

BISPECIFIC ANTIBODY-LIKE PROTEIN AND METHODS OF MAKING AND USING THEREOF**CROSS REFERENCE TO RELATED APPLICATIONS**

[0001] This application claims the benefit of the filing date of U.S. Provisional Application Ser. No. 63/604,077 filed November 29, 2023, under 35 U.S.C. 119(e), the entire disclosures of which are incorporated by reference herein.

TECHNICAL FIELD

[0002] The present disclosure generally relates to the technical field of antibody therapy for treating cancer, and more particularly relates to bispecific antibody-like proteins.

BACKGROUND

[0003] The human epidermal growth factor receptor (EGFR, also known as ErbB1, HER1) family has four members, EGFR, HER2, HER3, and HER4. Deregulation of each member by means of mutation, amplification, and overexpression plays an important role in tumorigenesis and metastasis in a wide variety of tumors. Interruption of EGFR family signaling, either by blocking binding sites on the extracellular domain of the receptor or by inhibiting intracellular tyrosine kinase activity, can prevent the growth of EGFR-family-expressing tumors and improve the patient's condition. For example, HER2 overexpression occurs in 30% of breast cancer patients, indicative of increased disease recurrence and a poor prognosis. HER2 overexpression is also known to occur in stomach, ovarian, and gastric cancer, adenocarcinoma of lung, aggressive forms of uterine cancer, and salivary duct carcinomas. HER2 mutations have been found in non-small-cell lung cancers. The underlying HER2 mutation and amplification produce aberrant growth signals that activate its downstream signaling pathway leading to tumorigenesis. Specifically, HER2 dimerizes with HER3 on the surface of tumor cells, which activates PI3K/AKT signalling that promotes tumor growth and survival.

[0004] Several therapeutic antibodies and small-molecule inhibitors directed against EGFR and HER2 have been approved for use in the treatment of cancer (Arteaga et al. 2012). The examples of therapeutic anti-HER2 antibodies include Trastuzumab and Pertuzumab that are approved for treating several forms of cancers, including breast cancer and gastric cancer (<https://www.herceptin.com/hcp/treating-HER2-cancer.html>). In particular, the monoclonal antibodies against either EGFR or HER2 have demonstrated good clinical responses in colon cancer (Price et al. 2014), squamous cell carcinoma of head and neck (Cohen, 2014), breast and gastric cancers (Arteaga et al. 2012).

[0005] Trastuzumab (Herceptin) and other agents targeting HER2 exerts antitumor efficacy in patients with HER2-expressing breast cancer and stomach cancer. Trastuzumab is a monoclonal antibody that binds to HER2 and the binding increases the activity of p27, a protein that halts cell proliferation. Trastuzumab is effective only in cancers where HER2 is overexpressed. One year of Trastuzumab therapy is recommended for all patients with HER2-positive breast cancer who are also receiving chemotherapy, and there is no additional benefit beyond 12 months.

Pertuzumab is another monoclonal antibody capable of inhibiting dimerization of HER2 with other receptors, such as HER3, and is a FDA-approved therapeutics for use in combination with Trastuzumab and Docetaxel, a chemotherapeutic agent, for the treatment of metastatic HER2-positive breast cancer (Durkee et al. 2016).

[0006] Despite of these success, the long-term benefit seems to be limited in some patients. Many tumors that initially respond to these therapeutic agents eventually progress due to an acquired resistance to the agents. The development of drug resistance reduces the efficacy of these treatments. In the case of HER2-targeted therapies, the resistance can occur via upregulation of HER3 or its ligand HRG. These findings imply that the current therapeutic approaches aiming at inhibiting the activation of HER2/HER3 signalling pathway have failed to provide meaningful clinical benefit (Geuijen et al. 2018; Yu et al. 2019).

[0007] In summary, currently approved antibody therapies targeting HER2 and/or HER3 have disadvantages of either a low response rate to the treatment or the patient developing resistance to the treatment. There is a need of a better treatment for these cancers.

SUMMARY

[0008] The present application generally relates to the technical field of immunotherapy, and more specifically relates to antibody therapeutic agents, and more particularly relates to antibody-like proteins against specific epitopes of HER2 and HER3. HER2 and HER3 often form a partnership in promoting cellular transformation that may ultimately leads to tumorigenesis and tumor metastasis. The application provides, among others, antibody-like proteins, the immunoconjugates of such antibody-like proteins, the method of making antibody-like proteins or their immunoconjugates, pharmaceutical compositions including the antibody-like proteins and/or their immunoconjugates, the method of using such antibody-like proteins or their immunoconjugates for treating diseases including, for example, cancers.

[0009] In one aspect, the application provides an antibody-like protein having a N terminal and a C terminal. In one embodiment, the protein includes a Fab region and a Fc region. The Fc region comprise a first Fc domain linked to a second Fc domain through a second hinge. The first Fc domain may pair with the second Fc domain to form the Fc region.

[0010] In one embodiment, the second hinge connecting the first Fc domain and the second Fc domain comprises a flexible GS linker having from about 10 to about 100, from about 20 to about 80, from about 20 to about 40 amino acids. In one embodiment, the second hinge comprises an amino acid sequence ((Gly-Gly-Gly-Gly-Ser)_n), and wherein n is an integer of at least 5. In one embodiment, n is from 5 to 10, from 6 to 12, from 5 to 20, or 5 to 15. In one embodiment, n is 5, 6, 7, 8, 9, 10, 11, 12, 15, 20. In one embodiment, n is 6.

[0011] In one embodiment, the antibody-like protein comprises a light chain and a heavy chain. The light chain comprises, from the N-terminus to the C-terminus, a VL domain and a CL domain. The heavy chain comprises, from the N-terminus to the C-terminus, a VH domain, a CH1 domain,

a first hinge, and the Fc region. In one embodiment, the VL domain and VH domain form the Fab region.

[0012] In one embodiment, the light chain and the heavy chain are covalently paired through at least one disulfide bond between the CH1 domain and the CL domain. In one embodiment, the CL domain may be C κ or C λ .

[0013] In one embodiment, the first Fc domain comprises a first CH2 domain and a first CH3 domain. In one embodiment, the second Fc domain comprises a second CH2 domain and a second CH3 domain. In one embodiment, the first Fc domain and the second Fc domain are covalently paired through 2 disulfide bonds between the first hinge and the second hinge.

[0014] The antibody-like protein may be monospecific, bispecific, or multispecific.

[0015] In one embodiment, the antibody-like protein may further comprise a first scFv domain having a scFv VH domain and a scFv VL domain. The first scFv domain may be linked, through a linker, to the heavy chain at the N terminal, the heavy chain at the C terminal, or the light chain at the N terminal. In one embodiment, the first scFv domain is linked to the N terminal of the heavy chain.

[0016] The antibody-like protein may further comprise a second scFv domain. In one embodiment, the second scFv domain may be linked to the first scFv domain in tandem. In one embodiment, the first scFv domain and the second scFv domain linked in tandem may be attached to the N terminal of the heavy chain through the linker.

[0017] In one embodiment, the first and the second scFv domain may be the same. In one embodiment, the first scFv domain may differ from the second scFv domain.

[0018] In one embodiment, the antibody-like protein may be bispecific. For example, the antibody-like protein may have a binding affinity to HER2 and HER3.

[0019] The Fab region may have a binding affinity to HER2, and the first scFv domain may have a binding affinity to HER3. In one embodiment, the antibody-like protein may include both the first and second scFv domain each having a binding affinity to HER3.

[0020] In one embodiment, the Fab region has a binding affinity to HER2 with a K_D from about 1pM to about 100 nM, about 1pM to about 10nM, about 2pM to about 5nM, or about 3pM to about 2nM. In one embodiment, the first or the second scFv domain has a binding affinity to HER3 with a K_D from about 1nM to about 1 μ M, about 50nM to about 500nM, about 60nM to about 200nM, about 80nM to about 300nM, about 80nM to about 150nM, about 100nM to about 500nM, about 100nM to about 400nM, or about 100nM to about 1 μ M.

[0021] The Fab region may have a binding affinity to HER3, and the first scFv domain may have a binding affinity to HER2. In one embodiment, the antibody-like protein may include both the first and second scFv domain each having a binding affinity to HER2.

[0022] In one embodiment, the Fab region has a binding affinity to HER3 with a K_D from about 1pM to about 1 μ M, about 1nM to about 120nM, about 50nM to about 500nM, about 100nM to about 500nM, about 100nM to about 400nM, or about 100nM to about 1 μ M. In one

embodiment, the first or the second scFv domain has a binding affinity to HER2 with a KD from about 1pM to about 100nM, about 1pM to about 10nM, about 3pM to about 5 nM, about 10pM to about 5nM, or about 3pM to about 2 nM.

[0023] In one embodiment, the heavy chain of the antibody-like protein comprises an amino acid sequence having at least 60%, 70% 80%, 90%, 95%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 6, 10, 18, 36, 40, 44. In one embodiment, the heavy chain VH domain comprises an amino acid sequence having at least 60%, 70% 80%, 90%, 95%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 22, 26. In one embodiment, the heavy chain VH domain comprises CDR-H1 having at least 98%, 99% or 100% sequence identity to SEQ ID NO: 47, 53, CDR-H2 having having at least 98%, 99% or 100% sequence identity to SEQ ID NO: 48, 54, and CDR-H3 having having at least 98%, 99% or 100% sequence identity to SEQ ID NO: 49, 55.

[0024] In one embodiment, the heavy chain VH domain comprises CDR-H1 having a SEQ ID NO: 47, CDR-H2 having a SEQ ID NO: 48, and CDR-H3 having a SEQ ID NO: 49. In one embodiment, the heavy chain VH domain comprises CDR-H1 having a SEQ ID NO: 53, CDR-H2 having a SEQ ID NO: 54, and CDR-H3 having a SEQ ID NO: 55.

[0025] In one embodiment, the light chain of the antibody-like protein comprises an amino acid sequence having at least 60%, 70% 80%, 90%, 95%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 8, 12, 20, 38, 42, 46. In one embodiment, the light chain VL domain comprises an amino acid sequence having at least 60%, 70% 80%, 90%, 95%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 24, 28. In one embodiment, the light chain VL domain comprises CDR-L1 having at least 98%, 99% or 100% sequence identity to SEQ ID NO: 50, 56, CDR-L2 having at least 98%, 99% or 100% sequence identity to SEQ ID NO: 51, 57, and CDR-L3 having at least 98%, 99% or 100% sequence identity to SEQ ID NO: 52, 58.

[0026] In one embodiment, the light chain VL domain comprises CDR-L1 having a SEQ ID NO: 50, CDR-L2 having a SEQ ID NO: 51, and CDR-L3 having a SEQ ID NO: 52. In one embodiment, the light chain VL domain comprises CDR-L1 having a SEQ ID NO: 56, CDR-L2 having a SEQ ID NO: 57, and CDR-L3 having a SEQ ID NO: 58.

[0027] In one embodiment, the Fab region may comprise CDR-H1 having a SEQ ID NO: 47, CDR-H2 having a SEQ ID NO: 48, and CDR-H3 having a SEQ ID NO: 49; and CDR-L1 having a SEQ ID NO: 50, CDR-L2 having a SEQ ID NO: 51, and CDR-L3 having a SEQ ID NO: 52.

[0028] In one embodiment, the Fab region may comprise CDR-H1 having a SEQ ID NO: 53, CDR-H2 having a SEQ ID NO: 54, and CDR-H3 having a SEQ ID NO: 55; and CDR-L1 having a SEQ ID NO: 56, CDR-L2 having a SEQ ID NO: 57, and CDR-L3 having a SEQ ID NO: 58.

[0029] In one embodiment, the antibody-like protein comprises the first scFv domain or the second scFv domain having an amino acid sequence with at least 60%, 70% 80%, 90%, 95%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 30, 32, 34.

[0030] In one embodiment, the first or the second scFv domain comprises an amino acid sequence having at least 60%, 70% 80%, 90%, 95%, 98%, 99%, or 100% sequence identity to SEQ

ID NO: 22, 26. In one embodiment, the first or the second scFv VH domain comprises CDR-H1 having at least 98%, 99% or 100% sequence identity to SEQ ID NO: 47, 53, CDR-H2 having at least 98%, 99% or 100% sequence identity to SEQ ID NO: 48, 54, and CDR-H3 having at least 98%, 99% or 100% sequence identity to SEQ ID NO: 49, 55.

[0031] In one embodiment, the first or the second scFv VH domain comprises CDR-H1 having a SEQ ID NO: 47, CDR-H2 having a SEQ ID NO: 48, and CDR-H3 having a SEQ ID NO: 49. In one embodiment, the first or the second scFv VH domain comprises CDR-H1 having a SEQ ID NO: 53, CDR-H2 having a SEQ ID NO: 54, and CDR-H3 having a SEQ ID NO: 55.

[0032] In one embodiment, the first or the second scFv VL domain comprises an amino acid sequence having at least 60%, 70% 80%, 90%, 95%, 98%, or 99% sequence identity to SEQ ID NO: 24, 28. In one embodiment, the first or the second scFv VL domain comprises CDR-L1 having at least 98%, 99% or 100% sequence identity to SEQ ID NO: 50, 56, CDR-L2 having at least 98%, 99% or 100% sequence identity to SEQ ID NO: 51, 57, and CDR-L3 having at least 98%, 99% or 100% sequence identity to SEQ ID NO: 52, 58.

[0033] In one embodiment, the first or the second scFv VL domain comprises CDR-L1 having a SEQ ID NO: 50, CDR-L2 having a SEQ ID NO: 51, and CDR-L3 having a SEQ ID NO: 52. In one embodiment, the first or the second scFv VL domain comprises CDR-L1 having a SEQ ID NO: 56, CDR-L2 having a SEQ ID NO: 57, and CDR-L3 having a SEQ ID NO: 58.

[0034] In one embodiment, the first or the second scFv domain comprises CDR-H1 having a SEQ ID NO: 47, CDR-H2 having a SEQ ID NO: 48, and CDR-H3 having a SEQ ID NO: 49; and CDR-L1 having a SEQ ID NO: 50, CDR-L2 having a SEQ ID NO: 51, and CDR-L3 having a SEQ ID NO: 52.

[0035] In one embodiment, the first or the second scFv domain comprises CDR-H1 having a SEQ ID NO: 53, CDR-H2 having a SEQ ID NO: 54, and CDR-H3 having a SEQ ID NO: 55; CDR-L1 having a SEQ ID NO: 56, CDR-L2 having a SEQ ID NO: 57, and CDR-L3 having a SEQ ID NO: 58.

[0036] In one embodiment, the linker connecting the first scFv domain and the second scFv domain, or the linker connecting the first scFv domain to the heavy chain or the light chain may be a flexible GS linker. The two linkers may be different or the same. Each linker may independently have from about 10 to about 20, about 15 to about 50, about 20 to about 40, or about 10 to about 50 amino acids. In one embodiment, each linker may independently comprise an amino acid sequence ((Gly-Gly-Gly-Gly-Ser)_m). In one embodiment, m is an integer of at least 3. In one embodiment, the linker may be from 3 to 5, from 2 to 10, or from 3 to 8. In one embodiment, m is 3, 4, 5, 6, 7, 8, 9, or 10. In one embodiment, m is 4.

[0037] In a second aspect, the application provides isolated nucleic acid sequences. In one embodiment, the isolated nucleic acid sequences encode the antibody-like proteins disclosed herein.

[0038] In a further aspect, the application provides expression vectors. In one embodiment, the expression vector may comprise the isolated nucleic acid sequences encoding the antibody-like protein disclosed herein.

[0039] In a further aspect, the application provides host cells. In one embodiment, the host cells may comprise the isolated nucleic acid sequence encoding the antibody-like protein as disclosed herein.

[0040] In a further aspect, application provides immunoconjugates. In one embodiment, the immunoconjugate may comprise the antibody-like protein conjugated to a cytotoxic agent. In one embodiment, the cytotoxic agent may comprise a radioisotope, a radionuclide, a therapeutic agent, a chemotherapeutic agent, or a combination thereof. In one embodiment, the immunoconjugate may comprise the antibody-like protein conjugated to an imaging agent.

[0041] In a further aspect, the application provides the pharmaceutical compositions.

[0042] In one embodiment, the pharmaceutical composition may comprise the antibody-like protein as disclosed herein. In one embodiment, the pharmaceutical composition may comprise the immunoconjugates as disclosed herein.

[0043] In one embodiment, the pharmaceutical composition may further and optionally comprise a pharmaceutically acceptable carrier. In one embodiment, the pharmaceutical composition may further comprise a cytotoxic agent. In one embodiment, the cytotoxic agent may be a radioisotope, a radionuclide, a therapeutic agent, a chemotherapeutic agent, or a combination thereof.

[0044] In a further aspect, the application provides the methods for treating or preventing cancer in a subject. In one embodiment, the method may include the step of administering to the subject the pharmaceutical composition comprising the antibody-like protein or its immunoconjugate.

[0045] In one embodiment, the method may include co-administering an effective amount of a therapeutic agent. In one embodiment, the therapeutic agent may be an antibody, a chemotherapy agent, an enzyme, or a combination thereof.

[0046] In one embodiment, the cancer comprises cells expressing HER2 and/or HER3. In one embodiment, the cancer comprises breast cancer, colorectal cancer, pancreatic cancer, head and neck cancer, melanoma, ovarian cancer, endometrial cancer, epidermal cancer, prostate cancer, non-small lung cell cancer, small cell lung cancer, glioma, esophageal cancer, nasopharyngeal cancer, kidney cancer, gastric cancer, liver cancer, bladder cancer, cervical cancer, brain cancer, lymphoma, leukaemia, or myeloma.

[0047] In one embodiment, the subject may be a human.

[0048] In a further aspect, the method provides methods of producing the protein-like antibody or its immunoconjugates.

[0049] In one embodiment, the application provides the method of making the antibody-like protein. In one embodiment, the method may include the steps of culturing a host cell such that the DNA sequence encoding the antibody-like protein is expressed, and purifying said antibody-like protein.

[0050] In one embodiment, the application provides the method for producing the immunoconjugate derived from the antibody-like protein. In one embodiment, the method may include the step of conjugating the antibody-like protein with a drug moiety or a cytotoxic agent. In one embodiment, the drug moiety or the cytotoxic agent may be a radioisotope, a radionuclide, a therapeutic agent, a chemotherapeutic agent, or a combination thereof. In one embodiment, the drug moiety or cytotoxic agent may be an imaging agent.

[0051] In a further aspect, the application provides a solution. In one embodiment, the solution comprising an effective concentration of the antibody-like protein, its immunoconjugates, or a combination thereof. In one embodiment, the solution is blood plasma in a subject.

BRIEF DESCRIPTION OF THE DRAWINGS

[0052] The foregoing and other features of this disclosure may become more fully apparent from the following description and appended claims, taken in conjunction with the accompanying drawings. Understanding that these drawings depict only several embodiments arranged in accordance with the disclosure and are, therefore, not to be considered limiting of its scope, the disclosure may be described with additional specificity and detail through use of the accompanying drawings, in which:

[0053] **Figure 1** shows diagrams of antibody-like proteins consisting of a monovalent-Fab as its first binding domain (D1) plus either one or two monovalent-scFv as its second binding domain (D2) linked to the N-terminal of a heavy chain monomer (**1A**) and anti-HER2/HER3 bispecific antibody-like proteins in various compositions and configurations as indicated (**1B**);

[0054] **Figure 2** depicts the VH-VL pairing of trastuzumab and MM-111 Fvs showing contacts between Q39 of the VH and Q38 of the VL (Kabat numbering) (**2A**), the correct pairing in favour of reversing the charge orientation (VH+ / VL- versus VH- / VL+) (**2B** upper panel), and the incorrect pairing (**2B** lower panel);

[0055] **Figure 3** shows Octet binding affinity of two monospecific control antibodies, SI-71MM4 (α HER2) and SI-71MM7 (α HER3) (**3A**); anti-HER2xHER3 bispecific bivalent and trivalent (bivalent to HER3) antibody-like proteins, SI-71XM21 and SI-71XM20, respectively (**3B**); anti-HER2 (D2)/HER3 (D1) bispecific antibody-like proteins with and without mutations for correct pairing (**3C**); anti-HER2 (D1)/HER3 (D2) bispecific antibody-like proteins with and without mutations for correct pairing (**3D**); and two monospecific antibodies against HER2 (SI-4C12) and HER3 (SI-1C16, respectively (**3E**).

[0056] **Figure 4** shows Octet binding avidity (HER3) of anti-HER2/HER3 monovalent-Fab antibody-like proteins, SI-71MX21 and SI-71MX21;

[0057] **Figure 5** shows the anti-proliferation effect of anti-HER2/HER3 monovalent-Fab antibody-like proteins to BT-474 cells (a breast cancer cell line expressing HER2 and HER3) in an Alamar-blue proliferation assay, indicating the positional effect of D1 and D2 binding domains in antibody-like proteins;

[0058] **Figure 6** shows the anti-proliferation effect of anti-HER2/HER3 monovalent-Fab antibody-like proteins to FaDu cells (a head and neck cancer cell line expressing HER2 and HER3) in an Alamar-blue proliferation assay, indicating the improvement by the anti-HER2/HER3 bi-specificity of SI-71XM40 (**6A**) and SI-71XM41 (**6B**) but not by the mutational effect for a correct pairing in either SI-71XM40 (**6C**) or SI-71XM41 (**6D**); and

[0059] **Figure 7** shows the average geometric mean fluorescence intensity (gMFI) signals of antibody or antibody-like proteins (10 ng of SI-71MX40, SI-71MX41) binding across 10 HER2/HER3-expressing cancer cell lines; bars represent average gMFI of anti-Fc AF647 APC channel; dots represent replicates (n=4); titles indicate tissue of origin, cell line name, HER2 antibody binding capacity, and HER3 antibody binding capacity.

DETAILED DESCRIPTION

[0060] This disclosure provides antibody-like proteins and their immunoconjugates with superior therapeutic properties or efficacies over the currently known antibodies targeting one or two members of EGFR family. In one embodiment, the antibody-like proteins are structurally configured to efficiently target two members of EGFR family, HER2 and HER3. The anti-proliferative activities of these bispecific antibody-like proteins may result from blocking or inhibiting different receptor-mediated oncogenic signaling simultaneously.

[0061] The terms “a”, “an” and “the” as used herein are defined to mean “one or more” and include the plural unless the context is inappropriate.

[0062] The terms “polypeptide”, “peptide”, and “protein”, as used herein, are interchangeable and are defined to mean a biomolecule composed of amino acids linked by a peptide bond.

[0063] The term “antigen” refers to an entity or fragment thereof which can induce an immune response in an organism, particularly an animal, more particularly a mammal including a human. The term includes immunogens and regions thereof responsible for antigenicity or antigenic determinants.

[0064] The terms “antigen- or epitope-binding portion or fragment”, “variable domain”, “variable region”, “variable region sequence”, or “binding domain” refer to fragments of an antibody that are capable of binding to an antigen (such as HER2 and HER3 in this application). These fragments may be capable of the antigen-binding function and additional functions of the intact antibody.

[0065] The terms “Fv” or “scFv” refers to the minimum antibody fragment which contains a complete antigen recognition and binding site. This region consists of a dimer of one heavy and one light chain variable domain in tight, non-covalent association. It is in this configuration that the three CDRs of each variable domain interact to define an antigen binding site on the surface of the VH-VL dimer. Collectively, the six CDRs confer antigen binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) can recognize and bind antigen, although at a lower affinity than the entire binding site. Examples of binding fragments include, but are not limited to, a single-chain Fv fragment

(scFv) consisting of variable light chain (VL) and variable heavy chain (VH) domains of a single arm of an antibody connected in a single polypeptide chain by a synthetic linker, or a Fab fragment which is a monovalent fragment consisting of VL, constant light (CL), VH and constant heavy 1 (CH1) domain. Antibody fragments can be even smaller sub-fragments and can consist of domains as small as a single CDR domain, in particular the CDR3 regions from either the VL and/or VH domains.

[0066] The term “VH–VL pairing” refers to the selection of suitable human germlines during the humanization process for the heavy and light chains to form a stable Fv. The mutual orientation of the VH and VL domains should correspond to that observed in the parental antibody.

[0067] Antibody fragments are produced using conventional methods known to those skilled in the art. The antibody fragments can be screened for utility using the same techniques employed with intact antibodies. Purified monoclonal antibodies can be cleaved with an enzyme, such as pepsin, and subjected to HPLC gel filtration. Papain digestion of antibodies produces two identical antigen binding fragments, called “Fab” fragments, each with a single antigen binding site, and a residual “Fc” fragment, whose name reflects its ability to crystallize readily. Pepsin treatment yields an $F(ab')_2$ fragment that has two antigen combining sites and is still capable of cross-linking antigen. The appropriate fraction containing Fab fragments can then be collected and concentrated by membrane filtration and the like. For further description of general techniques for the isolation of active fragments of antibodies.

[0068] The term “antibody” is used in the broadest sense and specifically covers single monoclonal antibodies and/or recombinant antibodies (including agonist and antagonist antibodies), antibody compositions with polyepitopic specificity, as well as antibody fragments (e.g., Fab, $F(ab')_2$, and Fv), so long as they exhibit the desired biological activity. In some embodiments, the antibody may be monoclonal, polyclonal, chimeric, single chain, multi-specific or multi-effective, human and humanized antibodies, as well as active fragments thereof. Examples of active fragments of molecules that bind to known antigens include Fab, $F(ab')_2$, scFv and Fv fragments, including the products of a Fab immunoglobulin expression library and epitope-binding fragments of any of the antibodies and fragments mentioned above.

[0069] The term “antibody-like protein” are proteins that are antibody-like and can specifically bind to antigens with high specificity and affinity. Sometimes, in this application, “antibody” and “antibody-like protein” may be used interchangeably.

[0070] In some embodiments, antibody may include immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e. molecules that contain a binding site and that immunospecifically bind an antigen. A typical antibody refers to heterotetrameric protein comprising typically of two heavy (H) chains and two light (L) chains. Each heavy chain is comprised of a heavy chain variable domain (abbreviated as VH) and a heavy chain constant domain. Each light chain is comprised of a light chain variable domain (abbreviated as VL) and a light chain constant domain. The light chains of antibodies

(immunoglobulins) from any vertebrate species can be assigned to one of two clearly distinct types, called kappa and lambda, based on the amino acid sequences of their constant domains. The VH and VL regions can be further subdivided into domains of hypervariable complementarity determining regions (CDR), and more conserved regions called framework regions (FR). Each variable domain (either VH or VL) is typically composed of three CDRs and four FRs, arranged in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4 from amino-terminus to carboxy-terminus. Within the variable regions of the light and heavy chains there are binding regions that interacts with the antigen.

[0071] Depending on the amino acid sequence of the constant domain of their heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG-1, IgG-2, IgG-3, and IgG-4; IgA-1 and IgA-2. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called alpha, delta, epsilon, gamma, and mu, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

[0072] The term “valency” refers to the number of antigenic determinants that an individual antibody molecule can bind. The valency of all natural antibodies is at least two, whereas the term “mono-valent” refers to an engineered antibody or antibody like protein with a single binding domain comprising the pairing of six hypervariable complementarity determining regions (CDRs), i.e., three HC-CDRs and three LC-CDR. In this context, “antibody affinity” refers to the tendency of an antibody to bind to a specific epitope at the surface of an antigen, i.e., to the strength of the interaction.

[0073] 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. In addition to their specificity, the monoclonal antibodies are advantageous in that they are synthesized by the hybridoma culture, uncontaminated by other immunoglobulins. The modifier “monoclonal” indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present disclosure may be made by the hybridoma method first described by Kohler & Milstein or may be made by recombinant DNA methods (see, e.g., U.S. Pat. No. 4,816,567). “Recombinant” means the antibodies are generated using recombinant nucleic acid techniques in exogeneous host cells. Monoclonal antibodies can be produced using various methods,

including without limitation, mouse hybridoma, phage display, recombinant DNA, molecular cloning of antibodies directly from primary B cells, and antibody discovery methods. Monoclonal antibodies may include “chimeric” antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity.

[0074] The term “humanized antibody” refers to a type of engineered antibody having its CDRs derived from a non-human donor immunoglobulin, the remaining immunoglobulin-derived parts of the molecule being derived from one (or more) human immunoglobulin(s). In addition, framework support residues may be altered to preserve binding affinity. Methods to obtain “humanized antibodies” are well known to those skilled in the art.

[0075] The terms “isolated” or “purified” refer to a biological molecule free from at least some of the components with which it naturally occurs. Either “isolated” or “purified,” when used to describe the various polypeptides disclosed herein, means a polypeptide that has been identified and separated and/or recovered from a cell or cell culture from which it was expressed. Ordinarily, a purified polypeptide will be prepared by at least one purification step. An “isolated” or a “purified” antibody refers to an antibody which is substantially free of other antibodies having different antigenic a binding specificity.

[0076] The term “immunogenic” refers to substances which elicit or enhance the production of antibodies, T-cells or other reactive immune cells directed against an immunogenic agent and contribute to an immune response in humans or animals. An immune response occurs when an individual produces sufficient antibodies, T-cells and other reactive immune cells against administered immunogenic compositions of the present disclosure to moderate or alleviate the disorder to be treated. While the immunogenic response generally includes both cellular (T cell) and humoral (antibody) arms of the immune response, antibodies directed against therapeutic proteins (anti-drug antibodies, ADA) may consist of IgM, IgG, IgE, and/or IgA isotypes.

[0077] The terms “specific binding”, “specifically binds to”, or “is specific for a particular antigen or an epitope” means that the binding is measurably different from a non-specific interaction. Specific binding can be measured, for example, by determining binding of a molecule compared to binding of a control molecule, which generally is a molecule of similar structure that does not have binding activity. For example, specific binding can be determined by competition with a control molecule that is similar to the target.

[0078] The term “affinity” refers to a measure of the attraction between two polypeptides, such as antibody/antigen, receptor/ligand, etc. The intrinsic attraction between two polypeptides can be expressed as the binding affinity equilibrium dissociation constant (KD) of a particular interaction. A KD binding affinity constant can be measured, e.g., by Bio-Layer Interferometry,

where KD is the ratio of k_{dis} (the dissociation rate constant) to k_{on} (the association rate constant), as $KD = k_{dis}/k_{on}$.

[0079] Specific binding for a particular antigen or an epitope can be exhibited, for example, by an antibody having a KD for an antigen or epitope of at least about 10^{-4} M, at least about 10^{-5} M, at least about 10^{-6} M, at least about 10^{-7} M, at least about 10^{-8} M, at least about 10^{-9} M, alternatively at least about 10^{-10} M, at least about 10^{-11} M, at least about 10^{-12} M, or greater, where KD refers to the equilibrium dissociation constant of a particular antibody-antigen interaction. Typically, an antibody that specifically binds an antigen will have a KD that is 20-, 50-, 100-, 500-, 1000-, 5,000-, 10,000- or more times greater for a control molecule relative to the antigen or epitope.

[0080] Also, specific binding for a particular antigen or an epitope can be exhibited, for example, by an antibody having a K_A or K_a for an antigen or epitope of at least 20-, 50-, 100-, 500-, 1000-, 5,000-, 10,000- or more times greater for the epitope relative to a control, where K_A or K_a refers to an association rate of a particular antibody-antigen interaction.

[0081] It is considered by the application that the antibody-like proteins potentially has the advantage over any combination therapy, which often has greater toxicity than a single agent treatment. Bispecific agents, such as bispecific antibody-like proteins as disclosed in the application, may act as a single agent targeting the same antigens as the combination therapy does but with the increased efficacy and response rate and reduced toxicity when compared to the combination therapy. In comparison to the combination therapy using two monoclonal antibodies, bispecific therapeutics such as antibody-like proteins disclosed herein can be less toxic to patients and/or more potent due to the increased binding specificity.

[0082] In one aspect, the application provides a bispecific antibody-like proteins having a N terminal and a C terminal, comprising at least two binding domains, wherein the binding domain comprises a Fab region and a scFv domain. The scFv domain may be attached to either the N terminal or the C terminal of the antibody. The Fab region and the scFv domain each independently have a binding specificity to different proteins in the EGFR family.

[0083] In some embodiments, scFv molecules described herein contain a linker of $(G_mS)_n$ that operably links the V_H and V_L , regardless of the V-region orientation (LH or HL). The remaining positions in the bispecific antibody-like protein may be consist of a human IgG Fc or IgG null Fc heavy chain, V_H -CH1-Hinge-CH2-CH3, and its corresponding kappa or lambda light chain, V_L -CL. Those scFv domains were genetically linked through a linker of $(SG_m)_n$ to either N-terminal or C-terminal of IgG heavy chain, resulting in a contiguous ~ 75 kDa heavy chain monomer peptide. When co-transfected with the appropriate light chain, the final symmetric bispecific molecule may be purified through the human IgG Fc (Protein A) and assayed to assess functional activity.

[0084] In one embodiment, the binding domain having the binding specificity to HER2 comprises Trastuzumab, which is given by intravenous infusion as a HER2 inhibitor medication used for the treatment of HER2+ breast cancer.

[0085] In one embodiment, the binding domain having the binding specificity to HER3 comprises MM-111, a bispecific HER2 and HER3 binding protein. MM-111 is a human serum albumin protein (HSA)-backed bispecific antibody fragment comprises one therapeutic binding to HER3, but its binding to HER2 alone is not sufficient to be considered as a therapeutic binding. In contrast, Trastuzumab comprises one single therapeutic binding to HER2.

[0086] The bispecific antibody-like protein may include an immunoglobulin G (IgG) moiety having at least a light chain and a heavy chain. In one embodiment, the antibody-like protein may include two scFv moieties being covalently connected to either C or N terminals of the heavy or light chains via a linker, such as (Ser-Gly-Gly-Gly-Gly)_n linkers, (Gly-Gly-Gly-Gly-Ser)_n linkers, (Gly-Gly-Gly-Ser)_n linkers, or (G_mS)_n or (SG_m)_n linkers.

[0087] It is known that having a single therapeutic agent poses significant challenges due to the selection of binding moieties and the backbone structure that may affect the binding efficiency *in vivo* and the therapeutic efficacy in patients. For example, ALM is a bispecific antibody targeting HER2/HER3, which has antiproliferative activity to tumor cells *in vitro*. But a short circulating half-life makes it an unlikely candidate drug due to rapid renal clearance. These bispecific antibodies may inhibit different receptor-mediated oncogenic signaling simultaneously therefore overcome resistance in EGFR family inhibitor or monoclonal antibody treatment.

[0088] In one embodiment, the binding domain having the binding specificity to HER2 comprises Trastuzumab. Trastuzumab inhibits HER2 phosphorylation and consequently, its downstream signaling pathways (Gijzen et al. 2010; Goel et al. 2015). But not all patients with HER2 overexpression in cancer cells can benefit from trastuzumab therapy due to the initial or acquired resistance (Luque-Cabal et al. 2016). The precise mechanism of the resistance to Trastuzumab remains unclear. Both the HER2/HER3 heterodimer and its downstream signaling play crucial roles in the tumor resistance and metastasis of HER2-positive cancer (Wang et al. 2016; Yang et al. 2017). Trastuzumab may inhibit ligand-independent HER3/HER2 interactions rather than blocking HER2 signaling. In fact, many forms of HER2-amplified metastatic cancer, which do not respond to or are eventually resistant to trastuzumab, often recover the phospho-HER3 and PI3K-Akt-mTOR downstream signals (Diaz-Serrano, et al. 2018).

[0089] HER2-targeted bispecific antibodies exhibit significant efficiency in preclinical studies for treating drug-resistant HER2-expressing malignant tumors. HER2-targeted bispecific antibodies include MM-111, ALM, PB4188, and MCLA-128 (McDonagh et al. 2012; Robinson et al. 2008; Geuijen et al. 2018; and Schram et al., 2022). MM-111 targets the HER2/HER3 heterodimer, blocks heregulin binding, and inhibits downstream signaling pathways. In contrast, Trastuzumab alone had no effect on heregulin-induced paclitaxel resistance. MM-111 has a higher-affinity HER2 arm to target HER2-amplified tumors as compared with its HER3 arm (McDonagh et al. 2012). Upon its binding to HER2-positive cells with high avidity, MM-111 can effectively block submaximal ligand-driven proliferation but not supramaximal activation. This is because the HER2 binding moiety of MM-111 does not have any sustained effect on tumor cell growth when

compared with that of Trastuzumab, which binds to a different epitope in the region IV of HER2 (Neve et al. 2001). Indeed, the combination therapy of Trastuzumab and MM-111 shows significantly greater activity than either antibody used alone (McDonagh et al. 2012).

[0090] MCLA-128 (Zenocutuzumab, Zeno) is a bispecific humanized immunoglobulin G1 (IgG1) containing two different Fab arms targeting the extracellular domains of HER2 and HER3. A recent clinical study (Schram et al., 2022) reveals that Zeno mediates durable clinical responses in a small group of patients with *NRG1* fusion-positive cancers.

[0091] Having a single therapeutic agent poses significant challenges due to the selection of binding moieties and the backbone structure that may affect the binding efficiency *in vivo* and the therapeutic efficacy in patients. The present application disclosed anti-HER2/HER3 antibodies characterized by their configurations and domain compositions (see **Table 1** and **Figure 1**). In some embodiments, the antibody-like proteins comprise a monovalent-Fab for binding either HER2 or HER3 as the first binding domain (D1). In some embodiments, one or two scFv domains are linked to the N-terminus of heavy chain variable domain as the second binding domain (D2). In one embodiment the antibody-like proteins comprise anti-HER2/HER3 monovalent-Fab antibodies having a second Fc linked to the C-terminus of the heavy chain.

EXAMPLES

Example 1. Anti-HER2 X HER3 Bispecific Monovalent-Fab Antibodies

Bispecific monovalent-Fab antibody-like proteins

[0092] A class of bispecific monovalent-Fab antibody-like proteins is characterized by a single heavy chain and a single light chain (**Figure 1A**). The single heavy chain contains a classic variable heavy (VH) domain linked to human IgG1 CH1 domain and the variable domains of the heavy (VH) and light chain (VL) form a monovalent Fab region that acts as the first binding domain (D1). The second binding domain (D2) may be a single (monovalent) or double (bivalent) scFv linked to the N-terminal of the heavy chain. The other characteristic feature of this class of antibody-like proteins is the linkage of two inverted IgG1 Fc elements that include hinge, CH2 domain, CH3 domain separated by a 30-residue flexible (GSSSS)₆ linker. The second hinge, immediately following the linker, has the N-terminal five residues removed so that it only contains the two cysteine residues involved in the typical interchain disulfide bonds found in normal human IgG1 molecules. In this way, the second Fc may stabilize the structure of this class of atypical antibodies by folding two Fc together for the formation of interchain disulfide bonds in the absence of heavy chain dimerization (**Figure 1A**). With its compact structure, the antibody-like protein may afford to be a bispecific antibody comprising a monovalent binding specificity (D1) and a monovalent or multivalent binding specificity (D2).

Anti-HER2 (IV) X HER3 antibody-like proteins

[0093] To identify potent therapeutics for treating HER2/HER3-expressing cancer, a group of 8 anti-HER2(IV)XHER3 antibody-like proteins was constructed on the monovalent-Fab platform (see **Table 1** and **Figure 1B**). The sequences encoding HER2 and HER3-binding domains were

adopted from that of Trastuzumab (specific to HER2 domain IV) and MM-111 (specific to HER3). The antibody-like protein heavy chain consists of VH, CH1, hinge, CH2, CH3, 30aa linker, hinge, CH3, and CH2 from human IgG1 or IgG2.

[0094] SI-71MM7 and SI71MM4 were created as anti-HER2/HER3 monospecific control antibodies, respectively. SI-71MX21 and SI-71MX20 were created to compare the effect of HER3 monovalent and bivalent binding. SI-71MX19 and SI-71MX41 were created to test the mutational effect of VH-VL pairing where D1 is HER3-specific and D2 is HER2-specific. Conversely, SI-71MX32 and SI-71MX40 were created to test the mutational effect of VH-VL pairing where D1 is HER2-specific and D2 is HER3-specific. From another perspective, SI-71MM4, SI-71XM21, and SI-71XM20 are characterized by having a monovalent anti-HER2 Fab region, whereas SI-71MM7, SI-71XM19, SI-71MX41, SI-71MX32, and SI-71MX40 are characterized by having a monovalent anti-HER3 Fab region (**Figure 1B**). Collectively, these anti-HER2/HER3 antibody-like proteins characterized by both monovalent-Fab and single chain Fc can be generated by co-transfecting appropriate a light chain.

Salt bridge engineering for antibody-like protein stabilization

[0095] The anti-HER2XHER3 bispecific antibody-like protein in the monovalent-Fab platform may be more highly aggregated following initial protein A purification than bispecific antibodies or antibody-like proteins in some other structural formula. It was postulated that the aggregation could be due to improper pairing of VH and VL domains during expression. The examination of Trastuzumab/MM-111 Fvs reveals that most VH/VL interface residues are bulky hydrophobics. Both Trastuzumab and MM-111 Fvs contain contacts between Q39 of the VH and Q38 of the VL (Kabat numbering, **Figure 2A**). Mutating these polar but uncharged Gln residues to charged residues could form salt bridge. Importantly, reversing the charge orientation (VH+/VL- versus VH-/VL+) could favor the correct pairing while disfavoring the incorrect chain pairing (**Figure 2B**). Used Discovery Studio to predict stability of all charge mutants to determine which are most likely to stabilize the correct pairings and destabilize the incorrect pairings. For this purpose, SI-71XM41 and SI-71XM40 were created to optimize SI-71XM19 and SI-71XM20 (i.e., SI-71XM32), respectively (**Figure 1B**).

Example 2. Generation of anti-HER2 X HER3 antibody-like proteins

[0096] Genes encoding antibody heavy and light chains (preceded by Kozak and secretory signal peptide) were cloned into pTT5 vector using standard molecular biology techniques. As shown in **Table 1** and **Figure 1**, this class of anti-HER2XHER3 monovalent-Fab antibody-like protein contains a heavy chain consisting of a standard variable heavy (VH) domain fused to human IgG1 CH1 domain and two IgG1 Fc elements (hinge, CH2 domain, CH3 domain) separated by a 30-residue flexible (GSSSS)₆ linker. The second hinge, immediately following the linker, has the N-terminal five residues removed so that it only contains the two cysteine residues involved in the typical interchain disulfide bonds found in normal human IgG1 molecules; thus, this single chain Fc (scFc) folds properly and binds to protein-A. The N-terminal cysteine in the first hinge is

involved in interchain disulfide bond between a light and scFc heavy chain. Co-transfection of a scFc heavy chain with a normal antibody light chain results in a monovalent Fab-Fc that may be purified by protein-A affinity chromatography. Single-chain variable fragments (scFv) may be fused to the N-terminus of the scFc heavy chain to create bispecific, monovalent-Fab antibody-like proteins. Resulting antibody-like proteins will be monovalent for the Fab binding domain and either monovalent or bivalent for the other binding domain depending on whether single or tandem scFv's are fused to the N-terminus. SI-71MM4, SI-71XM21, and SI-71XM20 were generated as exemplary anti-HER2 monovalent-Fab antibody-like proteins characterized by linking anti-HER3 scFv to the N-terminus of the heavy chain, whereas SI-71MM7, SI-71MX19, SI-71MX41, SI-71MX32, and SI-71MX40 were generated as exemplary anti-HER3 monovalent-Fab antibody-like proteins characterized by having a HER2 binding scFv domain linked to the heavy chain (**Figure 1B**).

[0097] The antibody-like protein were expressed by transiently transfecting the expression plasmids for heavy and light chains in the ExpiCHO system (Thermo Fisher). Briefly, 5.6µg of each expression plasmid was brought to 2.4ml with OptiPRO SFM medium containing 33.8µg sheared herring sperm carrier DNA. 2.2ml of OptiPRO SFM medium containing 192µl Expifectamine CHO reagent was added to the DNA and incubated at room temperature for 5 minutes. The resulting mixture was then added to 60ml ExpiCHO cells at 6×10^6 cells/ml in a 250ml Erlenmeyer flask and incubated at 37°C, 5% CO₂, 150rpm. Cells were fed with 21ml ExpiCHO feed and 360 µl of CHO enhancer at 24 hours post-transfection and shifted to 32°C, 5% CO₂, 150rpm. Cells were fed again at 48 hours post-transfection with 21ml ExpiCHO feed. Culture supernatant was harvested 9 days post-transfection, spun for 20min at 7500rpm to pellet the cells and then passed through a 0.2mm filter. Expression titer was quantitated using biolayer interferometry on an Octet384 system with protein A sensors and a standard curve prepared with purified antibody-like proteins.

[0098] Proteins were purified from the harvested supernatant using a 5-ml MabSelect Prisma protein-A column (Cytiva). The column was equilibrated with phosphate-buffered saline. The supernatant was then passed through the column at a flow rate of 5 ml/min. The column was washed with 25ml PBS. Protein was then eluted by passing 15ml of 50 mM sodium acetate, pH 3.5 through the column. The eluted protein was immediately neutralized by addition of 1/10th volume of 1M sodium acetate, pH7.0.

[0099] Immediately after first-step protein-A or His tag purification, proteins were analyzed by analytical SEC using Waters Acquity UPLC H-Class with ACQUITY UPLC® Protein BEH SEC 200Å, 4.6mm x 150mm, 1.7 µm column. PBS (125 mM sodium phosphate, 137 mM sodium chloride, pH 6.8) was used as mobile phase for 10-minute runs at 0.3 ml/min, injecting 15 µg protein. Proteins were further purified by preparative SEC using Superdex Increase 10/300 GL column in mobile phase of 25 mM sodium acetate, 125 mM NaCl, pH 5.5, ultimately to be buffer-exchanged

into 25 mM sodium acetate, 125 mM NaCl, 10% sucrose, pH 5.5. Final samples contained >95% protein of interest as assessed by analytical SEC and were used for subsequent assays.

Example 3. Characterization of anti-HER2 X HER3 bispecific antibody-like protein

[00100] Biolayer interferometry (Octet) binding assays were performed on an Octet384 instrument to quantify binding kinetics of antibody-like proteins to HER2 and HER3. For affinity experiments, the antibody-like protein was captured to anti-human Fc (AHC) sensor tips by loading for 150 seconds at 75nM. After a 60-second baseline step, a 180-second association phase with serial dilutions (0-100 nM for HER2; 0-400 nM for HER3; 1:2 dilution factor) of His-tagged HER2 or HER3 (expressed and purified in-house) in assay buffer (phosphate-buffered saline containing 1% BSA, 0.05% Tween20) was performed, followed by a 300-second dissociation phase in assay buffer. Regeneration was achieved using 10 mM glycine, pH 1.5. For avidity experiments, biotinylated HER3 was captured to streptavidin sensors by loading for 150 seconds at 2 µg/ml. After a 60-second baseline step, a 180-second association phase with serial dilutions (0-100 nM) of test antibody-like protein in assay buffer was performed, followed by a 300-second dissociation phase in assay buffer. Binding curves were globally fit to a 1:1 model to extract the dissociation constants, K_D , and kinetic association and dissociation rates.

[00101] Protein stability is a key parameter defined by the difference in free energy between the folded and unfolded states. For protein therapeutics, stability may impact immunogenicity, pharmacokinetics, and even efficacy, and reduction of aggregation can help to develop therapeutics that are easier to manufacture and safer for patients. In addition, expression efficiency and protein yield directly determine the cost of protein therapeutics. If proteins can be more efficiently expressed to reach higher titers and increased yield of purified protein, manufacturing costs can be reduced significantly.

[00102] After transient expression in ExpiCHO cells, titer of antibody-like proteins was quantitated using biolayer interferometry. The data shown in **Table 2** demonstrate that all proteins expressed in the ExpiCHO expression system, indicating they are stable enough to be efficiently produced. For the bispecific, monovalent Fab antibody-like proteins a general trend was observed in which decreasing titers correspond to increasing numbers of binding domains. For example, SI-71MM4 (anti-HER2 Fab only) had higher titer than SI-71XM21 (anti-HER2 Fab plus one anti-HER3 scFv). SI-71XM20, containing the anti-HER2 Fab and tandem anti-HER3 scFv's, had even lower titer. Even though the titer for SI-71XM41 (salt bridge variant) was slightly lower than SI-71XM19, introduction of salt bridge mutations did not dramatically change expression titers.

[00103] Immediately following first-step protein A purification, antibody-like proteins were evaluated for stability and aggregation by analytical size-exclusion chromatography on a Waters UPLC system. For the anti-HER2/HER3 monovalent-Fab antibody-like proteins containing a monovalent anti-HER2 Fab region, an unexpected phenomenon was observed. As shown in **Table 2**, SI-71XM20, which has tandem anti-HER3 scFv domains, had a larger percentage of the protein of interest (POI) after first step purification than SI-71XM21, which has a single anti-HER3

scFv domain. This larger %POI is due to lower amounts of aggregated high molecular weight (HMW) species. SI-71XM19 displayed higher percentage of high molecular weight species than SI-71XM41, its salt bridge mutation variant. SI-71XM32 contained over 65% high molecular weight species. Its salt bridge mutation variant, SI-71XM40 had a dramatically lower percentage of high molecular weight aggregate. The data suggests that the salt bridge mutations are indeed stabilizing the correct pairing of V domains during expression and initial purification.

Example 4. Octet binding affinity of anti-HER2 X HER3 antibody-like protein

[00104] The anti-HER2/HER3 antibody-like proteins share the characteristic structural features of monovalent Fab and single chain Fc region (**Figure 1B**). Under these unified features, the anti-HER2 X HER3 bispecific antibody-like proteins may be divided into two groups based on the location of HER2-binding domain at D1 (HER2/D1) and D2 (HER2/D2), respectively. To analyze the structure-function relationship, biolayer interferometry was used to evaluate the binding affinity of each antibody to HER2 and HER3. As expected, the anti-HER2 monospecific antibody-like protein, SI-71MM4, showed the binding specificity to HER2 but not HER3, whereas the anti-HER3 monospecific antibody-like protein, SI-71MM7, showed the binding specificity to HER3 but not HER2 (**Figure 3A**). All anti-HER2 X HER3 bispecific antibody-like proteins displayed their binding affinity toward both HER2 and HER3 (**Figure 3B, 3C, 3D**, and **Table 3**). To compare the affinity of monovalent and bivalent binding to HER2 and HER3, bivalent monospecific antibodies, Trastuzumab (SI-4C12) and MM111 (SI-1C16), were used as controls and the results were shown in **Figure 3E** and **Table 3**).

[00105] To quantify the positional effect of HER2 or HER3 binding domain, the binding affinity K_D values were listed in **Table 3A** and **3B**, respectively. Except SI-77XM20, the HER2/D1 antibody-like proteins, including SI-77MM4, SI-71XM21, SI-71XM32, SI-71XM40, and SI-4C12, showed the binding affinity to HER2 with K_D values between 0.967nM (SI-71MM4) and 1.34nM (SI-4C12). SI-71MM4 and SI-4C12 are anti-HER2 bivalent monospecific controls. The K_D values of SI-71XM21, SI-71XM32, SI-71XM40 were very close, indicating that HER2/D1 acts independently in this monovalent-Fab bispecific antibody platform as it does in controls. The K_D value of SI-71XM20 was 0.003nM, and this 30-fold increase was attributed to a significant decrease in the K_{OFF} value. On the other hand, these HER2/D1 antibody-like proteins showed the binding affinity to HER3 with K_D values between 91.8nM (SI-71XM20) and 236.7nM (SI-71XM21). The HER3/D1 antibody-like proteins, including SI-71MM7, SI-71XM19, SI-71XM41, and SI-1C16, showed the binding affinity to HER3 with K_D values between 147nM (SI-71XM19) and 364.7nM (SI-MM7), of which SI-71MM7 is the monovalent monospecific control, indicating the increase in valency, either HER2 or HER3, improves HER3 binding affinity. The HER3/D1 bispecific antibody-like proteins, SI-71XM19 and SI-71XM41, showed the K_D values of binding HER2 at 0.93nM and 1.1nM, respectively, which is comparable to that of the HER2/D1 antibody-like proteins except SI-77XM20. These data indicate that, while the MM-111 derived HER3 binding domain may exhibit low affinity in the monovalent-Fab structure (SI-71MM7), a bivalent antibody-like protein

significantly improves the binding affinity in two ways. First, a bivalent HER3/D1 in SI-1C16 and a bivalent of HER3/D1 and HER2/D2 improve the binding affinity to K_D value of 168nM and 0.93nM, respectively. And second, the synergy of HER2 and HER3 binding in the monovalent-Fab setting was unexpected but clearly demonstrated in SI-71XM20, which exhibits the lowest K_D values for both HER2 and HER3 binding.

Example 5. Octet binding avidity for anti-HER2 X HER3 antibody-like protein

[00106] To assess the avidity of SI-71XM21 and SI-71XM20 antibody-like proteins for HER3, biolayer interferometry was used. In contrast to the 'affinity' experiment, which used AHC sensors to measure binding of immobilized antibodies to HER3 in solution, this assay used streptavidin (SA) sensors to measure binding of immobilized biotinylated HER3 to the antibody in solution. Therefore, the experiment mimics the avidity effect that is observed when proteins are present on a surface such as the cell membrane. SI-71XM20 and SI-71XM21 are HER3/D2 antibody-like proteins with either one or two anti-HER3 scFv domains. SI-71XM21 showed relatively weak avidity (112.8nM) and low binding response (0.295nm) to immobilized HER3, whereas SI-71XM20 shows excellent avidity (1.7nM) and increased binding response (0.881nm) to immobilized HER3 (**Figure 4** and **Table 4**). This avidity effect might overcome the intrinsic low affinity of this MM-111-derived scFv allowing for tight binding to tumor cells that overexpress HER3, while binding less to cells with lower levels of HER3 surface expression.

Example 6. The anti-proliferation effect of anti-HER2 X HER3 antibody-like protein
Breast cancer cells (BT-474)

[00107] To test the effect of anti-HER2 X HER3 bispecific antibody-like proteins on growth of breast cancer cells, HER2- and HER3-expressing BT-474 cells were incubated with varying concentrations of test antibody-like proteins and proliferation was measured using Alamar blue.

[00108] The breast ductal carcinoma cell line BT-474 was purchased from ATCC (cat #HTB-20) and was maintained in Hybri-Care medium supplemented with 10% fetal bovine serum at 37°C with 5% CO₂. BT-474 cells were detached from flasks with trypsin and diluted to 1.2x10⁵ cells/ml in medium + 1% FBS. 50μl of cell suspension (6000 cells) was seeded to interior 60 wells of 96-well tissue culture plates. Outer wells were filled with 300μl sterile H₂O to minimize evaporation in interior wells. Cells were allowed to adhere for 4 hours at 37°C, 5% CO₂. Antibodies or antibody-like proteins to be tested were diluted to 2X final concentration in Hybri-Care medium + 1% FBS. 50μl of test antibodies were added to each well for a total volume of 100μl per well. Each antibody or antibody-like protein was tested in triplicate at the following final concentrations: 100nM, 25nM, 6.25nM, 1.563nM, 0.391nM, 0.098nM, 0.024nM, 0.006nM, and 0.0015nM. Each plate contained two antibodies or antibody-like proteins at those concentrations tested in triplicate. Six control wells per plate contained cells with medium only. Immediately following addition of test compounds, 10μl alamar blue (Thermo Fisher cat# DAL1100) was added to three of the medium only control wells on each plate. Cells were incubated for 2 hours at 37°C, 5% CO₂. Following the two-hour incubation, 110μl sample from each of the control wells was

removed and placed in a black, opaque 96-well plate. This plate was centrifuged for 5 minutes at 2000RPM to remove any bubbles. Fluorescence was then measured (excitation = 535nm, emission = 595nm) on a Molecular Devices FilterMax F5 microplate reader. Measured control fluorescence values (C_{start}) serve as the baseline to measure assay endpoint proliferation. Plates were returned to 37°C, 5% CO₂ for 7 days (168 hours). Following incubation, 10µl Alamar blue was added to each test well as well as the other three control (medium only) wells. Following 2 hours incubation at 37°C, 5% CO₂, fluorescence was measured as described above. Endpoint control fluorescence values (C_{end}) and test well fluorescence values (T_{end}) were used to calculate the % of control proliferation using the following formula:

$$\% \text{ of control proliferation} = ((T_{end} - C_{start}) / (C_{end} - C_{start})) * 100$$

[00109] Data points were analysed by GraphPad Prism and inhibition curves were fitted by nonlinear regression [log(inhibitor) vs. response, 4 parameters] and IC₅₀ values were calculated.

[00110] Given the difference in their binding affinity to HER2 and HER3, the structure and valency affect the function of SI-71XM21, SI-71XM20, and SI-71XM19. Specifically, the pairing of anti-HER2/D1 with anti-HER3/D2 or anti-HER2/D2 with anti-HER3/D1 led to the different degrees of effectiveness. Indeed, all three antibody-like proteins showed antiproliferative activity with double-digit IC₅₀ values and varying % efficacy. And, SI-71XM21 displayed higher efficacy, which is consistent with the synergy effect due to HER3 and HER2 binding (**Figure 5** and **Table 5**).

Head and neck squamous cell carcinoma cells (FaDu)

[00111] HER2 expression in breast cancer may be different from HER2 expression in head neck cancer. For example, gene amplification in breast cancer leads to high levels of HER2 overexpression, which is much less in head and neck cancer resulting in lower overall expression levels. HER2-targeting therapy has been effective in most malignancies, including breast cancer, where HER2 overexpression is present. However, HER2 mutations and gene amplifications are relatively rare in head and neck cancer. FaDu is a cell line isolated from a hypopharyngeal tumor of a squamous cell carcinoma patient and is widely used head and neck cancer cells for research. A comparative study was conducted to evaluate the effects of SI-71XM41 and SI-71XM40 on the proliferation of HER2/HER3 double positive FaDu cells. Both SI-71XM41 and SI-71XM40 achieved the maximum anti-proliferation activity at a drug concentration as low as 1nM and 0.1nM, respectively, while their parental antibodies, MM-111 and Trastuzumab showed the overlapping activity at approximately 100nM (**Figure 6A** and **6B**). Moreover, each pair of SI-71XM41 and SI-71XM19 or SI-71XM40 and SI-71XM32 (with the same amino acid sequence as that of SI-71XM20) displayed overlapping curves of anti-proliferation activities (**Figure 6C** and **6D**), indicating that the salt bridge mutation provides none or a minor advantage in terms of anti-proliferative activity. Thus, SI-71XM20 and SI-71XM40 were constructed based on the monovalent-Fab bispecific antibody-like protein platform and were optimized for inhibiting cell proliferation of HER2/HER3-expressing cancer cells, such as breast cancer and head and neck cancer.

Implication to other types of cancers

[00112] To evaluate the binding activity of SI-71XM41 and SI-71XM40 to other types of cancer, a panel of cancer cell lines were used for indications including breast cancer (SK-BR-3), pancreatic cancer (CFPAC-1), lung cancer (NCI-H358, Oka-C-1, and COR-L279), endometrial cancer (Ishikawa), liver cancer (HepG2), epidermal cancer (A-431), colon cancer (Colo-320). Each gMFI APC signal supposedly correlates with the density of antibody expression per cell. Collectively, there seems to be a consistent pattern of higher average gMFI signal by SI-71XM41 than the average gMFI signal by SI-71XM40 with Trastuzumab expressing an intermediate MFI signal. This pattern repeats for all indications even though the previously characterized two cell lines, BT-474 and FaDu, express highest and lowest average gMFI signals, respectively. Thus, both SI-71XM41 and SI-71XM40 are candidate therapeutics for treating the indications of these cancer cell lines.

TABLES

Table 1. Domain composition of bispecific, monovalent-Fab antibody-like proteins for binding either HER2 or HER3, comprising the first binding domain (D1, monovalent), the second binding domain (D2, monovalent or bivalent) containing one or two scFv linked to the N-terminus of heavy chain variable domain, and a single chain Fc region containing inverted CH2-CH3 repeats linked by two disulfide bridges (see **Figure 1**).

Protein	Heavy Chain				Light Chain
	N-scFv	D2 (scFv)	D1 (Fab)	Fc-C	
SI-71MM7	none	none	HER3	Inverted CH2-CH3	VL-C λ
SI-71MM4	none	none	HER2	Inverted CH2-CH3	VL-C λ
SI-71XM21	scFv	HER3	HER2	Inverted CH2-CH3	VL-C λ
SI-71XM20	scFv-scFv	(HER3) x 2	HER2	Inverted CH2-CH3	VL-C λ
SI-71XM19	scFv	HER2	HER3	Inverted CH2-CH3	VL-C λ
SI-71XM41	scFv	HER2 VH-Q39E/VL-Q38R	HER3 VH/Q39R/VLQ38E	Inverted CH2-CH3	VL-C λ
SI-71XM32	scFv	HER3	HER2	Inverted CH2-CH3	VL-C κ
SI-71XM40	scFv	HER3 VH-Q39R/VL-Q38E	HER2 VH-Q39E/VL-Q38R	Inverted CH2-CH3	VL-C κ

Table 2. Expression titer for antibody-like proteins targeting HER2 and HER3, and stability assessment of antibody-like proteins by analytical size-exclusion chromatography after first step protein A purification.

Protein	Titer (ug/ml)	POI %	HMW %	LMW %
SI-71MM7	229.4	70.9	29.1	0.0
SI-71MM4	196.3	63.8	36.2	0.0
SI-71XM21	151.9	43.7	56.0	0.0
SI-71XM20	127.8	73.1	26.9	0.0
SI-71XM19	121.9	79.5	20.5	0.0
SI-71XM41	99.0	91.6	8.4	0.0
SI-71XM32	175.9	34.8	65.2	0.0
SI-71XM40	176.9	93.9	6.1	0.0

Table 3A. The binding kinetics (affinity) of anti-HER2xHER3 antibody-like proteins to HER2 in solution.

Protein	Response (nm)	KD (nM)	kon ($M^{-1}s^{-1}$)	koff (s^{-1})
SI-71MM7	-0.005	no binding	no binding	no binding
SI-71MM4	0.367	0.967	2.09E+05	2.02E-04
SI-71XM21	0.242	1.478	1.35E+05	2.00E-04
SI-71XM20	0.078	0.003	1.64E+05	5.40E-07
SI-71XM19	0.0826	0.93	2.96E+05	2.75E-05
SI-71XM41	0.330	1.1	2.05E+05	2.29E-04
SI-71XM32	0.320	1.2	2.10E+05	2.55E-04
SI-71XM40	0.256	1.1	1.85E+05	2.03E-04
SI-4C12	0.578	1.34	2.75E+05	3.04E-04
SI-1C16	No binding	No binding	No binding	No binding

Table 3B. The binding kinetics (affinity) of anti-HER2/HER3 antibody-like proteins to HER3 in solution.

Protein	Response (nm)	KD (nM)	kon ($M^{-1}s^{-1}$)	koff (s^{-1})
SI-71MM7	0.196	364.7	1.79E+05	6.51E-02
SI-71MM4	0.008	no binding	no binding	no binding
SI-71XM21	0.219	236.7	1.57E+05	3.72E-02
SI-71XM20	0.327	91.8	3.11E+05	2.86E-02
SI-71XM19	0.073	147	3.28E+05	4.80E-02
SI-71XM41	0.223	207	2.23E+05	4.61E-02
SI-71XM32	0.276	129	3.01E+05	3.89E-02
SI-71XM40	0.233	158	3.16E+05	4.99E-02
SI-4C12	No binding	No binding	No binding	No binding
SI-1C16	0.351	168	3.24E+05	5.43E-02

Table 4. The binding kinetics (avidity) of anti-HER2xHER3 monovalent Fab antibody-like proteins to HER3 in solution.

Protein	Response (nm)	KD (nM)	kon ($M^{-1}s^{-1}$)	koff (s^{-1})
SI-71XM21	0.295	112.8	3.37E+05	3.80E-02
SI-71XM20	0.881	1.7	2.87E+05	4.87E-04

Table 5. Potency and efficacy parameters for BT-474 proliferation assay using anti-HER2xHER3 bispecific antibody-like proteins.

Protein	IC50 (nM)	Efficacy (%)
SI-71XM21	44.3	55.3
SI-71XM20	51.3	26.6
SI-71XM19	61.3	20.1

SEQUENCE LISTING

SEQ ID	Description
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SIBA075 - HER2 x HER3 asymmetric sequence listing

CDR's underlined in amino acid sequences

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CACCAAGGTGGACAAGAGAGTTGAGCCCAAATCTTGTGACAAAACCTCACACATGCCACCGTGCCAGCACCTGAAC
TCCTGGGGGACCGTCAGTCTTCTCTTCCCCCAAACCCAAAGGACACCCCTCATGATCTCCCGACCCCTGAGGTG
ACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCA
TAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCAGCTACCGTGTGCTCAGCGTCCCTACCGTCTCTGCAC
AGGACTGCTGATGGAAGGAGTACAAGTGAAGGTCTCCAACAAAGCCCTCCAGCCCCCATCGAGAAAACCATC
TCCAAGGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCA
GGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGG
AGAACAACCTACAAGACCACGCTCCCGTGTGACTCCGACGGCTCCTTCTTCTCTATAGCAAGCTCACCGTGGAC
AAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAA
GAGCCTCTCCCTGTCTCCGGTGGGGGAGGTGGTCCGGCGGTGGAGGCTGGAGGGTCTGGAGGCGGGGGTCCGGCGGAGGTG
GCTCAGGAGGCGGAGGGTCAAGGGGAGGTGGTCCGACAAGACCCACACCTGTCCACCTTGCCCCGCCCCCTGAACTG
CTGGGCGGCCCTTCCGTTTTTCTGTTCCCCCTAAGCCTAAGGACACCCCTGATGATCTCTCGGACACCCGAAGTGAC
CTGCGTGGTGGTGGACGTGTCCCATGAAGATCCTGAGGTGAAGTTCAACTGGTACGTGGACGGCGTGGAAAGTGACA
ACGCCAAGACCAAGCCAAGAGAAGAACAGTACAACCTCACTTACAGAGTTGTGTCCGTGCTGACCGTGTGCATCAA
GACTGGCTGAACGGCAAAGAATACAAGTGCAAGGTGAGTAACAAGGCTCTGCCTGCCCTATCGAGAAAACCATCTC
TAAGGCTAAGGGACAACCTAGAGAGCCTCAGGTGTACACCTGCCTCCTTCCCGGACGAGCTGACCAAGAACCAGG
TGTCCCTGACCTGTCTGGTGAAGGCTTCTACCCCTCTGACATCGCCGTGGAATGGGAGTGAACGGCCAGCCCGAG
AACAACCTACAAGACCACCCCTCCCGTGTGAGCAGCGACGGATCTTCTTCTCTGACTCCAAGCTGACCGTGCAGAA
GTCTAGATGGCAGCAGGGCAACGTGTTCTCTGCTGTGATGCACGAGGCTCTGCACAATCACTACACCCAGAAGT
CCCTGTCCCTATCGCCAGG

>SEQ ID 10 SI-71XM20 heavy chain amino acid sequence

QVQLQESGGGLVPGGSLRLSCAASGFTFSYWM^{SV}VRQAPGKLEWVANINRDGSASY^YYVDSVKGRFTISRDDAKN
SLYLQMN^{SL}RAEDTAVYYCARD^{RG}VGYFDLWGRGTLTVTVSSGGGSGGGSGGGGSSQ^SSALTQ^PPASVSGSPGQSITIS
CTGTSSDVGGYNFVSWYQQHPGKAPKLM^IYDVSDRPSGVSDRFSGSKSGNTASLIISGLQADDEADYYC^{SS}YSSST
HVIFGGGTKVTVLGGGSGGGGQVQLQESGGGLVPGGSLRLSCAASGFTFSYWM^{SV}VRQAPGKLEWVANINRD
GSASY^YYVDSVKGRFTISRDDAKNSLYLQMN^{SL}RAEDTAVYYCARD^{RG}VGYFDLWGRGTLTVTVSSGGGSGGGSGGG
GSQ^SSALTQ^PPASVSGSPGQSITISCTGTSSDVGGYNFVSWYQQHPGKAPKLM^IYDVSDRPSGVSDRFSGSKSGNTASLI

>SEQ ID 13 SI-71MM/ heavy chain nucleotide sequence

CAGGTGCAATTGCAGGAGTCGGGGGAGGCCTGCTCAAGCCTGGAGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGG
ATTCACCTTTAGTAGTTATTGGATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTGGCCACATAA
ACCGCGATGGAAGTGCAGATTACTATGTGAGACTCTGTGGAAGGGCCGATTACCATTCTCCAGAGCAGCGCCAAAGAC
TCACTGTATCTGCAAAAGACAGCCTGAGAGCTGAGGACCGGTGTGTTACTGTGCGAGAGATCCGTGGGTGGG
TCTACTTCGATCTCTGGGGCCGTGGCACCTGGTCAACCGTCTCGAGCGCTAGCACCAAGGGCCCATCGGTCTTCCCC
TGGCACCCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCGAACC
GTGACGTTGTGCTGGAACCTCAGGCGCCCTGACACAGCGGCTGCACACCTTCCCGGCTGTCTACAGTCTCTCAGGACT
CTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGACCTTGGGCGATCCAGACCTACATCTGCAACCGTAATCACA
AGCCACGACAACCAAGGTGGACAAGAGATTGAGCCAAATCTTGTGACAAAACTCACATACGCCACCGTGGCCCA
GCACCTGAACCTCTGGGGGACCGTCAGTCTTCTTCTTCCCCCAAACCAAGGACACCTCATGATCTCCGGAC
CCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCTGAGGTCAAGTTCAACTGGTACGTGGACGGCG
TGGAGGTGCATAATGCCAAGACAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCTCTACC
GTCTGTGACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGTCTCCAAACAAGCCCTCCAGCCCCCATCGA
GAAACCATTCTCAAAGCCAAAGGCGAGCCCGGAGAACCAACAGGTGTACACGCTGCCCCCATCCCGGGATGAGCTGA
CCAAAGAACCGTGCAGCCTGACCTGGCTGGTCAAAAGCTTCTATCCAGCGACATCGCCGTGGATGGGAGGACGAT
GGGAGCCGGAGAACAACTACAAGACCACGCTCCCGTGGTGGACTCCGACGGCTCCTTCTTCTATAGCAAGCT
CACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACT
ACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTGGGGAGGTGGCTCCGGCGGTGGAGGCTCTGGAGGCGGGGGTTCC
GGCGGAGGTGGCTCAGGAGCGGAGGGTCAGGGGGAGGTGGCTCCGACAAGACCCACACCTGTCCACCTTGCCCCG
CCCTGAAGTCTGGGCGGGCCTTCCGTTTTTCTGTCTCCCCCTAAGCCTAAGGACACCTTGATGATCTCTCGGACAC
CCGAAGTGACCTGGCTGGTGGTGGACGTGTCATGAAGATCTGAGGTGAAGTCAACTGGTACGTGGACGGCGTG
GAAGTGACAACGCGCAAGACAAGCCAAAGAGAAGAACAGTACAACCTCAACTACAGAGTTGTGTCCGTGCTGACCGT
GCTGCATCAAGACTGGCTGAACGGCAAGAATACAAGTGAAGGTGAGTAACAAGGCTCTGCCTGCCCTATCGAGA
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AAGAACCAGGTGTCCCTGACCTGTCTGGTGAAGGCTTCTACCCCTCTGACATCGCCGTGGAATGGGAGTCGAACGG
CCAGCCCCGAGAACAACTACAAGACACCCCTCCCGTGTGAGCAGCGACGGATCTTCTTCTGTACTCCAAGCTGA
CCGTGCAGAAGTCTAGATGGCAGCAGGGCAACGTTCTCTCTGCTCTGTGATGCACGAGGCTCTGCACAATCACTAC
ACCCGAAGTCCCTGTCCCTATCGCCAGGC

>SEQ ID 14 SI-71MM7 heavy chain amino acid sequence

QVQLQESGGGLVKPGGSLRLSCAASGFTFSSYWMWVRQAPGKGLEWVANINRDGSASYVDSVKGRFTISRDDAKN
SLYLQMNLSRAEDTAVYYCARDRGVGYFDLWGRGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP
VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRVPEKSCDKTHTCPPCP
APPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLT
VLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN
GQFPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPG

>SEQ ID 15 SI-71MM7 light chain nucleotide sequence

CAGTCTGCCCTGACTCAGCCTGCCTCCGTGTCTGGGTCTCCTGGACAGTCGATCACCATCTCCTGCACTGGAACCCAG
CAGTGACGTTGGTGGTTATAACTTTGTCTCCTGGTACCAACAACACCCAGGCAAAGCCCCAACTCATGATCTATG
ATGTCAGTGATCGGCCCTCAGGGGTGTCTGATCGCTTCTCCGGCTCCAAGTCTGGCAACACGGCTCCCTGATCATC
TCTGGCCTCCAGGCTGACGACGAGGCTGATTATTACTGCAGCTCATATGGGAGCAGCAGCACTCATGTGATTTTCGG
CGGAGGGACCAAGGTGACCGTCTTAGGCCAACCGAAAGCGGCGCCCTCGGTCACTCTGTCCCGCCCTCCTCTGAGG
AGCTTCAAGCCAACAAGGCCACACTGGTGTGTCTATAAGTGACTTCTACCCGGGAGCCGTGACAGTGGCCTGGAAG
GCAGATAGCAGCCCCGTCAAGGCGGGAGTGGAGACCACACCCCTCCAAACAAGCAACAACAAGTACGCGGCCAG
CAGCTATCTGAGCTGACGCTGAGCAGTGAAGTCCCACAGAAGCTACAGCTGCCAGGTACGCATGAAGGGAGCA
CCGTGGAGAAGACAGTGGCCCTACAGAATGTTCA

>SEQ ID 16 SI-71MM7 light chain amino acid sequence

QSALTQPASVSGSPGQSITISCTGTSSDVGGYNFVSWYQQHPGKAPKLMYDVSDRPSGVSDRFGSGSKSGNTASLII
SGLQADDEADYCYSSYSSSTHVI FGGGTKVTVLGGPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWK
ADSSPVKAGVETTTPSKQSNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS

>SEQ ID 17 SI-71XM19 heavy chain nucleotide sequence

GATATCCAGATGACCCAGTCCCCGAGCTCCCTGTCCGCCTCTGTGGGCGATAGGGTCACCATCACCTGCCGTGCCAG
TCAGGATGTGAATACTGCTGTAGCCTGGTATCAACAGAAACCAGGAAAAGCTCCGAAACTACTGATTTACTCGGCAT
CCTTCCTCTACTCTGGAGTCCCTTCTCGCTTCTCTGGCTCCAGATCTGGGACGGATTTCACTCTGACCATCAGCAGT
CTGCAGCCGGAAGACTTCGCAACTTATTACTGTCAGCAACATTATACTACTCCTCCACGTTCCGGACAGGGTACCAA
GGTGGAGATCAAAGCGGTGGCGGTAGTGGGGGAGCGGTTCTGGCGGTGGAGGGTCCGGCGGTGGAGGATCAGAGG
TTCAGCTGGTGGAGTCTGGCGGTGGCCTGGTGCAGCCAGGGGGCTCACTCCGTTTGTCTGTGCAGCTTCTGGCTTC
AACATTAAAGACACCTATATACACTGGGTGCGTCAGGCCCGGGTAAGGGCTGGAATGGGTGCAAGGATTATCC
TACGAATGGTTATACTAGATATGCCGATAGCGTCAAGGGCCGTTTCACTATAAGCGCAGACACATCCAAAAACACAG
CCTACCTGCAGATGAACAGCCTGCGTGCTGAGGACACTGCCGTCTATTATTGTTCTAGATGGGGAGGGGACGGCTTC
TATGCTATGGACTACTGGGGTCAAGGAACCTGGTCACCGTCTCCTCGTCCGGAGGCGGTGGTTCTGGCGGTGGAGG
GCAGGTGCAATTGCAGGAGTCCGGGGGAGGCCTGGTCAAGCCTGGAGGGTCCCTGAGACTCTCCTGTGCAGCCTCTG
GATTCACCTTTAGTAGTTATTGGATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTGGCCACATA
AACC CGATGGAAGTGCGAGTTACTATGTGGACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACGACGCCAAGAA
CTCACTGTATCTGCAAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCGAGAGATCGTGGGGTGG
GCTACTTCGATCTCTGGGGCCGTGGCACCTGGTACCCTCTCGAGCGCTAGCACCAAGGGCCCATCGGTCTTCCCC
CTGGCACCTCTCCAAGAGCACCTCTGGGGGCACAGCGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACC
GGTGACGGTGTCTGGAACCTCAGGCGCCCTGACCAGCGCGTGCACACCTTCCCGGCTGTCTACAGTCTCAGGAC
TCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCAC
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CCCCTGAGGTACATGCGTGGTGGTGGACGTGAGCCACGAAGACCTGAGGTCAAGTTCAACTGGTACGTGGACGGC
GTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAAGCTCTCAC
CGTCTGCACAGGACTGGCTGAATGGCAAGGAGTACAAGTGAAGGTCTCCAACAAAGCCCTCCAGCCCCCATCG
AGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCTGCCCCATCCCGGGATGAGCTG
ACCAAGAACCAGGTGACCTGACCTGCCTGGTCAAAGGCTTCTATCCAGCGACATCGCCGTGGAGTGGGAGAGCAA
TGGGCAGCCGAGAACAACTACAAGACCACGCCTCCCGTGTGGACTCCGACGGCTCCTTCTCTCTATAGCAAGC
TCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCAC
TACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTGGGGGAGGTGGCTCCGGCGGTGGAGGGTCTGGAGGCGGGGGTTC

CGGCGGAGGTGGCTCAGGAGGCGGAGGGTCAGGGGGAGGTGGCTCCGACAAGACCCACACCTGTCCACCTTGCCCCG
CCCCGAACTGCTGGGCGGCCCTTCCGTTTTCTGTCCCCCCTAAGCCTAAGGACACCCTGATGATCTCTCGGACA
CCCGAAGTGACCTGCGTGGTGGTGGACGTGTCCCATGAAGATCCTGAGGTGAAGTTCAACTGGTACGTGGACGGCGT
GGAAGTGCACAACGCCAAGACCAAGCCAAGAGAAGAACAGTACAACCTCCACTTACAGAGTTGTGTCCGTGCTGACCG
TGCTGCATCAAGACTGGCTGAACGGCAAAGAATACAAGTGCAAGGTGAGTAACAAGGCTCTGCCTGCCCTATCGAG
AAAACCATCTCTAAGGCTAAGGGACAACCTAGAGAGCCTCAGGTGTACACCTGCCTCCTTCCCGGGACGAGCTGAC
CAAGAACCAGGTGTCCCTGACCTGTCTGGTGAAAGGCTTCTACCCCTCTGACATCGCCGTGGAATGGGAGTCGAACG
GCCAGCCCGAGAACAACCTACAAGACCACCCCTCCCGTGTGGACAGCGACGGATCTTCTTCTTCTGACTCCAAGCTG
ACCGTCGACAAGTCTAGATGGCAGCAGGGCAACGTGTTCTCCTGCTCTGTGATGCACGAGGCTCTGCACAATCACTA
CACCCAGAAGTCCCTGTCCCTATCGCCAGGC

>SEQ ID 18 SI-71XM19 heavy chain amino acid sequence

DIQMTQSPSSLSASVGDRTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASF~~LYSGVPSRFS~~SGSRSGTDFTLTIS
LQPEDFATYYCQ~~QHY~~HTTPTFGQGTKEIKGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASG
FNI~~KD~~TYIHWVRQAPGKLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSR~~WGGD~~GF
YAMDYWGQGT~~LV~~TVSSSGGGGSGGGGQVQLQESGGGLVKPGGSLRLSCAASGFTFSSYWMSWVRQAPGKLEWVANI
NRDGSASYYVDSVKGRFTISRDDAKNSLYLQMNSLRAEDTAVYYCARDRGVGYFDLWGRGTLTVSSASTKGPSVF
P LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNH
KPSNTKVDKRVPEKSCDKHTHTCPPEPAPELLGGPSVFLFPPKPKDTLMISRTPEVTVCVVDVSHEDPEVKFNWYVDG
VEVHNAKTKPREEQYNSTYRVSVLT~~VL~~HQDWLNGKEYKCKVSNKALPAPIEKTI~~SKAKGQ~~PREPQVYTLPPSRDEL
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK~~TT~~PPVLDSDGSFFLYSKLTVDKSRWQQGNV~~F~~SCSVMH~~EAL~~HNH
YTQKSLSLSPGGGGSGGGGSGGGGSGGGGSGGGGSDKHTHTCPPEPAPELLGGPSVFLFPPKPKDTLMISRT
PEVTVCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLT~~VL~~HQDWLNGKEYKCKVSNKALPAPIE
KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK~~TT~~PPVLDSDGSFFLYSKL
TVDKSRWQQGNV~~F~~SCSVMH~~EAL~~HNHYTQKSLSLSPG

>SEQ ID 19 SI-71XM19 light chain nucleotide sequence

CAGTCTGCCCTGACTCAGCCTGCCTCCGTGCTCTGGGTCTCCTGGACAGTCGATCACCATCTCCTGCACTGGAACCA
CAGTGACGTTGGTGGTTATACTTTGTCTCCTGGTACCAACAACACCCAGGCAAAGCCCCAACTCATGATCTATG
ATGTCAGTGATCGGCCCTCAGGGGTGTCTGATCGCTTCTCCGGCTCCAAGTCTGGCAACACGGCCTCCCTGATCATC
TCTGGCCTCCAGGCTGACGACGAGGCTGATTATTACTGCAGCTCATATGGGAGCAGCAGCACTCATGTGATTTTCGG
CGGAGGGACCAAGGTGACCGTCTAGGCCAACCGAAAGCGGGCGCCCTCGGTCACTCTGTTCCCGCCCTCCTCTGAGG
AGCTTCAAGCCAACAAGGCCACACTGGTGTGTCTCATAAGTGACTTCTACCCGGGAGCCGTGACAGTGGCCCTGGAAG
GCAGATAGCAGCCCCGTCAAGCGCGGAGTGGAGACCACACACCCCTCCAACAACAAGCAACAAGTACCGCGGCCAG
CAGCTATCTGAGCCTGACGCCTGAGCAGTGAAGTCCACAGAAGCTACAGCTGCCAGGTACGCATGAAGGGAGCA
CCGTGGAGAAGACAGTGGCCCTACAGAATGTTCA

>SEQ ID 20 SI-71XM19 light chain amino acid sequence

QSALTQPASVSGSPGQSITISCTGTSSDVGGYNFVSWYQQHPGKAPKLMYDVS~~DRPSGVSDR~~FSGSKSGNTASLI
ISGLQADDEADYYC~~SS~~SYSSSTHVI~~FGGG~~TKVTVLQGPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWK
ADSSPVKAGVETTTPSKQSNKYAASSYLSLTPEQWKS~~SHRSY~~SCQVTHEGSTVEKTVAPTECS

>SEQ ID 21 anti-HER2 trastuzumab variable heavy (VH) domain nucleotide
sequence

GAGGTTCACTGGTGGAGTCTGGCGGTGGCCTGGTGCAGCCAGGGGGCTCACTCCGTTTGTCTGTGCAGCTTCTGG
CTTCAACATTAAAGACACCTATATACACTGGGTGCGTCAGGCCCCGGGTAAAGGCCTGGAATGGGTGCAAGGATTT
ATCCTACGAATGGTTATACTAGATATGCCGATAGCGTCAAGGGCCGTTTCACTATAAGCGCAGACACATCCAAAAAC
ACAGCCTACCTGCAGATGAACAGCCTGCGTGTGAGGACACTGCCGTCTATTATTGTTCTAGATGGGGAGGGGACGG
CTTCTATGCTATGGACTACTGGGGTCAAGGAACCTGGTCACCGTCTCCTCG

>SEQ ID 22 anti-HER2 trastuzumab variable heavy (VH) domain amino acid
sequence

EVQLVESGGGLVQPGGSLRLSCAASGFNI~~KD~~TYIHWVRQAPGKLEWVARIYPTNGYTRYADSVKGRFTISADTSKN
TAYLQMNSLRAEDTAVYYCSR~~WGGD~~GFYAMDYWGQGT~~LV~~TVSS

>SEQ ID 23 anti-HER2 trastuzumab variable light (VL) domain nucleotide
sequence

GATATCCAGATGACCCAGTCCCCGAGCTCCCTGTCCGCCTCTGTGGGCGATAGGGTCACCATCACCTGCCGTGCCAG
TCAGGATGTGAATACTGCTGTAGCCTGGTATCAACAGAAACCAGGAAAAGCTCCGAACTACTGATTTACTCGGCAT
CCTTCCTCTACTCTGGAGTCCCTTCTCGCTTCTCTGGCTCCAGATCTGGGACGGATTTCACCTCTGACCATCAGCAGT
CTGCAGCCGGAAGACTTCGCAACTTATTACTGTCAGCAACATTATACTACTCTCCACGTTCCGGACAGGGTACCAA
GGTGGAGATCAAA

>SEQ ID 24 anti-HER2 trastuzumab variable light (VL) domain nucleotide
sequence

DIQMTQSPSSLSASVGDRVITITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFRSGSRSGTDFLTIISS
LQPEDFATYYCQOHYTPPTFGQGTKVEIK

>SEQ ID 25 anti-HER3 MM-111 variable heavy (VH) domain nucleotide sequence
CAGGTGCAATTGCAGGAGTCGGGGGAGGCCTGGTCAAGCCTGGAGGTCCTGAGACTCTCCTGTGCAGCCTCTGG
ATTACCTTTAGTAGTTATTGGATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTGGCCAACATAA
ACCGCGATGGAAGTGCAGTTACTATGTGGACTCTGTGAAGGGCCGATTACCATCTCCAGAGACGACGCCAAGAAC
TCACTGTATCTGCAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCGAGAGATCGTGGGGTGGG
CTACTTCGATCTCTGGGGCCGTGGCACCTGGTCACCGTCTCGAGC

>SEQ ID 26 anti-HER3 MM-111 variable heavy (VH) domain amino acid sequence
QVQLQESGGGLVKPGGSLRLSCAASGFTFSSYWMWVRQAPGKLEWVANINRDGSASYVDSVKGRFTISRDDAKN
SLYLQMNSLRADTAVYYCARDRGVGYFDLWGRGTLVTVSS

>SEQ ID 27 anti-HER3 MM-111 variable light (VL) domain nucleotide sequence
CAGTCTGCCCTGACTCAGCCTGCCTCCGTGTCTGGGTCTCCTGGACAGTCGATACCATCTCCTGCACTGGAACAG
CAGTGACGTTGGTGGTTATACTTTGTCTCCTGGTACCAACAACACCCAGGCAAAGCCCCAACTCATGATCTATG
ATGTCAGTGATCGGCCCTCAGGGGTGTCTGATCGCTTCTCCGGCTCCAAGTCTGGCAACACGGCCTCCCTGATCATC
TCTGGCCTCCAGGCTGACGACGAGGCTGATTATTACTGCAGCTCATATGGGAGCAGCAGCACTCATGTGATTTTCGG
CGGAGGACCAAGGTGACCGTCCTA

>SEQ ID 28 anti-HER3 MM-111 variable light (VL) domain amino acid sequence
QSALTQPASVSGSPGQSITISCTGTSSDVGGYNFVSWYQQHPGKAPKLMIDVSDRPSGVSDRFRSGSKSGNTASLII
SGLQADDEADYYCSSYSSSTHVFEGGKTKVTVL

>SEQ ID 29 anti-HER2 trastuzumab scFv nucleotide sequence
GATATCCAGATGACCCAGTCCCCGAGCTCCCTGTCCGCCTCTGTGGGCGATAGGGTCACCATCACCTGCCGTGCCAG
TCAGGATGTGAATACTGCTGTAGCCTGGTATCAACAGAAACCAGGAAAAGCTCCGAACTACTGATTTACTCGGCAT
CCTTCCTCTACTCTGGAGTCCCTTCTCGCTTCTCTGGCTCCAGATCTGGGACGGATTTCACCTCTGACCATCAGCAGT
CTGCAGCCGGAAGACTTCGCAACTTATTACTGTCAGCAACATTATACTACTCTCCACGTTCCGGACAGGGTACCAA
GGTGGAGATCAAAGCGGTGGCGGTAGTGGGGGAGGCGGTTCTGGCGGTGGAGGGTCCGGCGGTGGAGGATCAGAGG
TTCAGCTGGTGGAGTCTGGCGGTGGCCTGGTGCAGCCAGGGGGCTCACTCCGTTTGTCTGTGCAGCTTCTGGCTTC
AACATTAAGACACCTATATACACTGGGTGCGTCAAGCCCCGGGTAAGGGCCTGGAATGGGTTGCAAGGATTTATCC
TACGAATGGTTATACTAGATATGCCGATAGCGTCAAGGGCCGTTTCACTATAAGCGCAGACACATCCAAAAACACAG
CCTACCTGCAGATGAACAGCCTGCGTGCTGAGGACACTGCCGTCTATTATTGTTCTAGATGGGAGGGGACGGCTTC
TATGCTATGGACTACTGGGTCAAGGAACCTGGTCACCGTCTCCTCG

>SEQ ID 30 anti-HER2 trastuzumab scFv amino acid sequence
DIQMTQSPSSLSASVGDRVITITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFRSGSRSGTDFLTIISS
LQPEDFATYYCQOHYTPPTFGQGTKVEIKGGGSGGGSGGGSGGGSEVQLVESGGGLVQPGGSLRLSCAASGF
NIKDTYIHWVRQAPGKLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRADTAVYYCSRWGGDGF
YAMDYWGQGTLLVTVSS

>SEQ ID 31 anti-HER3 MM-111 scFv nucleotide sequence
CAGGTGCAGCTGCAGGAGTCGGGGGAGGCCTGGTCAAGCCTGGAGGTCCTGAGACTCTCCTGTGCAGCCTCTGG
ATTCACCTTTAGTAGTTATTGGATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTGGCCAACATAA
ACCGCGATGGAAGTGCAGTTACTATGTGGACTCTGTGAAGGGCCGATTACCATCTCCAGAGACGACGCCAAGAAC
TCACTGTATCTGCAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCGAGAGATCGTGGGGTGGG
CTACTTCGATCTCTGGGGCCGTGGCACCTGGTCACCGTGTCTAGCGGTGGAGGCGGTTCAAGCGGAGGTTGGTCCG

GCGGTGGCGGCTCCAGTCTGCCCTGACTCAGCCTGCCTCCGTGTCTGGGTCTCCTGGACAGTCGATCACCATCTCC
TGCACCTGGAACAGCAGTGACGTTGGTGGTTATAACTTTGTCTCCTGGTACCAACAACACCCAGGCAAAGCCCCAA
ACTCATGATCTATGATGTAGTGATCGGCCCTCAGGGGTGTCTGATCGCTTCTCCGGCTCCAAGTCTGGCAACACGG
CCTCCCTGATCATCTCTGGCCTCCAGGCTGACGACGAGGCTGATTATTACTGCAGCTCATATGGGAGCAGCAGCACT
CATGTGATTTTCGGCGGAGGGACCAAGGTGACCGTCCTA

>SEQ ID 32 anti-HER3 MM-111 scFv amino acid sequence

QVQLQESGGGLVKPGGSLRLSCAASGFTFSSYWMSWVRQAPGKLEWVANINRDGSASYVDSVKGRFTISRDDAKN
SLYLQMSNLR AEDTAVYYCARDRGVGYFDLWGRGTLVTVSSGGGSGGGSGGGSGQSALTQPASVSGSPGQSITIS
CTGTSSDVGGYNFVSWYQHPGKAPKLMYDVDSDRPSGVSDRFSGSKSGNTASLIISGLQADDEADYYCSSYGSSST
HVIFFGGGTKVTVL

>SEQ ID 33 anti-HER3 MM-111 scFv nucleotide sequence

CAAGTGC AACTACAAGAAAGTGGTGGTGGTCTCGTGAAGCCCGAGGCTCTCTGCGGCTGTCTGTGCTGCTTCTGG
CTTTACATTCTCCTCTTACTGGATGTCCTGGGTGACAGAGGCTCCTGGCAAGGGCCTGGAATGGGTGGCCAAACATCA
ACCGGGATGGCTCCGCCTCTTATTACGTGGACTCCGTGAAAGGCAGATTACCATCTCTCTGGGACGACGCCAAGAAC
TCCCTGTACCTGCAGATGAATAGCCTGAGAGCCGAGGACACCGCTGTGTACTACTGCGCCAGAGATCGCGGCGTGGG
CTACTTCGACCTGTGGGGACGGGACCCCTGGTGACCGTGTCCAGCGGTGGCGGCGGCAGTGGAGGCGGCGGCTCTG
GCGGCGGGGCTCCCAAAGTGCACTAACTCAACCAGCAAGTGTGAGCGGATCTCCCGGCCAGTCCATCACCATCTCC
TGTACCGGCACATCCAGCGATGTCGGCGGCTACAACCTTCGTGTCTTGGTATCAACAGACCCCTGGCAAGGCCCTAA
GCTGATGATCTACGACGTGTCCGATAGACCTTCTGGCGTGTCCGACCGGTTAGCGGTTCCAAGTCCGGCAACACCG
CTTCTCTGATCATCTCTGGCCTGCAGGCCGACGACGAGGCTGACTACTGCTCCTCCTACGGCTCCTCTTCTACC
CACGTGATCTTCGGCGGAGGCACCAAAGTGACCGTGCTG

>SEQ ID 34 anti-HER3 MM-111 scFv amino acid sequence

QVQLQESGGGLVKPGGSLRLSCAASGFTFSSYWMSWVRQAPGKLEWVANINRDGSASYVDSVKGRFTISRDDAKN
SLYLQMSNLR AEDTAVYYCARDRGVGYFDLWGRGTLVTVSSGGGSGGGSGGGSGQSALTQPASVSGSPGQSITIS
CTGTSSDVGGYNFVSWYQHPGKAPKLMYDVDSDRPSGVSDRFSGSKSGNTASLIISGLQADDEADYYCSSYGSSST
HVIFFGGGTKVTVL

>SEQ ID 35 SI-71XM32 heavy chain nucleotide sequence

CAGGTGCAGCTGCAGGAGTCGGGGGAGGCCTGGTCAAGCCTGGAGGTCCTGAGACTCTCCTGTGCAGCCTCTGG
ATTCACCTTTAGTAGTTATTGGATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGCTGGAGTGGGTGGCCAAACATAA
ACCGCGATGGAAGTGGAGTTACTATGTGACTCTGTGAAGGGCCGATTACCATCTCCAGAGACGACGCCAAGAAC
TCACTGTATCTGCAAAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCGAGAGATCGTGGGGTGGG
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GCGGTGGCGGCTCCAGTCTGCCCTGACTCAGCCTGCCTCCGTGTCTGGGTCTCCTGGACAGTCGATCACCATCTCC
TGCACCTGGAACAGCAGTGACGTTGGTGGTTATAACTTTGTCTCCTGGTACCAACAACACCCAGGCAAAGCCCCAA
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CCTCCCTGATCATCTCTGGCCTCCAGGCTGACGACGAGGCTGATTATTACTGCAGCTCATATGGGAGCAGCAGCACT
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GCTGGTGGAGTCTGGCGGTGGCTGGTGCAGCCAGGGGGCTCACTCCGTTTGTCTCTGTGCAGCTTCTGGCTTCAACA
TTAAAGACACCTATATACACTGGGTGCGTCAGGCCCGGGTAAGGGCTGGAATGGGTGCAAGGATTTATCCTACG
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CCTGCAGATGAACAGCCTGCGTGCTGAGGACACTGCCGTCTATTATTGTTCTAGATGGGGAGGGGACGGCTTCTATG
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GCACCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGT
GACGGTGTCTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCTACAGTCTCAGGACTCT
ACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAG
CCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCCAAACTTGTGACAAAACCTCACACATGCCACCGTGGCCAGC
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AAGAACCAGGTGACCTGCCTGGTCAAAGGCTTCTATCCAGCGACATCGCGTGGAGTGGGAGAGCAATGG
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CGGAGGTGGCTCAGGAGGCGGAGGGTCAGGGGGAGGTGGCTCCGACAAGACCCACACCTGTCCACCTTGCCCGGCC
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GTCGACAAGTCTAGATGGCAGCAGGGCAACGTGTTCTCCTGCTCTGTGATGCACGAGGCTCTGCACAATCACTACAC
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>SEQ ID 36 SI-71XM32 heavy chain amino acid sequence

QVQLQESGGGLVPGGSLRLSCAASGFTFSSYWMSVWRQAPGKGLEWVANINRDGSASYVDSVKGRFTISRDDAKN
SLYLQMNSLRAEDTAVYYCARDRGVGYFDLWGRGTLTVTVSSGGGGSGGGSGGGGSQSALTQPASVSGSPGGQITIS
CTGTSSDVGGYNFVSWYQQHPGKAPKLMITYDVSDRPSGVSDRFSGSKSGNTASLIISGLQADDEADYYCSSYGSST
HVI FGGGTKVTVLSGGGGSGGGGEVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKLEWVARIYPT
NGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTTLTVTVSSASTKGPSVFPL
APSSKSTSGGTAAALGLVKDYFPEPVTVSWNSGALTSVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHNK
PSNTKVKDRVEPKSCDKHTHTCPCPAPELLGGPSVFLFPPPKPDKTLMISRTPEVTCVVDVDSHEDPEVKFNWYVDGV
EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDEL
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHY
TQKSLSLSPGGGGSGGGSGGGSGGGSGGGSGGGSGGGSDKHTHTCPCPAPELLGGPSVFLFPPPKPDKTLMISRTPE
VTCVVDVDSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK
TISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLT
VDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPG

>SEQ ID 38 SI-71XM32 light chain nucleotide sequence

GATATCCAGATGACCCAGTCCCCGAGTCCCTGTCCGCCTCTGTGGCGATAGGGTCACCATCACCTGCCGTGCCAG
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CCTTCCTCTACTCTGGAGTCCCTTCTCGCTTCTCTGGCTCCAGATCTGGGACGGATTTCACTCTGACCATCAGCAGT
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GGTGGAGATCAAAAGTACGGTGGCTGCACCATCTGTCTTCTCCGCCATCTGATGAGCAGTTGAAATCTGGAA
CTGCCTCTGTTGTGTGCCTGCTGAATACTTCTATCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTC
CAATCGGGTAACTCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCTGAC
GCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCA
CAAAGAGCTTCAACAGGGGAGAGTGT

>SEQ ID 38 SI-71XM32 light chain amino acid sequence

DIQMTQSPSSLSASVGRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFELYSGVPSRFSRSGRSGTDFTLTIS
LQPEDFATYYCQQHYTTPPTFGQGTKEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNAL
QSGNSQESVTEQDSKSTSYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

>SEQ ID 39 SI-71XM40 heavy chain nucleotide sequence

CAGGTGCAGCTGCAGGAGTCGGGGGAGGCCTGGTCAAGCCTGGAGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGG
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ACCGCGATGGAAGTGCAGTTACTATGTGGACTCTGTGAAGGGCCGATTACCATCTCCAGAGACGACGCCAAGAAC
TCACTGTATCTGCAAAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCGAGAGATCGTGGGGTGGG
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GCGGTGGCGGCTCCAGTCTGCCCTGACTCAGCCTGCCTCCGTGTCTGGGTCTCCTGGACAGTCGATCACCATCTCC
TGCACTGGAACCAGCAGTGACGTTGGTGGTTATAACTTTGTCTCCTGGTACCAAGAGCACCCAGGCAAAGCCCCAA
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AAGAACCAGGTGACCTGACCTGCCTGGTCAAAGGCTTCTATCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGG
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ACGCAGAGAGCCCTCTCCCTGTCTCCGGTGGGGGAGGTGGCTCCGGCGGTGGAGGGTCTGGAGCGGGGGTTCGGG
CGGAGGTGGCTCAGGAGCGGAGGGTCAAGGGGAGGTGGCTCCGACAAGACCCACACCTGTCCACCTGCCCCGGCC
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GAAGTGACCTGCGTGGTGGTGACGTGTCCCATGAAGATCCTGAGGTGAAGTTCAACTGGTACGTGGACGGCGTGGA
AGTGACAACGCCAAGACCAAGCAAGAGAAGAACAGTACAACCTCACTTACAGAGTTGTGTCCGTGCTGACCGTGC
TGCATCAAGACTGGCTGAACGGCAAAGAATACAAGTGCAAGGTGAGTAACAAGGCTCTGCCTGCCCTATCGAGAAA
ACCATCTCTAAGGCTAAGGGACAACCTAGAGAGCCTCAGGTGTACACCTGCCTCCTTCCCGGACGAGCTGACCAA
GAACAGGTGTCCGTGACCTGTCTGGTGAAGGCTTCTACCCCTCTGACATCGCCGTGGAATGGGAGTCGAACGGCC
AGCCCGAGAACAATAACAAGACCACCCCTCCCGTGTGGACAGCGACGGATCTTTCTTCTGTACTCCAAGCTGACC
GTCGACAAGTCTAGATGGCAGCAGGGCAACGTGTTCTCCTGCTGTGTATGCACGAGGCTCTGCACAATCACTACAC
CCAGAAGTCCCTGTCCCTATCGCCAGGC

>SEQ ID 40 SI-71XM40 heavy chain amino acid sequence

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SLYLQMNLSRAEDTAVYYCARDRGVGYFDLWGRGTLTVTVSSGGGGSGGGSGGGGSQSALTQPASVSGSPGQSITIS
CTGTSSDVGGYNFVSWYQEHFGKAPKLMYDVSDRPSGVSDRFSGSKSGNTASLIISGLQADDEADYYCSSYSSST
HVIFFGGGTKVTVLSGGGGSGGGGEVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVREAPGKLEWVARIYPT
NGYTRYADSVKGRFTISADTSKNTAYLQMNLSRAEDTAVYYCSRWGGDGFYAMDYWGQGLTVTVSSASTKGPSVFPL
APSSKSTSGGTAAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHK
PSNTKVDKRVEPKSCDKHTHTCPPCPAPELLGGPSVFLFPPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV
EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDEL
KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVVFSCSVMEALHNHY
TQKSLSLSPGGGGSGGGSGGGSGGGSGGGSGGGSGGGSDKHTHTCPPCPAPELLGGPSVFLFPPPKPKDTLMISRTPE
VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT
ISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLT
VDKSRWQQGNVVFSCSVMEALHNHYTQKSLSLSPG

>SEQ ID 41 SI-71XM40 light chain nucleotide sequence

GATATCCAGATGACCCAGTCCCCGAGCTCCCTGTCCGCCCTCTGTGGGCGATAGGGTACCATCACCTGCCGTGCCAG
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CCTTCTCTACTCTGGAGTCCCTTCTCGCTTCTCTGGCTCCAGATCTGGGACGGATTTCACTCTGACCATCAGCAGT
CTGCAGCCGGAAGACTTCGCAACTTATTACTGTACAGCAACATTATACTACTCTCTCCACGTTCCGACAGGGTACCAA
GGTGGAGATCAAACGTACGGTGGCTGCACCATCTGTCTTCTATCTTCCCGCATCTGATGAGCAGTTGAAATCTGGAA
CTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGAAGGTGGATAACGCCCTC
CAATCGGGTAACCTCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCTGAC
GCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCGCTCA
CAAAGAGCTTCAACAGGGGAGAGTGT

>SEQ ID 42 SI-71XM40 light chain amino acid sequence

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQRKPGKAPKLLIYSASFLYSGVPSRFSRSGSGTDFLTIS
LQPEDFATYYCQHHYTPPTFGQGTKEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPRQAKVQWKVDNAL
QSGNSQESVTEQDSKSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

>SEQ ID 43 SI-71XM41 heavy chain nucleotide sequence

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 CCTTCCTCTACTCTGGAGTCCCTTCTCGCTTCTCTGGCTCCAGATCTGGGACGGATTTACTCTGACCATCAGCAGT
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 GGTGGAGATCAAAGGCGGTGGCGGTAGTGGGGGAGGCGGTTCTGGCGGTGGAGGGTCCGGCGGTGGAGGATCAGAGG
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 AACATTAAGACACCTATATACACTGGGTGCGTGAGGCCCCGGGTAAGGGCCTGGAATGGGTTGCAAGGATTTATCC
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 TATGCTATGGACTACTGGGTCAAGGAACCCTGGTCACCGTCTCCTCGTCCGGAGGCGGTGGTTCTGGCGGTGGAGG
 GCAGGTGCAATTGACAGAGTCCGGGGGAGGCTGGTCAAGCCTGGAGGGTCCCTGAGACTCTCCTGTGCAGCCTCTG
 GATTCACCTTTAGTAGTTATTGGATGAGCTGGGTCCGCAGAGCTCCAGGGAAGGGGCTGGAGTGGGTGGCCAACATA
 AACCGCGATGGAAGTGGAGTTACTATGTGGACTCTGTGAAGGGCCGATTACCATCTCCAGAGACGACGCCAAGAA
 CTCACCTATCTGCAAAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCGAGAGATCGTGGGTGG
 GCTACTTCGATCTCTGGGGCCGTGGCACCTGGTCACCGTCTCGAGCGCTAGCACCAAGGGCCCATCGGTCTTCCCC
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 TCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCAC
 AAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCCAAATCTTGTGACAAAACCTACACATGCCACCGTGCCC
 AGCACCTGAACCTCTGGGGGACCGTCAGTCTTCTCTTCCCCCAAACCAAGGACACCCTCATGATCTCCCGGA
 CCCCTGAGGTACATGCGTGGTGGTGGAGCTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGC
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 CCCCTGAAGTGTGGGCGGCCCTTCCGTTTTCTGTTCCTCCCTAAGCCTAAGGACACCCTGATGATCTCTCGGACA
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 GGAAGTGACACAACGCCAAGACCAAGCCAAAGAGAAGACAGTACAACCTCCACTTACAGAGTTGTGTCCTGTGACCG
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 CAAGAACCAGGTGTCCCTGACCTGTCTGGTGAAAGGCTTCTACCCCTCTGACATCGCCGTGGAATGGGAGTCGAACG
 GCCAGCCCGAGAACAACTACAAGACCACCCTCCCGTGTGGACAGCGACGGATCTTCTCTCTGTACTCCAAGCTG
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 CACCCAGAAGTCCCTGTCCCTATCGCCAGGC

>SEQ ID 44 SI-71XM41 heavy chain amino acid sequence

DIQMTQSPSSLSASVGRVTITCRASQDVNTAVAWYQRKPGKAPKLLIYSASF¹LYSGVPSRFS²SGSRSGTDFTLT³ISS
 LQPEDFATYYCQ⁴QHYTTPPTFGQGTKEIKGGGSGGGGSGGGGSGGGGSEVQLV⁵ESGGGLVQPGGSLRLSCAASG⁶F
 NIKDTYIHWVREAPGKLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGF⁷
 YAMDYWGQGLTVTVSSSGGGGSGGGGQVQLQESGGGLVKPGGSLRLSCAASGFTFSSYWMSWVRRAPGKLEWVANI⁸
 NRDGSASYVDSVKGRFTISRDAKNSLYLQMNSLRAEDTAVYYCARDRVGYFDLWGRGTLTVTVSSASTKGPSVFP⁹
 LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTQYICNVNH¹⁰
 KPSNTKVDKRVEPKSCDKHTCTPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDG¹¹
 VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTI¹²SKAKGQPREPQVYTLPPSRDEL
 TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNV¹³FSCVMHEALHNH
 YTQKSLSLSPGGGGSGGGGSGGGGSGGGGSGGGGSDKHTCTPPCPAPELLGGPSVFLFPPKPKDTLMISRT¹⁴
 PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE¹⁵
 KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKL¹⁶
 TVDKSRWQQGNV¹⁷FSCVMHEALHNHYTQKSLSLSPG¹⁸

>SEQ ID 45 SI-71XM41 light chain nucleotide sequence
CAGTCTGCCCTGACTCAGCCTGCCTCCGTGTCTGGGTCTCCTGGACAGTCGATCACCATCTCCTGCACTGGAACCAG
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ATGTCAGTGATCGGCCCTCAGGGGTGTCTGATCGCTTCTCCGGCTCCAAGTCTGGCAACACGGCCTCCCTGATCATC
TCTGGCCTCCAGGCTGACGACGAGGCTGATTATTACTGCAGCTCATATGGGAGCAGCAGCACTCATGTGATTTTCGG
CGGAGGGACCAAGGTGACCGTCCTAGGCCAACCAGAAAGCGGCGCCCTCGGTCACTCTGTTCCCGCCCTCCTCTGAGG
AGCTTCAAGCCAACAAGGCCACACTGGTGTGTCTCATAAGTGACTTCTACCCGGGAGCCGTGACAGTGGCCTGGAAG
GCAGATAGCAGCCCCGTCAAGGCGGGAGTGGAGACCACACACCCTCCAAACAAAGCAACAACAAGTACGCGGCCAG
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CCGTGGAGAAGACAGTGGCCCTACAGAATGTTCA

>SEQ ID 46 SI-71XM41 light chain amino acid sequence
QSALTQPASVSGSPGQSITISCTGTSSDVGGYNFVSWYQEHGKAPKLMYDVSDRPSGVSDRFGSGSKSGNTASLII
SGLQADDEADYYCSSYGSSSTHVI FGGGTKVTVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWK
ADSSPVKAGVETTPSKQSNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS

>SEQ ID 47 anti-HER2 trastuzumab CDR-H1 amino acid sequence
DTYIH

>SEQ ID 48 anti-HER2 trastuzumab CDR-H2 amino acid sequence
RIYPTNGYTRYADSVKG

>SEQ ID 49 anti-HER2 trastuzumab CDR-H3 amino acid sequence
WGGDGFYAMDY

>SEQ ID 50 anti-HER2 trastuzumab CDR-L1 amino acid sequence
RASQDVNTAVA

>SEQ ID 51 anti-HER2 trastuzumab CDR-L2 amino acid sequence
SASFLYS

>SEQ ID 52 anti-HER2 trastuzumab CDR-L3 amino acid sequence
QQHYTTPPT

>SEQ ID 53 anti-HER3 MM-111 CDR-H1 amino acid sequence
SYWMS

>SEQ ID 54 anti-HER3 MM-111 CDR-H2 amino acid sequence
NINRDGSASYVDSVKG

>SEQ ID 55 anti-HER3 MM-111 CDR-H3 amino acid sequence
DRGVGYFDL

>SEQ ID 56 anti-HER3 MM-111 CDR-L1 amino acid sequence
TGTSSDVGGYNFVS

>SEQ ID 57 anti-HER3 MM-111 CDR-L2 amino acid sequence
DVSDRPS

>SEQ ID 58 anti-HER3 MM-111 CDR-L3 amino acid sequence
SSYGSSSTHVI

REFERENCES

Each of the references cited herein is incorporated by reference in its entirety. The incorporation of these references is not to be construed as an admission that any of the material constitutes prior art to the present invention.

1. Diaz-Serrano, A. et al. Genomic Profiling of HER2-Positive Gastric Cancer: PI3K/Akt/mTOR Pathway as Predictor of Outcomes in HER2-Positive Advanced Gastric Cancer Treated with Trastuzumab. *Oncologist*.23, 1092–1102 (2018).
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BISPECIFIC ANTIBODY-LIKE PROTEIN AND METHODS OF MAKING AND USING THEREOF**CLAIMS**

What is claimed is:

1. An antibody-like protein having a N terminal and a C terminal, comprising a Fab region and a Fc region, wherein the Fc region comprise a first Fc domain linked to a second Fc domain through a second hinge, wherein the first Fc domain and the second Fc domain pair to form the Fc region.
2. The antibody-like protein of Claim 1, comprising,
 - a light chain, comprising, from the N-terminus to the C-terminus, a VL domain and a CL domain,
 - a heavy chain, comprising, from the N-terminus to the C-terminus, a VH domain, a CH1 domain, a first hinge, and the Fc region, wherein the first Fc domain comprises a first CH2 domain and a first CH3 domain, and wherein the second Fc domain comprises a second CH2 domain and a second CH3 domainwherein the VL domain and VH domain form the Fab region,
wherein the light chain and the heavy chain are covalently paired through at least one disulfide bond between the CH1 domain and the CL domain.
3. The antibody-like protein of Claim 2, wherein the first Fc domain pairs with the second Fc domain to form the Fc region through 2 disulfide bonds between the first hinge and the second hinge.
4. The antibody-like protein of Claim 2, further comprises a first scFv domain having a scFv VH domain and a scFv VL domain, wherein the antibody-like protein has a binding affinity to HER2 and HER3.
5. The antibody-like protein of Claim 4, wherein the first scFv domain is linked through a linker to the heavy chain at the N terminal.
6. The antibody-like protein of Claim 4, further comprising a second scFv domain linked to the first scFv domain in tandem.
7. The antibody-like protein of Claim 6, wherein the first and the second scFv domain are the same.
8. The antibody-like protein of Claim 7, wherein the Fab region has a binding affinity to HER2, and each scFv domain has a binding affinity to HER3.
9. The antibody-like protein of Claim 4, wherein the Fab region has a binding affinity to HER2, and the first scFv domain has a binding affinity to HER3.
10. The antibody-like protein of Claim 9, wherein the Fab region has a binding affinity to HER2 with a KD from about 1 pM to about 100 nM, and wherein the first scFv domain has a binding affinity to HER3 with a KD from about 1 pM to about 1 μM.

11. The antibody-like protein of Claim 4, wherein the Fab region has a binding affinity to HER3, and the scFv domain has a binding affinity to HER2.
12. The antibody-like protein of Claim 11, wherein the Fab region has a binding affinity to HER3 with a KD from about 1 pM to about 1 μ M, and wherein the first scFv domain has a binding affinity to HER2 with a KD from about 1 pM to about 100 nM.
13. The antibody-like protein of Claim 2, wherein the heavy chain comprises an amino acid sequence having at least 98% sequence identity to SEQ ID NO: 6, 10, 18, 36, 40 or 44.
14. The antibody-like protein of Claim 2, wherein the VH domain comprises an amino acid sequence having at least 98% sequence identity to SEQ ID NO: 22 or 26.
15. The antibody-like protein of Claim 2, wherein the light chain comprises an amino acid sequence having at least 98% sequence identity to SEQ ID NO: 8, 12, 20, 38, 42 or 46.
16. The antibody-like protein of Claim 2, wherein the VL domain comprises an amino acid sequence having at least 98% sequence identity to SEQ ID NO: 24 or 28.
17. The antibody-like protein of Claim 2, wherein the Fab region comprises CDR-H1 having a SEQ ID NO: 47, CDR-H2 having a SEQ ID NO: 48, and CDR-H3 having a SEQ ID NO: 49; and CDR-L1 having a SEQ ID NO: 50, CDR-L2 having a SEQ ID NO: 51, and CDR-L3 having a SEQ ID NO: 52.
18. The antibody-like protein of Claim 2, wherein the Fab region comprises CDR-H1 having a SEQ ID NO: 47, CDR-H2 having a SEQ ID NO: 48, and CDR-H3 having a SEQ ID NO: 49; and CDR-L1 having a SEQ ID NO: 50, CDR-L2 having a SEQ ID NO: 51, and CDR-L3 having a SEQ ID NO: 52.
19. The antibody-like protein of Claim 4, wherein the first scFv domain comprises an amino acid sequence having at least 98% sequence identity to SEQ ID NO: 30, 32 or 34.
20. The antibody-like protein of Claim 4, wherein the first scFv VH domain comprises an amino acid sequence having at least 98% sequence identity to SEQ ID NO: 22 or 26, and the first scFv VL domain comprises an amino acid sequence having at least 98% sequence identity to SEQ ID NO: 24 or 28.
21. The antibody-like protein of Claim 4 or 6, , the first scFv domain or the second scFv domain comprises CDR-H1 having a SEQ ID NO: 47, CDR-H2 having a SEQ ID NO: 48, and CDR-H3 having a SEQ ID NO: 49; and CDR-L1 having a SEQ ID NO: 50, CDR-L2 having a SEQ ID NO: 51, and CDR-L3 having a SEQ ID NO: 52.
22. The antibody-like protein of Claim 4 or 6, the first scFv domain or the second scFv domain comprises CDR-H1 having a SEQ ID NO: 53, CDR-H2 having a SEQ ID NO: 54, and CDR-H3 having a SEQ ID NO: 55; CDR-L1 having a SEQ ID NO: 56, CDR-L2 having a SEQ ID NO: 57, and CDR-L3 having a SEQ ID NO: 58.
23. The antibody-like protein of Claim 1, wherein the second hinge comprises an amino acid sequence ((Gly-Gly-Gly-Gly-Ser)_n, and wherein n is an integer of at least 5.
24. The antibody-like protein of Claim 23, wherein n is 6.

25. The antibody-like protein of Claim 5, wherein the linker comprises an amino acid sequence ((Gly-Gly-Gly-Gly-Ser)_m, and wherein m is an integer of at least 3.
26. An isolated nucleic acid sequence encoding the antibody-like protein of Claim 1.
27. An expression vector comprising the isolated nucleic acid sequences of Claim 26.
28. A host cell comprising the isolated nucleic acid sequence of Claim 26.
29. An immunoconjugate comprising the antibody-like protein of Claim 1 and a cytotoxic agent, wherein the cytotoxic agent comprises a radioisotope, a radionuclide, a therapeutic agent, a chemotherapeutic agent, or a combination thereof.
30. A pharmaceutical composition, comprising the antibody-like protein of Claim 1 or the immunoconjugate of Claim 29, and optionally a pharmaceutically acceptable carrier.
31. The pharmaceutical composition of Claim 30, further comprises a cytotoxic agent, wherein the cytotoxic agent comprises a radioisotope, a radionuclide, a therapeutic agent, a chemotherapeutic agent, or a combination thereof.
32. A method for treating or preventing cancer in a subject, said method comprising administering to the subject a pharmaceutical composition comprising the antibody-like protein of Claim 1 or the immunoconjugate of Claim 29.
33. The method of Claim 32, further comprises co-administering an effective amount of a therapeutic agent, wherein the therapeutic agent comprises an antibody, a chemotherapy agent, an enzyme, or a combination thereof.
34. The method of Claim 33, wherein the cancer comprises cells expressing HER2 or HER3, and wherein the cancer comprises breast cancer, colorectal cancer, pancreatic cancer, head and neck cancer, melanoma, ovarian cancer, endometrial cancer, epidermal cancer, prostate cancer, non-small cell lung cancer, small cell lung cancer, glioma, esophageal cancer, nasopharyngeal cancer, kidney cancer, gastric cancer, liver cancer, bladder cancer, cervical cancer, brain cancer, lymphoma, leukaemia, or myeloma.
35. The method of Claim 32, wherein the subject is a human.
36. A method for producing the antibody-like protein of Claim 1, comprising
culturing a host cell such that the DNA sequence encoding the antibody-like protein of Claim 1 is expressed, and
purifying said antibody-like protein.
37. A method for producing the immunoconjugate of Claim 29, comprising conjugating the antibody-like protein of Claim 1 with a cytotoxic moiety, wherein the cytotoxic moiety comprises a radioisotope, a radionuclide, a therapeutic agent, a chemotherapeutic agent, or a combination thereof.
38. A solution comprising an effective concentration of the antibody-like protein of Claim 1 or the immunoconjugate of Claim 29, wherein the solution is blood plasma in a subject.