

CLAIMS

1. A protein comprising a first moiety and a second moiety and a peptide linker between the first moiety and the second moiety,

wherein the first moiety comprises a Fab, wherein the first moiety is capable of specifically binding to a molecule expressed in a disease tissue;

wherein the peptide linker is cleavable by a protease expressed in a diseased tissue;

wherein the second moiety comprises a Fab, wherein the second moiety is capable of specifically binding to a molecule expressed in the diseased tissue;

wherein the protein comprises from N-terminus to C-terminus: the first moiety, the peptide linker, and the second moiety;

wherein the binding of the second moiety to the molecule expressed in the diseased tissue is reduced or inhibited when the peptide linker is uncleaved; and

wherein the peptide linker comprises an amino acid sequence of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:81, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:86, or SEQ ID NO:87.

2. The protein of claim 1, wherein the peptide linker is between 5 and 15 amino acids in length.

3. The protein of claim 1 or 2, wherein the protease is a human matrix metalloprotease (MMP), a human cathepsin, a human enterokinase, a human thrombin, a human tPA, a human Granzyme B, a human uPA, or a human ADAMTs-5.

4. The protein of any one of claims 1-3, wherein the peptide linker comprises a human MMP cleavage site, a human cathepsin cleavage site, a human enterokinase cleavage site, a human thrombin cleavage site, a human tPA cleavage site, a human Granzyme B cleavage site, a human uPA cleavage site, or a human ADAMTs-5 cleavage site.

5. The protein of claim 3 or 4, wherein the human MMP is MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-10, MMP-12, MMP-13 or MMP14.

6. The protein of any one of claims 3-5, wherein the human cathepsin is Cathepsin A, Cathepsin C, Cathepsin D, Cathepsin G, Cathepsin L or Cathepsin K.

7. The protein of any one of claims 1-6, wherein the first moiety specifically binds to a first molecule expressed in a diseased tissue and the second moiety is capable of specifically binding to a second molecule expressed in a diseased tissue, wherein the first molecule expressed in a diseased tissue and the second molecule expressed in a diseased tissue are different molecules; optionally wherein:

(a) the first molecule expressed in a diseased tissue and the second molecule expressed in a diseased tissue are expressed by the same cell;

(b) the first molecule expressed in a diseased tissue and the second molecule expressed in a diseased tissue are expressed by different cells;

(c) the first molecule expressed in a diseased tissue and/or the second molecule expressed in a diseased tissue is expressed on the surface of a cell; or

(d) the first molecule expressed in a diseased tissue and/or the second molecule expressed in a diseased tissue is a soluble molecule.

8. The protein of any one of claims 1-7, wherein the first moiety binds specifically to human EGFR, human HER2, human HER3, human CD105, human C-KIT, human PD1, human PD-L1, human PSMA, human EpCAM, human Trop2, human EphA2, human CD20, human BCMA, human GITR, human OX40, human CSF1R, human Lag3 or human cMET; or

the second moiety binds specifically to human CD3, human CD16A, human CD16B, human CD28, human CD89, human CTLA4, human NKG2D, human SIRP α , human SIRP γ , human PD1, human Lag3, human 4-1BB, human OX40, or human GITR.

9. The protein of any one of claims 1-8, wherein the first moiety comprises a heavy chain variable (VH) region and a light chain variable (VL) region.

10. The protein of any one of claims 1-9, wherein the first moiety comprises an immunoglobulin constant region or a portion of an immunoglobulin constant region; optionally wherein the immunoglobulin constant region:

(a) is IgG, IgE, IgM, IgD, IgA, or IgY; or

(b) is IgG1, IgG2, IgG3, IgG4, IgA1, or IgA2; or

(c) is immunologically inert; or

(d) is a wild-type human IgG4 constant region, a human IgG4 constant region comprising the amino acid substitution S228P, a wild-type human IgG1 constant region, a human IgG1 constant region comprising the amino acid substitutions L234A and L235A, a human IgG1 constant region comprising the amino acid substitutions L234A, L235A and G237A, a human IgG1 constant region comprising the amino acid substitutions L234A, L235A, G237A and P331S, or a wild-type human IgG2 constant region.

11. The protein of any one of claims 1-10, wherein the second moiety comprises a heavy chain variable (VH) region and a light chain variable (VL) region.

12. The protein of any one of claims 1-11, wherein the second moiety comprises an immunoglobulin constant region or a portion of an immunoglobulin constant region; optionally wherein the immunoglobulin constant region:

(a) is IgG, IgE, IgM, IgD, IgA or IgY; or

(b) is IgG1, IgG2, IgG3, IgG4, IgA1, or IgA2; or

(c) is immunologically inert; or

(d) is a wild-type human IgG4 constant region, a human IgG4 constant region comprising the amino acid substitution S228P, a wild-type human IgG1 constant region, a human IgG1 constant region comprising the amino acid substitutions L234A and L235A, a human IgG1 constant region comprising the amino acid substitutions L234A, L235A and G237A, a human IgG1 constant region comprising the amino acid substitutions L234A, L235A, G237A and P331S, or a wild-type human IgG2 constant region.

13. The protein of claim 1, wherein the protein has an immune effector function or two, three or more immune effector functions; optionally wherein the immune effector function is ADCC, CDC or ADCP.

14. The protein of any one of claims 1-13, wherein the first moiety prevents or reduces specific binding of the second moiety to the molecule expressed in the diseased tissue.

15. The protein of any one of claims 1-14, wherein the peptide linker is cleaved in the vicinity of the diseased tissue or inside the diseased tissue; optionally wherein the diseased tissue is a tumour or an inflamed tissue.

16. The protein of claim 1, wherein:

(a) the first moiety binds specifically to human cMET, wherein the second moiety binds specifically to human CD47, and wherein the protein comprises a first polypeptide chain comprising or consisting of the amino acid sequence of SEQ ID NO:16 and a second polypeptide chain comprising or consisting of the amino acid sequence of SEQ ID NO:17; or

(b) the first moiety binds specifically to human HER2, wherein the second moiety binds specifically to human CD3, and wherein the protein comprises a first polypeptide chain comprising or consisting of the amino acid sequence of SEQ ID NO:26 and a second polypeptide chain comprising or consisting of the amino acid sequence of SEQ ID NO:27; or

(c) the first moiety binds specifically to human HER2, wherein the second moiety binds specifically to human CD47, and wherein the protein comprises a first polypeptide chain comprising or consisting of the amino acid sequence of SEQ ID NO:34 and a second polypeptide chain comprising or consisting of the amino acid sequence of SEQ ID NO:35; or

(d) the first moiety binds specifically to human cMET, wherein the second moiety binds specifically to human CD47, and wherein the protein comprises a first polypeptide chain comprising or consisting of the amino acid sequence of SEQ ID NO:36 and a second polypeptide chain comprising or consisting of the amino acid sequence of SEQ ID NO:37; or

(e) the first moiety binds specifically to human Her2, wherein the second moiety binds specifically to human CD3, and wherein the protein comprises a first polypeptide chain comprising or consisting of the amino acid sequence of SEQ ID NO:38 and a second polypeptide chain comprising or consisting of the amino acid sequence of SEQ ID NO:39; or

(f) the first moiety binds specifically to human Her2, wherein the second moiety binds specifically to human CD3, and wherein the protein comprises a first polypeptide chain comprising or consisting of the amino acid sequence of SEQ ID NO:73 and a second polypeptide chain comprising or consisting of the amino acid sequence of SEQ ID NO:74; or

(g) the first moiety binds specifically to human cMET, wherein the second moiety binds specifically to human cMET, and wherein the protein comprises a first polypeptide chain comprising or consisting of the amino acid sequence of SEQ ID NO:75 and a second polypeptide chain comprising or consisting of the amino acid sequence of SEQ ID NO:76; or

(f) the first moiety binds specifically to human Her2, wherein the second moiety binds specifically to human CD3, and wherein the protein comprises a first polypeptide chain comprising or consisting of the amino acid sequence of SEQ ID NO:40 and a second polypeptide chain comprising or consisting of the amino acid sequence of SEQ ID NO:41.

17. The protein of claim 1, wherein the first moiety binds specifically to human Her2, wherein the second moiety binds specifically to human CD47, and wherein the protein comprises a first polypeptide chain and a second polypeptide chain, wherein:

(a) the first polypeptide chain comprises or consists of the amino acid sequence of SEQ ID NO:42, and the second polypeptide chain comprises or consists of the amino acid sequence of SEQ ID NO:43; or

(b) the first polypeptide chain comprises or consists of the amino acid sequence of SEQ ID NO:44, and the second polypeptide chain comprises or consists of the amino acid sequence of SEQ ID NO:45; or

(c) the first polypeptide chain comprises or consists of the amino acid sequence of SEQ ID NO:46, and the second polypeptide chain comprises or consists of the amino acid sequence of SEQ ID NO:47; or

(d) the first polypeptide chain comprises or consists of the amino acid sequence of SEQ ID NO:48, and the second polypeptide chain comprises or consists of the amino acid sequence of SEQ ID NO:49; or

(e) the first polypeptide chain comprises or consists of the amino acid sequence of SEQ ID NO:50, and the second polypeptide chain comprises or consists of the amino acid sequence of SEQ ID NO:51; or

(f) the first polypeptide chain comprises or consists of the amino acid sequence of SEQ ID NO:52, and the second polypeptide chain comprises or consists of the amino acid sequence of SEQ ID NO:53; or

(g) the first polypeptide chain comprises or consists of the amino acid sequence of SEQ ID NO:54, and the second polypeptide chain comprises or consists of the amino acid sequence of SEQ ID NO:55; or

(h) the first polypeptide chain comprises or consists of the amino acid sequence of SEQ ID NO:88, and the second polypeptide chain comprises or consists of the amino acid sequence of SEQ ID NO:89; or

(i) the first polypeptide chain comprises or consists of the amino acid sequence of SEQ ID NO:90, and the second polypeptide chain comprises or consists of the amino acid sequence of SEQ ID NO:91; or

(j) the first polypeptide chain comprises or consists of the amino acid sequence of SEQ ID NO:44, and the second polypeptide chain comprises or consists of the amino acid sequence of SEQ ID NO:92; or

(k) the first polypeptide chain comprises or consists of the amino acid sequence of SEQ ID NO:44, and the second polypeptide chain comprises or consists of the amino acid sequence of SEQ ID NO:93; or

(l) the first polypeptide chain comprises or consists of the amino acid sequence of SEQ ID NO:44, and the second polypeptide chain comprises or consists of the amino acid sequence of SEQ ID NO:94; or

(m) the first polypeptide chain comprises or consists of the amino acid sequence of SEQ ID NO:44, and the second polypeptide chain comprises or consists of the amino acid sequence of SEQ ID NO:95; or

(n) the first polypeptide chain comprises or consists of the amino acid sequence of SEQ ID NO:44, and the second polypeptide chain comprises or consists of the amino acid sequence of SEQ ID NO:96; or

(o) the first polypeptide chain comprises or consists of the amino acid sequence of SEQ ID NO:44, and the second polypeptide chain comprises or consists of the amino acid sequence of SEQ ID NO:97.

18. The protein of claim 1, wherein the first moiety binds specifically to human Her2, wherein the second moiety binds specifically to human CD3, and wherein the protein comprises a first polypeptide chain and a second polypeptide chain, wherein:

(a) the first polypeptide chain comprises or consists of the amino acid sequence of SEQ ID NO:98, and the second polypeptide chain comprises or consists of the amino acid sequence of SEQ ID NO:99; or

(b) the first polypeptide chain comprises or consists of the amino acid sequence of SEQ ID NO:100, and the second polypeptide chain comprises or consists of the amino acid sequence of SEQ ID NO:101; or

(c) the first polypeptide chain comprises or consists of the amino acid sequence of SEQ ID NO:102, and the second polypeptide chain comprises or consists of the amino acid sequence of SEQ ID NO:103; or

(d) the first polypeptide chain comprises or consists of the amino acid sequence of SEQ ID NO:104, and the second polypeptide chain comprises or consists of the amino acid sequence of SEQ ID NO:105.

19. An immunoconjugate comprising the protein of any one of claims 1-18 linked to a therapeutic agent; optionally wherein the therapeutic agent is a cytotoxin, a radioisotope, a chemotherapeutic agent, an immunomodulatory agent, an anti-angiogenic agent, an antiproliferative agent, a pro-apoptotic agent, a cytostatic enzyme, a cytolytic enzymes, a therapeutic nucleic acid, an anti-angiogenic agent, an anti-proliferative agent, or a pro-apoptotic agent.

20. A pharmaceutical composition comprising the protein of any one of claims 1-18 or the immunoconjugate of claim 19, and a pharmaceutically acceptable carrier, diluent or excipient.

21. A nucleic acid molecule encoding the protein of any one of claims 1-18.

22. The protein of any one of claims 1-18, the immunoconjugate of claim 19, or the pharmaceutical composition of claim 20 for use in therapy.

23. Use of the protein of any one of claims 1-18, the immunoconjugate of claim 19, or the pharmaceutical composition of claim 20, in the manufacture of a medicament for the treatment of cancer, an autoimmune disease, an inflammatory disease, a cardiovascular disease or a fibrotic disease in a subject, wherein the medicament is to be administered to the subject.

24. The use of claim 23, wherein:

(a) the cancer is Gastrointestinal Stromal cancer (GIST), pancreatic cancer, skin cancer, melanoma, breast cancer, lung cancer, bronchial cancer, colorectal cancer, prostate cancer, stomach cancer, ovarian cancer, urinary bladder cancer, brain or central nervous system cancer, peripheral nervous system cancer, esophageal cancer, cervical cancer, uterine or endometrial cancer, cancer of the oral cavity or pharynx, liver cancer, kidney cancer, testicular cancer, biliary tract cancer, small bowel or appendix cancer, salivary gland cancer, thyroid gland cancer, adrenal gland cancer, osteosarcoma, chondrosarcoma or cancer of hematological tissues;

(b) the autoimmune disease or the inflammatory disease is arthritis, asthma, multiple sclerosis, psoriasis, Crohn's disease, inflammatory bowel disease, lupus, Grave's disease, Hashimoto's thyroiditis or ankylosing spondylitis;

(c) the cardiovascular disease is coronary heart disease, atherosclerosis, or stroke; or

(d) the fibrotic disease is myocardial infarction, angina, osteoarthritis, pulmonary fibrosis, cystic fibrosis, bronchitis or asthma.