESLKISCKGSGYSFTSYWIA
WVRQMPGKGLEYMGLIYPGDSDTKYSPSFQGQVTISVDKSVSTAYLQWSSLKPSDSAV
YFCARHDVGYCTDRTCAKWPEWLGVWGQGTLVTVSSGGGGSSGGGSGGGGSQSVLT
QPPSVSAAPGQKVTISCSGSSSNIGNNYVSWYQQLPGTAPKLLIYDHTNRPAGVPDRFSG
SKSGTSASLAISGFRSEDEADYYCASWDYTLSGWVFGGGTKLTVLG

SEQ ID NO:25
B2B3-10 (H3-mHSA-F5B6H2)

QVQLQESGGGLVKPGGSLRLSCAASGFTFSSYWMSWVRQAPGKGLEWVANINRDGSA
SYYVDSVKGRFTISRDDAKNSLYLQMNSLRAEDTAVYYCARDRGVGYFDLWGRGTLV
TVSSASTGGGGSGGGGSGGGGSQSALTQPASVSGSPGQSITISCTGTSSDVGGYNFVSWY
QQHPGKAPKLMIYDVSDRPSGVSDRFSGSKSGNTASLIISGLQADDEADYYCSSYGSSST
HVIFGGGTKVTVLGAASDAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQSPFEDHVKL
VNEVTEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNE
CFLQHKDDNPNLPRLVRPEVDVMCTAFHDNEETFLKKYLYEIARRHPYFYAPELLFFAK
RYKAAFTECCQAADKAACLLPKLDELRDEGKASSAKQRLKCASLQKFGERAFKAWAV
ARLSQRFPKAEFAEVSKLVTDLTKVHTECCHGDLLECADDRADLAKYICENQDSISSKL
KECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNYAEAKDVFLGMFLYE
YARRHPDYSVVLLLRLAKTYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNC
ELFEQLGEYKFQNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCAE

DYLSVVLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFQAETFTF
HADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCCKADDKETC
FAEEGKKLVAASQAALGLAAALQVQLVESGGGLVQPGGSLRLSCAASGFTFRSYAMSW
VRQAPGKGLEWVSAISGRGDNTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVY
YCAKMTSNAVGFDYWGQGTLVTVSSGGGGSGGGSGGGGSGQSVLTQPPSVSGAPGQR
VTISCTGRHSNIGLGYGVHWYQQLPGTAPKLLIYGNTNRPSGVPDRFSGFKSGTSASLAI
TGLQAEDEADYYCQSYDRRTPGWVFGGGTKLTVLG

SEQ ID NO:26
H3 (anti-ErbB3) scFv

QVQLQESGGGLVKPGGSLRLSCAASGFTFSSYWMSWVRQAPGKGLEWVANINRDGSA
SYYVDSVKGRFTISRDDAKNSLYLQMNSLRAEDTAVYYCARDRGVGYFDLWGRGTLV
TVSSASTGGGGSGGGGSGGGGSQSALTQPASVSGSPGQSITISCTGTSSDVGGYNFVSWY
QQHPGKAPKLMIYDVSDRPSGVSDRFSGSKSGNTASLIISGLQADDEADYYCSSYGSSST
HVIFGGGTKVTVLG

SEQ ID NO:27
B1D2 (anti-ErbB2)

QVQLVQSGAEVKKPGESLKISCKGSGYSFTSYWIAWVRQMPGKGLEYMGLIYPGDSDT
KYSPSFQGQVTISVDKSVSTAYLQWSSLKPSDSAVYFCARHDVGYCTDRTCAKWPEWL
GVWGQGTLVTVSSGGGGSSGGGSGGGGSQSVLTQPPSVSAAPGQKVTISCSGSSSNIGN
NYVSWYQQLPGTAPKLLIYDHTNRPAGVPDRFSGSKSGTSASLAISGFRSEDEADYYCA
SWDYTLSGWVFGGGTKLTVLG

SEQ ID NO:28
A5 (anti-ErbB3) scFv

QVQLVQSGGGLVKPGGSLRLSCAASGFSFNTYDMNWVRQAPGKGLEWVSSISSSSSYIY
YADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARDGVATTPFDYWGQGTLVT
VSSGGGGSGGGGSGGGGSQSVLTQPPSVSGAPGQRVTISCTGSSSNIGAGYDVHWYQQL
PGTAPKLLIYGNSNRPSGVPDRFSGSKSGTSASLAITGLQAEDEADYYCQSYDSSLSALF
GGGTKLTVLG

SEQ ID NO:29
ML3.9 (anti-ErbB2) scFv

QVQLVQSGAEVKKPGESLKISCKGSGYSFTSYWIAWVRQMPGKGLEYMGLIYPGDSDT
KYSPSFQGQVTISVDKSVSTAYLQWSSLKPSDSAVYFCARHDVGYCSSSNCAKWPEYFQ
HWGQGTLVTVSSGGGGSSGGGSGGGGSQSVLTQPPSVSAAPGQKVTISCSGSSSNIGNN
YVSWYQQLPGTAPKLLIYDHTNRPAGVPDRFSGSKSGTSASLAISGFRSEDEADYYCAS
WDYTLSGWVFGGGTKLTVLG

SEQ ID NO:30
B12 (anti-ErbB3) scFv

QVQLVQSGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSGISWNSGSI
GYADSVKGRFTISRDNAKNSLYLQMNSLRPEDTAVYYCARDLGAKQWLEGFDYWGQG
TLVTVSSASTGGGGSGGGGSGGGGSSYELTQDPAVSVALGQTVRITCQGDSLRSYYAS
WYQQKPGQAPVLVIYGKNNRPSGIPDRFSGSTSGNSASLTITGAQAEDEADYYCNSRDS
SGNHWVFGGGTKVTVLG

SEQ ID NO:31
F4 (anti-ErbB3) scFv

QVQLQESGGGLVKPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSTISGSGGST
YYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKGYSSSWSEVASGYWGQG
TLVTVSSASTGGGGSGGGGSGGGGSAIVMTQSPSSLSASVGDRVTITCRASQGIRNDLG
WYQQKAGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPDDFATYFCQQAHSF
PPTFGGGTKVEIKRG

SEQ ID NO:32
F5B6H2 (anti-ErbB2) scFv

QVQLVESGGGLVQPGGSLRLSCAASGFTFRSYAMSWVRQAPGKGLEWVSAISGRGDNT
YYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKMTSNAVGFDYWGQGTLV
TVSSGGGGSGGGSGGGGSGQSVLTQPPSVSGAPGQRVTISCTGRHSNIGLGYGVHWYQ
QLPGTAPKLLIYGNTNRPSGVPDRFSGFKSGTSASLAITGLQAEDEADYYCQSYDRRTPG
WVFGGGTKLTVLG

SEQ ID NO:33
c-myc
EQKLISEEDL

SEQ ID NO:34
hemagglutinin
YPYDVPDYA

SEQ ID NO:35
Histidine tag (HIS_6)
HHHHHH

SEQ ID NO:36
Caspase 3
Homo sapiens
CAC88866

MENTENSVDSKSIKNLEPKIIHGSESMDSGMSWDTGYKMDYPEMGLCIIINNKNFHKST
GMTSRSGTDVDAANLRETFRNLKYEVRNKNDLTREEIVELMRDVSKEDHSKRSSFVCV

LLSHGEEGIIFGTNGPVDLKKITNFFRGDRCRSLTGKPKLFIIQACRGTELDCGIETDSGVD
DDMACHKIPVDADFLYAYSTAPGYYSWRNSKDGSWFIQSLCAMLKQYADKLEFMHILT
RVNRKVATEFESFSFDATFHAKKQIPCIVSMLTKELYFYH

SEQ ID NO:37
Caspase 8
Homo sapiens
AAD24962

MDFSRNLYDIGEQLDSEDLASLKFLSLDYIPQRKQEPIKDALMLFQRLQEKRMLEESNLS
FLKELLFRINRLDLLITYLNTRKEEMERELQTPGRAQISAYRVMLYQISEEVSRSELRSFK
FLLQEEISKCKLDDDMNLLDIFIEMEKRVILGEGKLDILKRVCAQINKSLLKIINDYEEFSK
ERSSSLEGSPDEFSNGEELCGVMTISDSPREQDSESQTLDKVYQMKSKPRGYCLIINNHN
FAKAREKVPKLHSIRDRNGTHLDAGALTTTFEELHFEIKPHDDCTVEQIYDILKIYQLMD
HSNMDCFICCILSHGDKGIIYGTDGQEPPIYELTSQFTGLKCPSLAGKPKVFFIQACQGDN
YQKGIPVETDSEEQPYLEMDLSSPQTRYIPDEADFLLGMATVNNCVSYRNPAEGTWYIQ
SLCQSLRERCPRGDDILTILTEVNYEVSNKDDKKNMGKQMPQPTFTLRKKLVFPSD

SEQ ID NO:38
Granzyme B
Homo sapiens
AAA75490

MQPILLLLAFLLLPRADAGEIIGGHEAKPHSRPYMAYLMIWDQKSLKRCGGFLIQDDFVL
TAAHCWGSSINVTLGAHNIKEQEPTQQFIPVKRAIPHPAYNPKNFSNDIMLLQLERKAKR
TRAVQPLRLPSNKAQVKPGQTCSVAGWGQTAPLGKHSHTLQEVKMTVQEDRKCESDL
RHYYDSTIELCVGDPEIKKTSFKGDSGGPLVCNKVAQGIVSYGRNNGMPPRACTKVSSF
VHWIKKTMKRY

SEQ ID NO:39
Cytochrome c
Homo sapiens
NP_061820

MGDVEKGKKIFIMKCSQCHTVEKGGKHKTGPNLHGLFGRKTGQAPGYSYTAANKNKGI
IWGEDTLMEYLENPKKYIPGTKMIFVGIKKKEERADLIAYLKKATNE

SEQ ID NO:40
Tumor Necrosis Factor Alpha (TNFα)
Homo sapiens
CAA26669

MSTESMIRDVELAEEALPKKTGGPQGSRRCLFLSLFSFLIVAGATTLFCLLHFGVIGPQRE
EFPRDLSLISPLAQAVRSSSRTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRET
PEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLDFAESGQVYFGIIAL

SEQ ID NO:41
Fas
Homo sapiens
CAI13871

MLGIWTLLPLVLTSVARLSSKSVNAQVTDINSKGLELRKTVTTVETQNLEGLHHDGQFC
HKPCPPGERKARDCTVNGDEPDCVPCQEGKEYTDKAHFSSKCRRCRLCDEGHGLEVEI
NCTRTQNTKCRCKPNFFCNSTVCEHCDPCTKCEHGIIKECTLTSNTKCKEEVKRKEVQK
TCRKHRKENQGSHESPTLNPETVAINLSDVDLSKYITTIAGVMTLSQVKGFVRKNGVNE
AKIDEIKNDNVQDTAEQKVQLLRNWHQLHGKKEAYDTLIKDLKKANLCTLAEKIQTIIL
KDITSDSENSNFRNEIQSLV

SEQ ID NO:42
Bad (Bcl2 antagonist of cell death)
Homo sapiens
CAG46757

MFQIPEFEPSEQEDSSSAERGLGPSPAGDGPSGSGKHHRQAPGLLWDASHQQEQPTSSSH
HGGAGAVEIRSRHSSYPAGTEDDEGMGEEPSPFRGRSRSAPPNLWAAQRYGRELRRMS
DEFVDSFKKGLPRPKSAGTATQMRQSSSWTRVFQSWWDRNLGRGSSAPSQ

SEQ ID NO:43
Apoptosis Inducing Protein (AIP)
Homo sapiens
AAK67626

MVDHLANTEINSQRIAAVESCFGASGQPLALPGRVLLGEGVLTKECRKKAKPRIFFLFND
ILVYGSIVLNKRKYRSQHIIPLEEVTLELLPETLQAKNRWMIKTAKKSFVVSAASATERQ
EWISHIEECVRRQLKATGRPPSTEHAAPWIPDKATDICMRCTQTRFSALTRRHHCRKCGF
VVCAECSRQRFLLPRLSPKPVRVCSLCYRELAAQQRQEEAEEQGAGSPRQPAHLARPIC
GASSGDDDDSDEDKEGSRDGDWPSSVEFYASGVAWSAFHS

SEQ ID NO:44
Pierisin-1
Pieris rapae
Q9U8Q4

MADRQPYMTNGIQAAVVEWIRALDLEIISLLLSRAWPMALLATSELRWRPTVLTDTDN
VVRLDRRQRLVRWDRRPPNEIFLDGFVPIVTRENPDWEETDLYGFAKNNHPSIFVSTTKT
QRNKKKYVWTPRNANRGIVYQYEIYAPGGVDVNDSFSDASPWPNQMEVAFPGGIQNIY
IRSARELHNGRIQRIWINPNFLDPGDLEPIVSSSRTPQVIWRMNHPDGGHRDQRSERSASS
YDDLMYGGTGNVQEDTFGDEPNNPKPIAAGEFMIESIKDKNSFLDLSKNVNGGVIHSNL
YSGGDNQIWVFSYDDNKKAYRIQSYQNSYLYLSWDSNASSKEMILRGYTNSGSNNQY
WQIEQTGKNYRLRNLLNLDMIITAQDKPSAFGGKEVIVNTEISNSNTKISQEWKMIPFDF
RPIIDGDYNIFNVDLSNQVVDFSNQPDLLVHGHIFCDNENQTWHFTYNSTYHAYKIWSG
RKSNLLLTWDSNAASKEMVVRAYTESRSKNQYWRIEQTGSKSYKVRNLENSSMILGLT

RVSTPYGGLNLMVEDDSDGHSDLHSDWDIKPIFYQDIPDGDYNIFNDNFPNIAIDFTNQE
GSLIHGHNFCSNNNQKWSFVFDGKRKAYRIKSGVRSNLWLSWDSNASSKEMVLRAYTE
SGSSNQYWRLDEANDGSYRIRNLQDYYKLIALTNKNTPYGGKELIVSDNKESGNTWYL
KKLGEVPLPNRKFRIATKLNYKKVIDSSTSYNLIITHDLNFASSIWELVYDSSKKAYNIYS
SDINNLGWIYQNKNFFVKLGNIDGPDHGDLRYFWTIEYSMQTGCYLIRSLHDPANAVGY
TDSESVITDTSTYSDNQLFHFILM

SEQ ID NO:45
TRAIL (TNF-related apoptosis-inducing ligand)
Homo sapiens
P50591

MAMMEVQGGPSLGQTCVLIVIFTVLLQSLCVAVTYVYFTNELKQMQDKYSKSGIACFL
KEDDSYWDPNDEESMNSPCWQVKWQLRQLVRKMILRTSEETISTVQEKQQNISPLVRE
RGPQRVAAHITGTRGRSNTLSSPNSKNEKALGRKINSWESSRSGHSFLSNLHLRNGELVI
HEKGFYYIYSQTYFRFQEEIKENTKNDKQMVQYIYKYTSYPDPILLMKSARNSCWSKDA
EYGLYSIYQGGIFELKENDRIFVSVTNEHLIDMDHEASFFGAFLVG

SEQ ID NO:46
Bax
Homo sapiens
Q07812

MDGSGEQPRGGGPTSSEQIMKTGALLLQGFIQDRAGRMGGEAPELALDPVPQDASTKK
LSECLKRIGDELDSNMELQRMIAAVDTDSPREVFFRVAADMFSDGNFNWGRVVALFYF
ASKLVLKALCTKVPELIRTIMGWTLDFLRERLLGWIQDQGGWDGLLSYFGTPTWQTVTI
FVAGVLTASLTIWKKMG

SEQ ID NO:47
Green Fluorescent Protein (GFP)
Aequorea victoria
P42212

MSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWPT
LVTTFSYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGD
TLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQ
LADHYQQNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMVLLEFVTAAGITHGMDE
LYK

SEQ ID NO:48
Yellow Fluorescent Protein (YFP)
Aliivibrio fischeri
P21578

MFKGIVEGIGIIEKIDIYTDLDKYAIRFPENMLNGIKKESSIMFNGCFLTVTSVNSNIVWFD
IFEKEARKLDTFREYKVGDRVNLGTFPKFGAASGGHILSARISCVASIIEIIENEDYQQMW
IQIPENFTEFLIDKDYIAVDGISLTIDTIKNNQFFISLPLKIAQNTNMKWRKKGDKVNVELS
NKINANQCW

SEQ ID NO:49
Cyan Fluorescent Protein (CFP)
Montastraea cavernosa
AAL17905

MSVIKSVMKIKLRMDGIVNGHKFMITGEGEGKPFEGTHTIILKVKEGGPLPFAYDILTTAF
QYGNRVFTKYPKDIPDYFKQSFPEGYSWERSMTFEDQGVCTVTSDIKLEGDCFFYEIRFY
GVNFPSSGPVMQKKTLKWEPSTENMYVRDGVLLGDVSRTLLLEGDKHHRCNFRSTYG
AKKGVVLPEYHFVDHRIEILSHDKDYNTVEVYENAVARPSMLPVKAK

SEQ ID NO:50
Red Fluorescent Protein (RFP)
Discosoma sp. SSAL-2000
AAG16224

MSCSKNVIKEFMRFKVRMEGTVNGHEFEIKGEGEGRPYEGHCSVKLMVTKGGPLPFAF
DILSPQFQYGSKVYVKHPADIPDYKKLSFPEGFKWERVMNFEDGGVVTVSQDSSLKDG
CFIYEVKFIGVNFPSDGPVMQRRTRGWEASSERLYPRDGVLKGDIHMALRLEGGGHYL
VEFKSIYMVKKPSVQLPGYYYVDSKLDMTSHNEDYTVVEQYEKTQGRHHPFIKPLQ

SEQ ID NO:51
Luciferase
Photinus pyralis
CAA59282

MEDAKNIKKGPAPFYPLEDGTAGEQLHKAMKRYALVPGTIAFTDAHIEVNITYAEYFEM
SVRLAEAMKRYGLNTNHRIVVCSENSLQFFMPVLGALFIGVAVAPANDIYNERELLNSM
NISQPTVVFVSKKGLQKILNVQKKLPIIQKIIIMDSKTDYQGFQSMYTFVTSHLPPGFNEY
DFVPESFDRDKTIALIMNSSGSTGSPKGVALPHRTACVRFSHARDPIFGNQIIPDTAILSVV
PFHHGFGMFTTLGYLICGFRVVLMYRFEEELFLRSLQDYKIQSALLVPTLFSFFAKSTLID
KYDLSNLHEIASGGAPLSKEVGEAVAKRFHLPGIRQGYGLTETTSAILITPEGDDKPGAV
GKVVPFFEAKVVDLDTGKTLGVNQRGELCVRGPMIMSGYVNDPEATNALIDKDGWLH
SGDIAYWDEDEHFFIVDRLKSLIKYKGCQVAPAELESILLQHPNIFDAGVAGLPGDDAGE
LPAAVVVLEHGKTMTEKEIVDYVASQVTTAKKLRGGVVFVDEVPKGLTGKLDARKIRE
ILIKAKKGGKSKL

SEQ ID NO:52
Luciferase
Renilla reniformis
AAA29804

MTSKVYDPEQRKRMITGPQWWARCKQMNVLDSFINYYDSEKHAENAVIFLHGNAASS
YLWRHVVPHIEPVARCIIPDLIGMGKSGKSGNGSYRLLDHYKYLTAWFELLNLPKKIIFV
GHDWGACLAFHYSYEHQDKIKAIVHAESVVDVIESWDEWPDIEEDIALIKSEEGEKMVL
ENNFFVETMLPSKIMRKLEPEEFAAYLEPFKEKGEVRRPTLSWPREIPLVKGGKPDVVQI
VRNYNAYLRASDDLPKMFIESDPGFFSNAIVEGAKKFPNTEFVKVKGLHFSQEDAPDEM
GKYIKSFVERVLKNEQ

SEQ ID NO:53
Human Serum Albumin (HSA) Linker with C34S substitution, Domain I
Amino Acid Sequence

DAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQSPFEDHVKLVNEVTEFAKTCVADESAE
NCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECFLQHKDDNPNLPRLVRPEV
DVMCTAFHDNEETFLKKYLYEIARRHPYFYAPELLFFAKRYKAAFTECCQAADKAACLLP
KLDELRDEGKASSAKQR

SEQ ID NO:54
Human Serum Albumin (HSA) Linker, Domain II
Amino Acid Sequence

GKASSAKQRLKCASLQKFGERAFKAWAVARLSQRFPKAEFAEVSKLVTDLTKVHTECC
HGDLLECADDRADLAKYICENQDSISSKLKECCEKPLLEKSHCIAEVENDEMPADLPSLA
ADFVESKDVCKNYAEAKDVFLGMFLYEYARRHPDYSVVLLLRLAKTYETTLEKCCAA
ADPHECYAKVFDEFKPLVEEPQ

SEQ ID NO:55
Human Serum Albumin (HSA) Linker with N503Q substitution, Domain III
Amino Acid Sequence

VEEPQNLIKQNCELFEQLGEYKFQNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCCK
HPEAKRMPCAEDYLSVVLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETY
VPKEFQAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDDFAAFVE
KCCKADDKETCFAEEGKKLVAASQAALGL

SEQ ID NO:56
Human Serum Albumin (HSA) Linker, Domain I
Amino Acid Sequence

DAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQCPFEDHVKLVNEVTEFAKTCVADES
AENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECFLQHKDDNPNLPRLV

RPEVDVMCTAFHDNEETFLKKYLYEIARRHPYFYAPELLFFAKRYKAAFTECCQAADK
AACLLPKLDELRDEGKASSAKQR

SEQ ID NO:57
Human Serum Albumin (HSA) Linker, Domain III
Amino Acid Sequence

VEEPQNLIKQNCELFEQLGEYKFQNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCCK
HPEAKRMPCAEDYLSVVLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETY
VPKEFNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDDFAAFVE
KCCKADDKETCFAEEGKKLVAASQAALGL

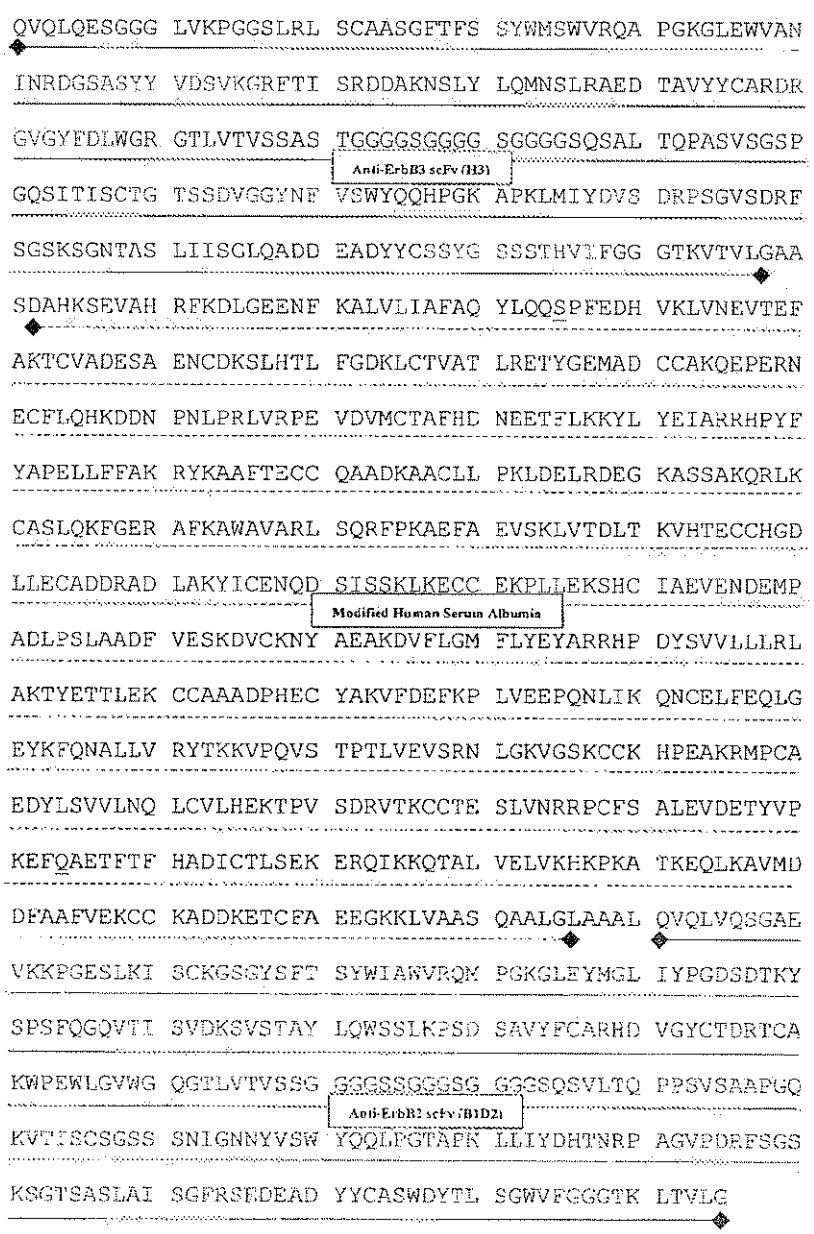
SEQ ID NO:58
Human Alpha-Fetoprotein
Amino Acid Sequence

MKWVESIFLIFLLNFTESRTLHRNEYGIASILDSYQCTAEISLADLATIFFAQFVQEATYKE
VSKMVKDALTAIEKPTGDEQSSGCLENQLPAFLEELCHEKEILEKYGHSDCCSQSEEGRH
NCFLAHKKPTPASIPLFQVPEPVTSCEAYEEDRETFMNKFIYEIARRHPFLYAPTILLWAA
RYDKIIPSCCKAENAVECFQTKAATVTKELRESSLLNQHACAVMKNFGTRTFQAITVTK
LSQKFTKVNFTEIQKLVLDVAHVHEHCCRGDVLDCLQDGEKIMSYICSQQDTLSNKITE
CCKLTTLERGQCIIHAENDEKPEGLSPNLNRFLGDRDFNQFSSGEKNIFLASFVHEYSRRH
PQLAVSVILRVAKGYQELLEKCFQTENPLECQDKGEEELQKYIQESQALAKRSCGLFQK
LGEYYLQNAFLVAYTKKAPQLTSSELMAITRKMAATAATCCQLSEDKLLACGEGAADII
IGHLCIRHEMTPVNPGVGQCCTSSYANRRPCFSSLVVDETYVPPAFSDDKFIFHKDLCQA
QGVALQTMKQEFLINLVKQKPQITEEQLEAVIADFSGLLEKCCQGQEQEVCFAEEGQKLI
SKTRAALGV

APPENDIX B

SEQUENCE ALIGNMENTS

B2B3-1 (H3-mHSA-B1D2)



CDR loops are highlighted within H3 (blue with pink CDRs) and B1D2 (pink with cyan CDRs). Connectors to modified HSA are shown in red.

Sequence alignments for B2B3/mHSA variants

```
                                1                                   45
      A5-mHSA-ML3.9      (1)  QVQLVQSGGGLVKPGGSLRLSCAASGFSFNTYDMNWVRQAPGKGL
        A5-mHSA-B1D2     (1)  QVQLVQSGGGLVKPGGSLRLSCAASGFSFNTYDMNWVRQAPGKGL
      A5-mHSA-F5B6H2     (1)  QVQLVQSGGGLVKPGGSLRLSCAASGFSFNTYDMNWVRQAPGKGL
        B12-mHSA-B1D2    (1)  QVQLVQSGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGL
      B12-mHSA-F5B6H2    (1)  QVQLVQSGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGL
        F4-mHSA-B1D2     (1)  QVQLQESGGGLVKPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGL
      F4-mHSA-F5B6H2     (1)  QVQLQESGGGLVKPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGL
        H3-mHSA-B1D2     (1)  QVQLQESGGGLVKPGGSLRLSCAASGFTFSSYWMSWVRQAPGKGL
      H3-mHSA-F5B6H2     (1)  QVQLQESGGGLVKPGGSLRLSCAASGFTFSSYWMSWVRQAPGKGL

                                46                                  90
      A5-mHSA-ML3.9     (46)  EWVSSISSSSSYIYYADSVKGRFTISRDNAKNSLYLQMNSLRAED
        A5-mHSA-B1D2    (46)  EWVSSISSSSSYIYYADSVKGRFTISRDNAKNSLYLQMNSLRAED
      A5-mHSA-F5B6H2    (46)  EWVSSISSSSSYIYYADSVKGRFTISRDNAKNSLYLQMNSLRAED
        B12-mHSA-B1D2   (46)  EWVSGISWNSGSIGYADSVKGRFTISRDNAKNSLYLQMNSLRPED
      B12-mHSA-F5B6H2   (46)  EWVSGISWNSGSIGYADSVKGRFTISRDNAKNSLYLQMNSLRPED
        F4-mHSA-B1D2    (46)  EWVSTISGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAED
      F4-mHSA-F5B6H2    (46)  EWVSTISGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAED
        H3-mHSA-B1D2    (46)  EWVANINRDGSASYYVDSVKGRFTISRDDAKNSLYLQMNSLRAED
      H3-mHSA-F5B6H2    (46)  EWVANINRDGSASYYVDSVKGRFTISRDDAKNSLYLQMNSLRAED

                                91                                 135
      A5-mHSA-ML3.9     (91)  TAVYYCARDG---VATTPFDYWGQGTLVTVS---SGGGGSGGGGS
        A5-mHSA-B1D2    (91)  TAVYYCARDG---VATTPFDYWGQGTLVTVS---SGGGGSGGGGS
      A5-mHSA-F5B6H2    (91)  TAVYYCARDG---VATTPFDYWGQGTLVTVS---SGGGGSGGGGS
        B12-mHSA-B1D2   (91)  TAVYYCARDLGAKQWLEGFDYWGQGTLVTVSSASTGGGGSGGGGS
      B12-mHSA-F5B6H2   (91)  TAVYYCARDLGAKQWLEGFDYWGQGTLVTVSSASTGGGGSGGGGS
        F4-mHSA-B1D2    (91)  TAVYYCAKGYSSSWSEVASGYWGQGTLVTVSSASTGGGGSGGGGS
      F4-mHSA-F5B6H2    (91)  TAVYYCAKGYSSSWSEVASGYWGQGTLVTVSSASTGGGGSGGGGS
        H3-mHSA-B1D2    (91)  TAVYYCARDR----GVGYFDLWGRGTLVTVSSASTGGGGSGGGGS
      H3-mHSA-F5B6H2    (91)  TAVYYCARDR----GVGYFDLWGRGTLVTVSSASTGGGGSGGGGS

                               136                                 180
      A5-mHSA-ML3.9    (130)  GGGGSQSVLTQPPS-VSGAPGQRVTISCTGSSSNIGAGYDVHWYQ
        A5-mHSA-B1D2   (130)  GGGGSQSVLTQPPS-VSGAPGQRVTISCTGSSSNIGAGYDVHWYQ
      A5-mHSA-F5B6H2   (130)  GGGGSQSVLTQPPS-VSGAPGQRVTISCTGSSSNIGAGYDVHWYQ
        B12-mHSA-B1D2  (136)  GGGGSSYELTQDPA-VSVALGQTVRITCQGDSLRS---YYASWYQ
      B12-mHSA-F5B6H2  (136)  GGGGSSYELTQDPA-VSVALGQTVRITCQGDSLRS---YYASWYQ
        F4-mHSA-B1D2   (136)  GGGGSAIVMTQSPSSLSASVGDRVTITCRASQGIR---NDLGWYQ
      F4-mHSA-F5B6H2   (136)  GGGGSAIVMTQSPSSLSASVGDRVTITCRASQGIR---NDLGWYQ
        H3-mHSA-B1D2   (132)  GGGGSQSALTQPAS-VSGSPGQSITISCTGTSSDVGGYNFVSWYQ
      H3-mHSA-F5B6H2   (132)  GGGGSQSALTQPAS-VSGSPGQSITISCTGTSSDVGGYNFVSWYQ

                               181                                 225
      A5-mHSA-ML3.9    (174)  QLPGTAPKLLIYGNSNRPSGVPDRFSGSKSGTSASLAITGLQAED
        A5-mHSA-B1D2   (174)  QLPGTAPKLLIYGNSNRPSGVPDRFSGSKSGTSASLAITGLQAED
      A5-mHSA-F5B6H2   (174)  QLPGTAPKLLIYGNSNRPSGVPDRFSGSKSGTSASLAITGLQAED
        B12-mHSA-B1D2  (177)  QKPGQAPVLVIYGKNNRPSGIPDRFSGSTSGNSASLTITGAQAED
      B12-mHSA-F5B6H2  (177)  QKPGQAPVLVIYGKNNRPSGIPDRFSGSTSGNSASLTITGAQAED
        F4-mHSA-B1D2   (178)  QKAGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPDD
      F4-mHSA-F5B6H2   (178)  QKAGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPDD
        H3-mHSA-B1D2   (176)  QHPGKAPKLMIYDVSDRPSGVSDRFSGSKSGNTASLIISGLQADD
      H3-mHSA-F5B6H2   (176)  QHPGKAPKLMIYDVSDRPSGVSDRFSGSKSGNTASLIISGLQADD
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                                226                                 270
      A5-mHSA-ML3.9   (219) EADYYCQSYDSS-LSALFGGGTKLTVLG-AASDAHKSEVAHRFKD
        A5-mHSA-B1D2  (219) EADYYCQSYDSS-LSALFGGGTKLTVLG-AASDAHKSEVAHRFKD
      A5-mHSA-F5B6H2  (219) EADYYCQSYDSS-LSALFGGGTKLTVLG-AASDAHKSEVAHRFKD
       B12-mHSA-B1D2  (222) EADYYCNSRDSSGNHWVFGGGTKVTVLG-AASDAHKSEVAHRFKD
     B12-mHSA-F5B6H2  (222) EADYYCNSRDSSGNHWVFGGGTKVTVLG-AASDAHKSEVAHRFKD
        F4-mHSA-B1D2  (223) FATYFCQQAHSF--PPTFGGGTKVEIKRGAASDAHKSEVAHRFKD
      F4-mHSA-F5B6H2  (223) FATYFCQQAHSF--PPTFGGGTKVEIKRGAASDAHKSEVAHRFKD
        H3-mHSA-B1D2  (221) EADYYCSSYGSSSTHVIFGGGTKVTVLG-AASDAHKSEVAHRFKD
      H3-mHSA-F5B6H2  (221) EADYYCSSYGSSSTHVIFGGGTKVTVLG-AASDAHKSEVAHRFKD

                                271                                 315
      A5-mHSA-ML3.9   (262) LGEENFKALVLIAFAQYLQQSPFEDHVKLVNEVTEFAKTCVADES
        A5-mHSA-B1D2  (262) LGEENFKALVLIAFAQYLQQSPFEDHVKLVNEVTEFAKTCVADES
      A5-mHSA-F5B6H2  (262) LGEENFKALVLIAFAQYLQQSPFEDHVKLVNEVTEFAKTCVADES
       B12-mHSA-B1D2  (266) LGEENFKALVLIAFAQYLQQSPFEDHVKLVNEVTEFAKTCVADES
     B12-mHSA-F5B6H2  (266) LGEENFKALVLIAFAQYLQQSPFEDHVKLVNEVTEFAKTCVADES
        F4-mHSA-B1D2  (266) LGEENFKALVLIAFAQYLQQSPFEDHVKLVNEVTEFAKTCVADES
      F4-mHSA-F5B6H2  (266) LGEENFKALVLIAFAQYLQQSPFEDHVKLVNEVTEFAKTCVADES
        H3-mHSA-B1D2  (265) LGEENFKALVLIAFAQYLQQSPFEDHVKLVNEVTEFAKTCVADES
      H3-mHSA-F5B6H2  (265) LGEENFKALVLIAFAQYLQQSPFEDHVKLVNEVTEFAKTCVADES

                                316                                 360
      A5-mHSA-ML3.9   (307) AENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECFL
        A5-mHSA-B1D2  (307) AENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECFL
      A5-mHSA-F5B6H2  (307) AENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECFL
       B12-mHSA-B1D2  (311) AENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECFL
     B12-mHSA-F5B6H2  (311) AENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECFL
        F4-mHSA-B1D2  (311) AENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECFL
      F4-mHSA-F5B6H2  (311) AENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECFL
        H3-mHSA-B1D2  (310) AENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECFL
      H3-mHSA-F5B6H2  (310) AENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECFL

                                361                                 405
      A5-mHSA-ML3.9   (352) QHKDDNPNLPRLVRPEVDVMCTAFHDNEETFLKKYLYEIARRHPY
        A5-mHSA-B1D2  (352) QHKDDNPNLPRLVRPEVDVMCTAFHDNEETFLKKYLYEIARRHPY
      A5-mHSA-F5B6H2  (352) QHKDDNPNLPRLVRPEVDVMCTAFHDNEETFLKKYLYEIARRHPY
       B12-mHSA-B1D2  (356) QHKDDNPNLPRLVRPEVDVMCTAFHDNEETFLKKYLYEIARRHPY
     B12-mHSA-F5B6H2  (356) QHKDDNPNLPRLVRPEVDVMCTAFHDNEETFLKKYLYEIARRHPY
        F4-mHSA-B1D2  (356) QHKDDNPNLPRLVRPEVDVMCTAFHDNEETFLKKYLYEIARRHPY
      F4-mHSA-F5B6H2  (356) QHKDDNPNLPRLVRPEVDVMCTAFHDNEETFLKKYLYEIARRHPY
        H3-mHSA-B1D2  (355) QHKDDNPNLPRLVRPEVDVMCTAFHDNEETFLKKYLYEIARRHPY
      H3-mHSA-F5B6H2  (355) QHKDDNPNLPRLVRPEVDVMCTAFHDNEETFLKKYLYEIARRHPY

                                406                                 450
      A5-mHSA-ML3.9   (397) FYAPELLFFAKRYKAAFTECCQAADKAACLLPKLDELRDEGKASS
        A5-mHSA-B1D2  (397) FYAPELLFFAKRYKAAFTECCQAADKAACLLPKLDELRDEGKASS
      A5-mHSA-F5B6H2  (397) FYAPELLFFAKRYKAAFTECCQAADKAACLLPKLDELRDEGKASS
       B12-mHSA-B1D2  (401) FYAPELLFFAKRYKAAFTECCQAADKAACLLPKLDELRDEGKASS
     B12-mHSA-F5B6H2  (401) FYAPELLFFAKRYKAAFTECCQAADKAACLLPKLDELRDEGKASS
        F4-mHSA-B1D2  (401) FYAPELLFFAKRYKAAFTECCQAADKAACLLPKLDELRDEGKASS
      F4-mHSA-F5B6H2  (401) FYAPELLFFAKRYKAAFTECCQAADKAACLLPKLDELRDEGKASS
        H3-mHSA-B1D2  (400) FYAPELLFFAKRYKAAFTECCQAADKAACLLPKLDELRDEGKASS
      H3-mHSA-F5B6H2  (400) FYAPELLFFAKRYKAAFTECCQAADKAACLLPKLDELRDEGKASS

                                451                                 495
      A5-mHSA-ML3.9   (442) AKQRLKCASLQKFGERAFKAWAVARLSQRFPKAEFAEVSKLVTDL
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A5-mHSA-B1D2    (442) AKQRLKCASLQKFGERAFKAWAVARLSQRFPKAEFAEVSKLVTDL
A5-mHSA-F5B6H2  (442) AKQRLKCASLQKFGERAFKAWAVARLSQRFPKAEFAEVSKLVTDL
B12-mHSA-B1D2   (446) AKQRLKCASLQKFGERAFKAWAVARLSQRFPKAEFAEVSKLVTDL
B12-mHSA-F5B6H2 (446) AKQRLKCASLQKFGERAFKAWAVARLSQRFPKAEFAEVSKLVTDL
F4-mHSA-B1D2    (446) AKQRLKCASLQKFGERAFKAWAVARLSQRFPKAEFAEVSKLVTDL
F4-mHSA-F5B6H2  (446) AKQRLKCASLQKFGERAFKAWAVARLSQRFPKAEFAEVSKLVTDL
H3-mHSA-B1D2    (445) AKQRLKCASLQKFGERAFKAWAVARLSQRFPKAEFAEVSKLVTDL
H3-mHSA-F5B6H2  (445) AKQRLKCASLQKFGERAFKAWAVARLSQRFPKAEFAEVSKLVTDL

                        496                                       540
A5-mHSA-ML3.9   (487) TKVHTECCHGDLLECADDRADLAKYICENQDSISSKLKECCEKPL
A5-mHSA-B1D2    (487) TKVHTECCHGDLLECADDRADLAKYICENQDSISSKLKECCEKPL
A5-mHSA-F5B6H2  (487) TKVHTECCHGDLLECADDRADLAKYICENQDSISSKLKECCEKPL
B12-mHSA-B1D2   (491) TKVHTECCHGDLLECADDRADLAKYICENQDSISSKLKECCEKPL
B12-mHSA-F5B6H2 (491) TKVHTECCHGDLLECADDRADLAKYICENQDSISSKLKECCEKPL
F4-mHSA-B1D2    (491) TKVHTECCHGDLLECADDRADLAKYICENQDSISSKLKECCEKPL
F4-mHSA-F5B6H2  (491) TKVHTECCHGDLLECADDRADLAKYICENQDSISSKLKECCEKPL
H3-mHSA-B1D2    (490) TKVHTECCHGDLLECADDRADLAKYICENQDSISSKLKECCEKPL
H3-mHSA-F5B6H2  (490) TKVHTECCHGDLLECADDRADLAKYICENQDSISSKLKECCEKPL

                        541                                       585
A5-mHSA-ML3.9   (532) LEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNYAEAKDVFLG
A5-mHSA-B1D2    (532) LEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNYAEAKDVFLG
A5-mHSA-F5B6H2  (532) LEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNYAEAKDVFLG
B12-mHSA-B1D2   (536) LEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNYAEAKDVFLG
B12-mHSA-F5B6H2 (536) LEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNYAEAKDVFLG
F4-mHSA-B1D2    (536) LEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNYAEAKDVFLG
F4-mHSA-F5B6H2  (536) LEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNYAEAKDVFLG
H3-mHSA-B1D2    (535) LEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNYAEAKDVFLG
H3-mHSA-F5B6H2  (535) LEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNYAEAKDVFLG

                        586                                       630
A5-mHSA-ML3.9   (577) MFLYEYARRHPDYSVVLLLRLAKTYETTLEKCCAAADPHECYAKV
A5-mHSA-B1D2    (577) MFLYEYARRHPDYSVVLLLRLAKTYETTLEKCCAAADPHECYAKV
A5-mHSA-F5B6H2  (577) MFLYEYARRHPDYSVVLLLRLAKTYETTLEKCCAAADPHECYAKV
B12-mHSA-B1D2   (581) MFLYEYARRHPDYSVVLLLRLAKTYETTLEKCCAAADPHECYAKV
B12-mHSA-F5B6H2 (581) MFLYEYARRHPDYSVVLLLRLAKTYETTLEKCCAAADPHECYAKV
F4-mHSA-B1D2    (581) MFLYEYARRHPDYSVVLLLRLAKTYETTLEKCCAAADPHECYAKV
F4-mHSA-F5B6H2  (581) MFLYEYARRHPDYSVVLLLRLAKTYETTLEKCCAAADPHECYAKV
H3-mHSA-B1D2    (580) MFLYEYARRHPDYSVVLLLRLAKTYETTLEKCCAAADPHECYAKV
H3-mHSA-F5B6H2  (580) MFLYEYARRHPDYSVVLLLRLAKTYETTLEKCCAAADPHECYAKV

                        631                                       675
A5-mHSA-ML3.9   (622) FDEFKPLVEEPQNLIKQNCELFEQLGEYKFQNALLVRYTKKVPQV
A5-mHSA-B1D2    (622) FDEFKPLVEEPQNLIKQNCELFEQLGEYKFQNALLVRYTKKVPQV
A5-mHSA-F5B6H2  (622) FDEFKPLVEEPQNLIKQNCELFEQLGEYKFQNALLVRYTKKVPQV
B12-mHSA-B1D2   (626) FDEFKPLVEEPQNLIKQNCELFEQLGEYKFQNALLVRYTKKVPQV
B12-mHSA-F5B6H2 (626) FDEFKPLVEEPQNLIKQNCELFEQLGEYKFQNALLVRYTKKVPQV
F4-mHSA-B1D2    (626) FDEFKPLVEEPQNLIKQNCELFEQLGEYKFQNALLVRYTKKVPQV
F4-mHSA-F5B6H2  (626) FDEFKPLVEEPQNLIKQNCELFEQLGEYKFQNALLVRYTKKVPQV
H3-mHSA-B1D2    (625) FDEFKPLVEEPQNLIKQNCELFEQLGEYKFQNALLVRYTKKVPQV
H3-mHSA-F5B6H2  (625) FDEFKPLVEEPQNLIKQNCELFEQLGEYKFQNALLVRYTKKVPQV

                        676                                       720
A5-mHSA-ML3.9   (667) STPTLVEVSRNLGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLCVL
A5-mHSA-B1D2    (667) STPTLVEVSRNLGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLCVL
A5-mHSA-F5B6H2  (667) STPTLVEVSRNLGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLCVL
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B12-mHSA-B1D2    (671) STPTLVEVSRNLGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLCVL
B12-mHSA-F5B6H2  (671) STPTLVEVSRNLGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLCVL
F4-mHSA-B1D2     (671) STPTLVEVSRNLGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLCVL
F4-mHSA-F5B6H2   (671) STPTLVEVSRNLGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLCVL
H3-mHSA-B1D2     (670) STPTLVEVSRNLGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLCVL
H3-mHSA-F5B6H2   (670) STPTLVEVSRNLGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLCVL

                       721                                       765
A5-mHSA-ML3.9    (712) HEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFQAETFT
A5-mHSA-B1D2     (712) HEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFQAETFT
A5-mHSA-F5B6H2   (712) HEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFQAETFT
B12-mHSA-B1D2    (716) HEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFQAETFT
B12-mHSA-F5B6H2  (716) HEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFQAETFT
F4-mHSA-B1D2     (716) HEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFQAETFT
F4-mHSA-F5B6H2   (716) HEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFQAETFT
H3-mHSA-B1D2     (715) HEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFQAETFT
H3-mHSA-F5B6H2   (715) HEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFQAETFT

                       766                                       810
A5-mHSA-ML3.9    (757) FHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDDFAA
A5-mHSA-B1D2     (757) FHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDDFAA
A5-mHSA-F5B6H2   (757) FHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDDFAA
B12-mHSA-B1D2    (761) FHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDDFAA
B12-mHSA-F5B6H2  (761) FHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDDFAA
F4-mHSA-B1D2     (761) FHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDDFAA
F4-mHSA-F5B6H2   (761) FHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDDFAA
H3-mHSA-B1D2     (760) FHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDDFAA
H3-mHSA-F5B6H2   (760) FHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDDFAA

                       811                                       855
A5-mHSA-ML3.9    (802) FVEKCCKADDKETCFAEEGKKLVAASQAALGLAAALQVQLVQSGA
A5-mHSA-B1D2     (802) FVEKCCKADDKETCFAEEGKKLVAASQAALGLAAALQVQLVQSGA
A5-mHSA-F5B6H2   (802) FVEKCCKADDKETCFAEEGKKLVAASQAALGLAAALQVQLVESGG
B12-mHSA-B1D2    (806) FVEKCCKADDKETCFAEEGKKLVAASQAALGLAAALQVQLVQSGA
B12-mHSA-F5B6H2  (806) FVEKCCKADDKETCFAEEGKKLVAASQAALGLAAALQVQLVESGG
F4-mHSA-B1D2     (806) FVEKCCKADDKETCFAEEGKKLVAASQAALGLAAALQVQLVQSGA
F4-mHSA-F5B6H2   (806) FVEKCCKADDKETCFAEEGKKLVAASQAALGLAAALQVQLVESGG
H3-mHSA-B1D2     (805) FVEKCCKADDKETCFAEEGKKLVAASQAALGLAAALQVQLVQSGA
H3-mHSA-F5B6H2   (805) FVEKCCKADDKETCFAEEGKKLVAASQAALGLAAALQVQLVESGG

                       856                                       900
A5-mHSA-ML3.9    (847) EVKKPGESLKISCKGSGYSFTSYWIAWVRQMPGKGLEYMGLIYPG
A5-mHSA-B1D2     (847) EVKKPGESLKISCKGSGYSFTSYWIAWVRQMPGKGLEYMGLIYPG
A5-mHSA-F5B6H2   (847) GLVQPGGSLRLSCAASGFTFRSYAMSWVRQAPGKGLEWVSAISGR
B12-mHSA-B1D2    (851) EVKKPGESLKISCKGSGYSFTSYWIAWVRQMPGKGLEYMGLIYPG
B12-mHSA-F5B6H2  (851) GLVQPGGSLRLSCAASGFTFRSYAMSWVRQAPGKGLEWVSAISGR
F4-mHSA-B1D2     (851) EVKKPGESLKISCKGSGYSFTSYWIAWVRQMPGKGLEYMGLIYPG
F4-mHSA-F5B6H2   (851) GLVQPGGSLRLSCAASGFTFRSYAMSWVRQAPGKGLEWVSAISGR
H3-mHSA-B1D2     (850) EVKKPGESLKISCKGSGYSFTSYWIAWVRQMPGKGLEYMGLIYPG
H3-mHSA-F5B6H2   (850) GLVQPGGSLRLSCAASGFTFRSYAMSWVRQAPGKGLEWVSAISGR

                       901                                       945
A5-mHSA-ML3.9    (892) DSDTKYSPSFQGQVTISVDKSVSTAYLQWSSLKPSDSAVYFCARH
A5-mHSA-B1D2     (892) DSDTKYSPSFQGQVTISVDKSVSTAYLQWSSLKPSDSAVYFCARH
A5-mHSA-F5B6H2   (892) GDNTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKM
B12-mHSA-B1D2    (896) DSDTKYSPSFQGQVTISVDKSVSTAYLQWSSLKPSDSAVYFCARH
B12-mHSA-F5B6H2  (896) GDNTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKM
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F4-mHSA-B1D2     (896) DSDTKYSPSFQGQVTISVDKSVSTAYLQWSSLKPSDSAVYFCARH
F4-mHSA-F5B6H2   (896) GDNTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKM
H3-mHSA-B1D2     (895) DSDTKYSPSFQGQVTISVDKSVSTAYLQWSSLKPSDSAVYFCARH
H3-mHSA-F5B6H2   (895) GDNTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKM

                        946                                        990
A5-mHSA-ML3.9    (937) DVGYCSSSNCAKWPEYFQHWGQGTLVTVSSGGGGSSGGGSGGGGS
A5-mHSA-B1D2     (937) DVGYCTDRTCAKWPEWLGVWGQGTLVTVSSGGGGSSGGGSGGGGS
A5-mHSA-F5B6H2   (937) TSNAVG----------FDYWGQGTLVTVSSGGGGSGGGSGGGGSG
B12-mHSA-B1D2    (941) DVGYCTDRTCAKWPEWLGVWGQGTLVTVSSGGGGSSGGGSGGGGS
B12-mHSA-F5B6H2  (941) TSNAVG----------FDYWGQGTLVTVSSGGGGSGGGSGGGGSG
F4-mHSA-B1D2     (941) DVGYCTDRTCAKWPEWLGVWGQGTLVTVSSGGGGSSGGGSGGGGS
F4-mHSA-F5B6H2   (941) TS----------NAVGFDYWGQGTLVTVSSGGGGSGGGSGGGGSG
H3-mHSA-B1D2     (940) DVGYCTDRTCAKWPEWLGVWGQGTLVTVSSGGGGSSGGGSGGGGS
H3-mHSA-F5B6H2   (940) TSNAVG----------FDYWGQGTLVTVSSGGGGSGGGSGGGGSG

                        991                                       1035
A5-mHSA-ML3.9    (982) QSVLTQPPSVSAAPGQKVTISCSGSSSNIGNNY-VSWYQQLPGTA
A5-mHSA-B1D2     (982) QSVLTQPPSVSAAPGQKVTISCSGSSSNIGNNY-VSWYQQLPGTA
A5-mHSA-F5B6H2   (972) QSVLTQPPSVSGAPGQRVTISCTGRHSNIGLGYGVHWYQQLPGTA
B12-mHSA-B1D2    (986) QSVLTQPPSVSAAPGQKVTISCSGSSSNIGNNY-VSWYQQLPGTA
B12-mHSA-F5B6H2  (976) QSVLTQPPSVSGAPGQRVTISCTGRHSNIGLGYGVHWYQQLPGTA
F4-mHSA-B1D2     (986) QSVLTQPPSVSAAPGQKVTISCSGSSSNIGNNY-VSWYQQLPGTA
F4-mHSA-F5B6H2   (976) QSVLTQPPSVSGAPGQRVTISCTGRHSNIGLGYGVHWYQQLPGTA
H3-mHSA-B1D2     (985) QSVLTQPPSVSAAPGQKVTISCSGSSSNIGNNY-VSWYQQLPGTA
H3-mHSA-F5B6H2   (975) QSVLTQPPSVSGAPGQRVTISCTGRHSNIGLGYGVHWYQQLPGTA

                       1036                                       1080
A5-mHSA-ML3.9   (1026) PKLLIYDHTNRPAGVPDRFSGSKSGTSASLAISGFRSEDEADYYC
A5-mHSA-B1D2    (1026) PKLLIYDHTNRPAGVPDRFSGSKSGTSASLAISGFRSEDEADYYC
A5-mHSA-F5B6H2  (1017) PKLLIYGNTNRPSGVPDRFSGFKSGTSASLAITGLQAEDEADYYC
B12-mHSA-B1D2   (1030) PKLLIYDHTNRPAGVPDRFSGSKSGTSASLAISGFRSEDEADYYC
B12-mHSA-F5B6H2 (1021) PKLLIYGNTNRPSGVPDRFSGFKSGTSASLAITGLQAEDEADYYC
F4-mHSA-B1D2    (1030) PKLLIYDHTNRPAGVPDRFSGSKSGTSASLAISGFRSEDEADYYC
F4-mHSA-F5B6H2  (1021) PKLLIYGNTNRPSGVPDRFSGFKSGTSASLAITGLQAEDEADYYC
H3-mHSA-B1D2    (1029) PKLLIYDHTNRPAGVPDRFSGSKSGTSASLAISGFRSEDEADYYC
H3-mHSA-F5B6H2  (1020) PKLLIYGNTNRPSGVPDRFSGFKSGTSASLAITGLQAEDEADYYC

                       1081                1104
A5-mHSA-ML3.9   (1071) ASWDYTLSGWVFGGGTKLTVLG--
A5-mHSA-B1D2    (1071) ASWDYTLSGWVFGGGTKLTVLG--
A5-mHSA-F5B6H2  (1062) QSYDRRTPGWVFGGGTKLTVLG--
B12-mHSA-B1D2   (1075) ASWDYTLSGWVFGGGTKLTVLG--
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APPENDIX C

ANTICANCER AGENTS

Anticancer agents for combination with B2B3-1	Brand Name(s)	Manufacturer/Proprietor
Anti-IGF1R Antibodies		
AMG 479 (fully humanized mAb)		Amgen
IMCA12 (fully humanized mAb)		ImClone
NSC-742460		Dyax
19D12 (fully humanized mAb)		
CP751-871 (fully humanized mAb)		Pfizer
H7C10 (humanized mAb)		
alphaIR3 (mouse)		
scFV/FC (mouse/human chimera)		
EM/164 (mouse)		
MK-0646, F50035		Pierre Fabre Medicament, Merck
Small Molecules Targeting IGF1R		
NVP-AEW541		Novartis
BMS-536,924 (1H-benzoimidazol-2-yl)-1H-pyridin-2-one)		Bristol-Myers Squibb
BMS-554,417		Bristol-Myers Squibb
Cycloligan		
TAE226		
PQ401		
Anti-EGFR Monoclonal Antibodies		
INCB7839		Incyte
Bevacizumab	Avastin	Genentech
Cetuximab	Erbitux®	IMCLONE
mAb 806		
Matuzumab (EMD72000)		
Nimotuzumab (TheraCIM)		
Panitumumab	Vectibix®	Amgen
Anti-ErbB3 Therapeutics		
U3-1287 / AMG888		U3 Pharma/Amgen
MM-121		Merrimack Pharmaceuticals
Anti-ErbB2 Therapeutics		
Trastuzumab	Herceptin	Genentech
HKI-272	Neratinib	Wyeth

KOS-953	Tanespimycin	Kosan Biosciences
Her/ErbB Dimerization Inhibitors		
2C4, R1273	Pertuzumab, Omintarg	Genentech, Roche
Small Molecules Targeting EGFR		
CI-1033 (PD 183805)		Pzifer, Inc.
EKB-569		
Gefitinib	IRessa®	AstraZeneca
Lapatinib (GW572016)		GlaxoSmithKline
Lapatinib Ditosylate	Tykerb®	SmithKline Beecham
Erlotinib HCl (OSI-774)	Tarceva®	OSI Pharms
PD158780		
PKI-166		Novartis
Tyrphostin AG 1478 (4-(3-Chloroanillino)-6,7-dimethoxyquinazoline)		
Anti-cmet Antibody Therapies		
AVEO (AV299)		AVEO
AMG102		Amgen
5D5 (OA-5D5)		Genentech
Small Molecules Targeting cmet		
PHA665752		
ARQ-650RP		ArQule
ARQ 197		ArQule
Alkylating Agents		
BCNU→ 1 ,3-bis t2-chloroethyl)-nitrosourea		
Bendamustine		
Busulfan	Myleran	GlaxoSmithKline
Carboplatin	Paraplatin	Bristol-Myers Squibb
Carboquone		
Carmustine		
CCNU→ 1,-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (methyl CCNU)		
Chlorambucil	Leukeran®	Smithkline Beecham
Chlormethine		
Cisplatin (Cisplatinum, CDDP)	Platinol	Bristol-Myers
Cyclophosphamide	Cytoxan	Bristol-Myers Squibb
	Neosar	Teva Parenteral
Dacarbazine (DTIC)		

Fotemustine		
Hexamethylmelamine (Altretamine, HMM)	Hexalen®	MGI Pharma, Inc.
Ifosfamide	Mitoxana®	ASTA Medica
Lomustine		
Mannosulfan		
Melphalan	Alkeran®	GlaxoSmithKline
Nedaplatin		
Nimustine		
Oxaliplatin	Eloxatin®	Sanofi-Aventis US
Prednimustine,		
Procarbazine HCL	Matulane	Sigma-Tau Pharmaceuticals, Inc.
Ribonucleotide Reductase Inhibitor (RNR)		
Ranimustine		
Satraplatin		
Semustine		
Streptozocin		
Temozolomide		
Treosulfan		
Triaziquone		
Triethylene Melamine		
ThioTEPA		Bedford, Abraxis, Teva
Triplatin tetranitrate		
Trofosfamide		
Uramustine		
Antimetabolites		
5-azacytidine		
Flourouracil (5-FU)/Capecitabine		
6-mercaptopurine (Mercaptopurine, 6-MP)		
6-Thioguanine (6-TG)	Purinethol®	Teva
Cytosine Arabinoside (Cytarabine, Ara-C)	Thioguanine®	GlaxoSmithKline
Azathioprine	Azasan®	AAIPHARMA LLC
Capecitabine	XELODA®	HLR (Roche)
Cladribine (2-CdA, 2-chlorodeoxyadenosine)	Leustatin®	Ortho Biotech
5-Trifluoromethyl-2'-deoxyuridine		
Fludarabine phosphate	Fludara®	Bayer Health Care
Floxuridine (5-fluoro-2)	FUDR®	Hospira, Inc.
Methotrexate sodium	Trexall	Barr

Pemetrexed	Alimta®	Lilly
Pentostatin	Nipent®	Hospira, Inc.
Raltitrexed	Tomudex®	AstraZeneca
Tegafur		
Aromatose Inhibitor		
Ketoconazole		
Glucocorticoids		
Dexamethasone	Decadron® Dexasone, Diodex, Hexadrol, Maxidex	Wyeth, Inc.
Prednisolone		
Prednisone	Deltasone, Orasone, Liquid Pred, Sterapred®	
Immunotherapeutics		
Alpha interferon		
Angiogenesis Inhibitor	Avastin®	Genentech
IL-12→ Interleukin 12		
IL-2→ Interleukin 2 (Aldesleukin)	Proleukin ®	Chiron
Kinase Inhibitors		
AMG 386		Amgen
Axitinib ((AG-013736)		Pfizer, Inc
Bosutinib (SKI-606)		Wyeth
Brivanib alalinate (BMS-582664)		BMS
Cediranib (AZD2171)	Recentin	AstraVeneca
Dasatinib (BMS-354825)	Sprycel®	Bristol-Myers Squibb
Imatinib mesylate	Gleevec	Novartis
Lestaurtinib (CEP-701)		Cephalon
Motesanib diphosphate (AMG-706)		Amgen/Takeda
Nilotinib hydrochloride monohydrate	Tasigna®	Novartis
Pazopanib HCL (GW786034)	Armala	GSK
Semaxanib (SU5416)		Pharmacia,
Sorafenib tosylate	Nexavar®	Bayer
Sunitinib malate	Sutent®	Pfizer, Inc.
Vandetanib (AZD647)	Zactima	AstraZeneca
Vatalanib; PTK-787		Novartis; Bayer Schering Pharma
XL184, NSC718781		Exelixis, GSK
Microtubule-Targeting Agents		
Docetaxel	Taxotere®	Sanofi-Aventis US
Ixabepilone	IXEMPRA™	Bristol-Myers Squibb

Larotaxel		
Ortataxel		
Nanoparticle paclitaxel (ABI-007)	Abraxane®	Abraxis BioScience, Inc.
Paclitaxel	Taxol®	Bristol-Myers Squibb
Tesetaxel		
Vinblastine sulfate	Velban®	Lilly
Vincristine	Oncovin®	Lilly
Vindesine sulphate	Eldisine®	Lilly
Vinflunine		
Vinorelbine tartrate	Navelbine®	Pierre Fabre
mTOR Inhibitors		
Deforolimus (AP23573, MK 8669)		ARIAD Pharmaceuticals, Inc
Everolimus (RAD001, RAD001C)	Certican®, Afinitor	Novartis
Sirolimus (Rapamycin)	Rapamune®	Wyeth Pharama
Temsirolimus (CCI-779)	Torisel®	Wyeth Pharama
Protein Synthesis Inhibitor		
L-asparaginase	Elspar®	Merck & Co.
Somatostatin Analogue		
Octreotide acetate	Sandostatin®	Novartis
Topoisomerase Inhibitors		
Actinomycin D		
Camptothecin (CPT)		
Belotecan		
Daunorubicin citrate	Daunoxome®	Gilead
Doxorubicin hydrochloride	Doxil®	Alza
Etoposide	Vepesid®	Bristol-Myers Squibb
	Etopophos	Hospira, Bedford, Teva Parenteral, Etc.
Irinotecan HCL (CPT-11)	Camptosar®	Pharmacia & Upjohn
Mitoxantrone HCL	Novantrone	EMD Serono
Rubitecan		
Teniposide (VM-26)	Vumon®	Bristol-Myers Squibb
Topotecan HCL	Hycamtin®	GlaxoSmithKline

Chemotherapeutic Agents Adriamycin, 5-Fluorouracil, Cytoxin, Bleomycin, Mitomycin C, Daunomycin, Carminomycin, Aminopterin, Dactinomycin, Mitomycins, Esperamicins Clofarabine, Mercaptopurine, Pentostatin, Thioguanine, Cytarabine, Decitabine, Floxuridine, Gemcitabine (Gemzar), Enocitabine, Sapacitabine		
Hormonal Therapy		
Abarelix	Plenaxis™	Amgen
Abiraterone acetate	CB7630	BTG plc
Afimoxifene	TamoGel	Ascend Therapeutics, Inc.
Anastrazole	Arimidex®	AstraZeneca
Aromatase inhibitor	Atamestane plus toremifene	Intarcia Therapeutics, Inc.
	Arzoxifene	Eli Lilly & Co.
Asentar; DN-101		Novartis; Oregon Health & Science Univ.
Bicalutamide	Casodex®	AstraZeneca
Buserelin	Suprefact®	Sanofi Aventis
Cetrorelix	Cetrotide®	EMD Serono
Exemestane	Aromasin®	Pfizer
Exemestane	Xtane	Natco Pharma, Ltd.
Fadrozole (CGS 16949A)		
Flutamide	Eulexin®	Schering
Flutamide	Prostacur	Laboratorios Almirall, S.A.
Fulvestrant	Faslodex®	AstraZeneca
Goserelin acetate	Zoladex®	AstraZeneca
Letrozole	Femara®	Novartis
Letrozole (CGS20267)	Femara	Chugai Pharmaceutical Co., Ltd.
Letrozole	Estrochek	Jagsonpal Pharmaceuticals, Ltd.
Letrozole	Letrozole	Indchemie Health Specialities
Leuprolide acetate	Eligard®	Sanofi Aventis
Leuprolide acetate	Leopril	VHB Life Sciences, Inc.
Leuprolide acetate	Lupron®/Lupron Depot	TAP Pharma
Leuprolide acetate	Viador	Bayer AG
Megestrol acetate	Megace®	Bristol-Myers Squibb
Magestrol acetate	Estradiol Valerate (Delestrogen)	Jagsonpal Pharmaceuticals, Ltd.
Medroxyprogesterone acetate	Veraplex	Combiphar

MT206		Medisyn Technologies, Inc.
Nafarelin		
Nandrolone decanoate	Zestabolin	Mankind Pharma, Ltd.
Nilutamide	Nilandron®	Aventis Pharmaceuticals
Raloxifene HCL	Evista®	Lilly
Tamoxifen	Taxifen	Yung Shin Pharmaceutical
Tamoxifen	Tomifen	Alkem Laboratories, Ltd.
Tamoxifen citrate	Nolvadex	AstraZeneca
Tamoxifen citrate	Soltamox	EUSA Pharma, Inc.
Tamoxifen citrate	Tamoxifen citrate SOPHARMA	Sopharma JSCo.
Toremifene citrate	Fareston®	GTX, Inc.
Triptorelin pamoate	Trelstar®	Watson Labs
Triptorelin pamoate	Trelstar Depot	Paladin Labs, Inc.
Protein Kinase B (PKB) Inhibitor		
Akt Inhibitor ASTEX		Astex Therapeutics
Akt Inhibitors NERVIANO		Nerviano Medical Sciences
AKT Kinase Inhibitor TELIK		Telik, Inc.
AKT DECIPHERA		Deciphera Pharmaceuticals, LLC
Perifosine (KRX0401, D-21266)		Keryx Biopharmaceuticals, Inc., AEterna Zentaris, Inc.
Perifosine with Docetaxel		Keryx Biopharmaceuticals, Inc., AEterna Zentaris, Inc.
Perifosine with Gemcitabine		AEterna Zentaris, Inc.
Perifosine with Paclitaxel		Keryx Biopharmaceuticals, Inc, AEterna Zentaris, Inc.
Protein Kinase-B inhibitor DEVELOGEN		DeveloGen AG
PX316		Oncothyreon, Inc.
RX0183		Rexahn Pharmaceuticals, Inc.
RX0201		Rexahn Pharmaceuticals, Inc.
VQD002		VioQuest Pharmaceuticals, Inc.
XL418		Exelixis, Inc.
ZEN027		AEterna Zentaris, Inc.
Phosphatidylinositol 3-Kinase (PI3K) Inhibitor		
BEZ235		Novartis AG
BGT226		Novartis AG
CAL101		Calistoga Pharmaceuticals, Inc.
CHR4432		Chroma Therapeutics, Ltd.
Erk/PI3K Inhibitors ETERNA		AEterna Zentaris, Inc.

GDC0941		Genentech Inc./Piramed Limited/Roche Holdings, Ltd.
Enzastaurin HCL (LY317615)	Enzastaurin	Eli Lilly
LY294002/Wortmannin		
PI3K Inhibitors SEMAFORE		Semafore Pharmaceuticals
PX866		Oncothyreon, Inc.
SF1126		Semafore Pharmaceuticals
VMD-8000		VM Discovery, Inc.
XL147		Exelixis, Inc.
XL147 with XL647		Exelixis, Inc.
XL765		Exelixis, Inc.
PI-103		Roche/Piramed
Cyclin-dependent kinase inhibitors		
CYC200, r-roscovitine	Seliciclib	Cyclacel Pharma
NSC-649890, L86-8275, HMR-1275	Alvocidib	NCI
TLr9, CD289		
IMOxine		Merck KGaA
HYB2055		Idera
IMO-2055		Isis Pharma
1018 ISS		Dynavax Technologies/UCSF
PF-3512676		Pfizer
Enzyme Inhibitor		
Lonafarnib (SCH66336)	Sarasar	SuperGen, U Arizona
Anti-TRAIL		
AMG-655		Aeterna Zentaris, Keryx Biopharma
Apo2L/TRAIL, AMG951		Genentech, Amgen
Apomab (fully humanized mAb		Genentech

Anticancer agents for combination with B2B3-1	Target	Manufacturer/Proprietor
Other		
Imprime PGG		Biothera
CHR-2797	AminopeptidaseM1	Chroma Therapeutics
E7820, NSC 719239	Integrin-alpha2	Eisai
INCB007839	ADAM 17, TACE	Incyte
CNF2024, BIIB021	Hsp90	Biogen Idec
MP470, HPK-56	Kit/Met/Ret	Shering-Plough

SNDX-275/MS-275	HDAC	Syndax
Zarnestra, Tipifarnib, R115777	Ras	Janssen Pharma
Volociximab; Eos 200-4, M200	alpha581 integrin	Biogen Idec; Eli Lilly/UCSF/PDL BioPharma
Apricoxib (TP2001)	COX-2 Inhibitor	Daiichi Sankyo; Tragara Pharma

Other Embodiments

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure that come within known or customary practice within the art to which the invention pertains and may be applied to the essential features hereinbefore set forth.

All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each independent publication or patent application was specifically and individually indicated to be incorporated by reference in their entirety.

What is claimed is:

Claims

1. An agent comprising a human serum albumin (HSA) linker comprising an amino acid sequence having at least 95% sequence identity to the sequence set forth in SEQ ID NO: 1 and comprising a first binding moiety bonded to the amino terminus of said HSA linker and a second binding moiety bonded to the carboxy terminus of said HSA linker, wherein each of said first and second binding moieties specifically binds ErbB2 or ErbB3 and wherein:

i) if said first binding moiety specifically binds ErbB3, said second binding moiety specifically binds ErbB2; and

ii) if said first binding moiety specifically binds ErbB2, said second binding moiety specifically binds ErbB3.

2. The agent of claim 1, wherein said amino acid sequence comprises the amino acid sequence of SEQ ID NO: 1.

3. The agent of claim 1 or 2 further comprising polypeptide connectors at the amino and/or carboxy terminus of said HSA linker that covalently bond said first and/or second binding moiety to said HSA linker, wherein preferably each of said polypeptide connectors comprises 2 to 20 amino acid residues.

4. The agent of any one of claims 1 to 3, wherein said first binding moiety specifically binds ErbB2 and said second binding moiety specifically binds ErbB3.

5. The agent of any one of claims 1 to 3, wherein said first binding moiety specifically binds ErbB3 and said second binding moiety specifically binds ErbB2.

6. The agent of any one of claims 1 to 5, wherein said first or second binding moiety is a single-chain Fv molecule.

7. The agent of claim 6, wherein said single-chain Fv molecule is human or humanized.

8. The agent of claim 5, wherein said first binding moiety binds ErbB3 with a K_d in the range of about 1 nm to about 50 nm.

9. The agent of claim 5, wherein said second binding moiety binds ErbB2 with a K_d in the range of about 0.01 nm to about 10 nm.

10. The agent of claim 8, wherein said K_d is about 16 nM.

11. The agent of claim 9, wherein said K_d is about 0.3 nM.

12. The agent of any one of claims 1 to 11, wherein said first binding moiety has the amino acid sequence of SEQ ID NO: 26 and said second binding moiety has the amino acid sequence of SEQ ID NO: 27.

13. The agent of any one of claims 1 to 11, wherein said first binding moiety specifically binds ErbB2 and is an scFv comprising the amino acid sequence of any one of SEQ ID NOs: 27, 29, and 32 and wherein said second binding moiety specifically binds ErbB3 and is an scFV comprising the sequence of any one of SEQ ID NOs: 26, 28, 30, and 31.

14. The agent of any one of claims 1 to 11, wherein said first binding moiety specifically binds ErbB3 and is an scFv comprising the amino acid sequence of any one of SEQ ID NOs: 26, 28, 30, and 31 and wherein said second binding moiety specifically binds ErbB2 and is an scFV comprising the sequence of any one of SEQ ID NOs: 27, 29, and 32.

15. The agent of any one of claims 1 to 14, wherein said agent is capable of binding to and killing a tumor cell or inhibiting tumor cell proliferation.

16. The agent of any one of claims 1 to 15, wherein said agent exhibits an *in vivo* half-life of between 6 hours and 7 days.

17. The agent of any one of claims 1 to 15, wherein said agent exhibits an *in vivo* half-life of greater than 8 hours.

18. The agent of any one of claims 1 to 17, wherein said agent is formulated for administration with one or more therapeutic agents selected from the group consisting of cyclophosphamide, camptothecin, homocamptothecin, colchicine, combrestatin, combrestatin, rhizoxin, dolistatin, ansamitocin p3, maytansinoid, auristatin, caleachimicin, methotrexate, 5-fluorouracil (5-FU), doxorubicin, paclitaxel, docetaxel, cisplatin, carboplatin, tamoxifen, raloxifene, letrozole, epirubicin, bevacizumab, pertuzumab, trastuzumab.

19. The agent of any one of claims 1 to 18, wherein said HSA linker comprises amino acid residues 25-44 and amino acid residues 494-513 of SEQ ID NO: 1.

20. The agent of claim 19, wherein said HSA linker comprises amino acid residues 25-70 and amino acid residues 450-513 of SEQ ID NO: 1.

21. The agent of claim 20, wherein said HSA linker comprises amino acid residues 15-100 and amino acid residues 400-520 of SEQ ID NO: 1.

22. The agent of claim 21, wherein said HSA linker comprises amino acid residues 10-200 and amino acid residues 300-575 of SEQ ID NO: 1.

23. The agent of claim 22, wherein said HSA linker comprises amino acid residues 5-250 and 275-580 of SEQ ID NO: 1.

24. The agent of any one of claims 1 to 23, wherein said agent is admixed with a pharmaceutically acceptable carrier, excipient, or diluent.

25. The agent of any one of claims 1 to 24, wherein the agent remains stable, retaining comparable ability to bind both ErbB2 and ErbB3 before and after incubation in human serum at 37°C for 120 hours.

26. A kit comprising the agent of any one of claims 1 to 24 and instructions for administering said agent to a patient.

27. Use of the agent of any one of claims 1 to 25 in the manufacture of a medicament for treating a mammal having a proliferative disease.

28. The use of claim 27, wherein said proliferative disease is associated with cellular signaling through a cell surface receptor.

29. The use of claim 27, wherein said proliferative disease is melanoma, clear cell sarcoma, head and neck cancer, bladder cancer, breast cancer, colon cancer, ovarian cancer, endometrial cancer, gastric cancer, pancreatic cancer, renal cancer, prostate cancer, salivary gland cancer, lung cancer, liver cancer, skin cancer, or brain cancer.

30. The use of any one of claims 27 to 29, wherein said agent is formulated for administration with one or more therapeutic agents selected from the group consisting of cyclophosphamide, camptothecin, homocamptothecin, colchicine, combrestatin, rhizoxin, dolistatin, ansamitocin p3, maytansinoid, auristatin, caleachimicin, methotrexate, 5-fluorouracil (5-FU), doxorubicin, paclitaxel, docetaxel, cisplatin, carboplatin, tamoxifen, raloxifene, letrozole, epirubicin, bevacizumab, pertuzumab, trastuzumab.

31. The use of claim 30, wherein said agent is formulated for administration with trastuzumab.

32. The use of any one of claims 27 to 31, wherein said mammal is a human.

33. A method for making the agent of any one of claims 1 to 25, comprising expressing a polynucleotide molecule encoding said agent in a bacterial, insect, yeast, or mammalian cell, or in a non-human mammalian tissue, organ, or organism.

34. A medicament comprising the agent of any one of claims 1 to 25 admixed with a pharmaceutically acceptable carrier, excipient, or diluent.

35. The medicament of claim 34 for use in a method for treating a mammal having a proliferative disease.

36. The medicament of claim 34, wherein said proliferative disease is associated with cellular signaling through a cell surface receptor.

37. The medicament of claim 35 or 36, wherein said proliferative disease is a solid tumor.

38. The medicament of claim 35 or 36, wherein said proliferative disease is melanoma, clear cell sarcoma, head and neck cancer, bladder cancer, breast cancer, colon cancer, ovarian cancer, endometrial cancer, gastric cancer, pancreatic cancer, renal cancer, prostate cancer, salivary gland cancer, lung cancer, liver cancer, skin cancer, or brain cancer.

39. The medicament of any one of claims 34 to 38, wherein said use comprises administration in combination with one or more therapeutic agents selected from the group consisting of cyclophosphamide, camptothecin, homocamptothecin, colchicine, combrestatin, rhizoxin, dolistatin, ansamitocin p3, maytansinoid, auristatin, caleachimicin, methotrexate, 5-

fluorouracil (5-FU), doxorubicin, paclitaxel, docetaxel, cisplatin, carboplatin, tamoxifen, raloxifene, letrozole, epirubicin, bevacizumab, pertuzumab, trastuzumab, natalizumab, infliximab, adalimumab, rituximab, alemtuzumab, daclizumab, efalizumab, golimumab, certolizumab, abatacept, etanercept, cetuximab, panitumumab, anakinra, daunorubicin, etoposide, irinotecan, mitoxantrone, teniposide, topotecan, gefitinib, lapatinib, lapatinib ditosylate, erlotinib, neratinib, tanespimycin, busulfan, chlorambucil, hexamethylmelamine, ifosfamide, melphalan, procarbazine, 6-thioguanine, cytosine arabinoside, azathioprine, capecitabine, cladribine, fludarabine, floxuridine, pemetrexed, pentostatin, raltitrexed, dexamethasone, prednisone, aldesleukin, cediranib, dasatinib, imatinib, nilotinib, pazopanib, sorafenib, sunitinib, vandetanib, ixabepilone, nanoparticle paclitaxel, vinblastine, vincristine, vindesine, vinorelbine, everolimus, sirolimus, temsirolimus, l-asparaginase, octreotide, abarelix, afimoxifene, anastrozole, bicalutamide, buserelin, cetrorelix, exemestane, flutamide, fulvestrant, goserelin, leuprolide, megestrol, estradiol, medroxyprogesterone, nandrolone, nilutamide, toremifene, triptorelin pamoate, enzastaurin, roscovitine, alvocidib, and lonafarnib.

40. The medicament of claim 39, wherein said one or more therapeutic agent is trastuzumab.

41. The medicament of any one of claims 34 to 40, wherein said mammal is a human.