such as EGFR and HER2, have demonstrated good clinical responses in colon cancer (Price et al., *The Lancet Oncology* 15(6), Pages 569-579, May 2014), squamous cell carcinoma of head and neck (Cohen, *Cancer Treatment Reviews* 40 (2014) 567–577), breast and gastric cancers (Arteaga et al., *Nature Reviews Clinical Oncology* 9 16-32, January 2012). Several therapeutic anti-EGFR antibodies, including cetuximab, panitumumab and nimotuzumab are approved therapeutics for several cancers including metastatic colorectal cancer, head and neck squamous cell carcinoma and glioma (Price and Cohen*, Curr Treat Options Oncol.* 2012 Mar;13(1):35-46; Bode et al*., Expert Opin Biol Ther.* 2012 Dec;12(12):1649-59). Unfortunately, many tumors that initially respond to these therapeutic agents eventually progress due to an acquired resistance to the agents (Jackman et al. *J Clin Oncol* 2010; 28:357–60). Therefore, there exists a need for better cancer therapeutics.

SUMMARY

In one aspect, the present disclosure refers to a bispecific tetravalent antibody, said bispecific tetravalent antibody comprising two IgG1 heavy chains; two kappa light chains; and two single chain Fv (scFv) domains; wherein the two IgG1 heavy chains and kappa light chains form an IgG moiety with a binding specificity to a first member of the EGFR family; wherein the two scFv domains have a binding specificity to a second member of the EGFR family, and each scFv domain is connected to the C-terminus of either of the IgG1 heavy chains by a connector with an amino acid sequence of (gly-gly-gly-gly-ser)n, to provide a IgG1-connector connection, wherein n is an integral of at least 1; and wherein each scFv domain has a structure order of N terminus – variable heavy chain – linker – variable light chain – C terminus or N-terminus – variable light chain– linker– variable heavy chain– C-terminus, and wherein the linker is comprised of amino acid sequence of (gly-gly-gly-gly-ser)m, wherein m is an integral of at least 3. In another aspect, the present invention refers to an isolated nucleic acid encoding the antibody of as disclosed herein, the IgG1 heavy chain comprising an amino acid sequences selected from SEQ ID NO: 7, 15, 23, 31, 39, 47, and 127, the kappa light chain as disclosed herein, the variable light chain comprising an amino acid sequence selected from SEQ ID NO: 4, 12, 20, 28, 36, 44, 52, 62, 72, 82, 93, 104, 114, 124, and 132; or the variable heavy chain comprising an amino acid sequence selected from SEQ ID NO: 8, 16, 24, 32, 40, 48, 57, 67, 77, 87, 99, 109, 119, 128, and 137. In yet another aspect, the present disclosure refers to an expression vector comprising the isolated nucleic acid disclosed herein. In a further aspect, the present disclosure refers to a host cell comprising the nucleic acid disclosed herein. In one aspect, the present disclosure refers to a host cell

comprising the expression vector disclosed herein; optionally wherein the host cell is a prokaryotic cell or a eukaryotic cell. In another aspect, the present disclosure refers to an immunoconjugate comprising the antibody disclosed herein and a cytotoxic agent. In yet another aspect, the present disclosure refers to a pharmaceutical composition, comprising the bispecific tetravalent antibody disclosed herein and a pharmaceutically acceptable carrier. In a further aspect, the present disclosure refers to a pharmaceutical composition, comprising the immunoconjugate disclosed herein and a pharmaceutically acceptable carrier. In a further aspect, the present disclosure refers to use of the bispecific tetravalent antibody disclosed herein, the expression vector as disclosed herein, or the pharmaceutical composition as disclosed herein in the preparation of a medicament for the treatment of a subject with a cancer. In a further aspect, the present disclosure refers to use of the bispecific tetravalent antibody disclosed herein, the expression vector as disclosed herein, or the pharmaceutical composition as disclosed herein in the preparation of a medicament to inhibit a biological activity of a HER receptor in a subject. In another aspect, the present disclosure refers to a solution comprising an effective concentration of the bispecific tetravalent antibody disclosed herein, wherein the solution is blood plasma.

The disclosure describes bispecific tetravalent antibodies. The bispecific tetravalent antibodies may include an immunoglobulin G (IgG) moiety with two heavy chains and two light chains, and two scFv moieties being covalently connected to either C or N terminals of the heavy or light chains. The IgG moiety may have a binding specificity to a first member of EGFR family. The scFv moiety may have a binding specificity to a second member of the EGFR family. The IgG moiety and two scFv moieties are covalently connected to be functional as a bispecific tetravalent antibody. The objectives and advantages of the disclosure will become apparent from the following detailed description of preferred embodiments thereof in connection with the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

Preferred embodiments according to the present disclosure will now be described with reference to the FIGs, in which like reference numerals denote

CLAIMS

What is claimed is:

1. A bispecific tetravalent antibody, said bispecific tetravalent antibody comprising:

two IgG1 heavy chains;

two kappa light chains; and two single chain Fv (scFv) domains;

wherein the two IgG1 heavy chains and kappa light chains form an IgG moiety with a binding specificity to a first member of the EGFR family;

wherein the two scFv domains have a binding specificity to a second member of the EGFR family, and each scFv domain is connected to the C-terminus of either of the IgG1 heavy chains by a connector with an amino acid sequence of $(gly\text{-}gly\text{-}gly\text{-}gly\text{-}ser)_n$, to provide a IgG1-connector connection, wherein n is an integral of at least 1; and

wherein each scFv domain has a structure order of N terminus - variable heavy chain - linker - variable light chain - C terminus or N-terminus - variable light chain- linker- variable heavy chain- C-terminus, and wherein the linker is comprised of amino acid sequence of $(gly\text{-}gly\text{-}gly\text{-}gly\text{-}ser)_m$, wherein m is an integral of at least 3.

2. The bispecific tetravalent antibody of Claim 1, wherein at least one of the IgG1 heavy chains is a humanized or human IgG1 heavy chain.

3. The bispecific tetravalent antibody of claim 1, wherein both IgG1 heavy chains are humanized or human IgG1 heavy chains.

4. The bispecific tetravalent antibody of any one of claims 1 to 3, wherein at least one of the kappa light chains is a humanized or human kappa light chain.

5. The bispecific tetravalent antibody of any one of claims 1 to 3, wherein both kappa light chains are humanized or human kappa light chains.

6. The bispecific tetravalent antibody of any one of claims 1 to 5, wherein the first or the second member of the EGFR family comprises HER3, EGFR, a fragment or a derivative thereof.

7. The bispecific tetravalent antibody of any one of claims 1 to 5, wherein the first or the second member of the EGFR family is independently selected from a group consisting of HER3, EGFR, a fragment or a derivative thereof.

8. The bispecific tetravalent antibody of any one of claims 1 to 7, wherein the IgG moiety has a binding specificity for HER3.

9. The bispecific tetravalent antibody of any one of claims 1 to 8, wherein the scFv domains have a binding specificity for EGFR.

10. The bispecific tetravalent antibody of any one of claims 1 to 7, wherein the IgG moiety has a binding specificity for HER3 and the scFv domains have a binding specificity for EGFR simultaneously.

11. The bispecific tetravalent antibody of any one of claims 1 to 7, wherein the IgG moiety has a binding specificity for EGFR.

12. The bispecific tetravalent antibody of any one of claims 1 to 7, wherein the scFv domains have a binding specificity for HER3.

13. The bispecific tetravalent antibody of any one of claims 1 to 7, wherein the IgG moiety has a binding specificity for EGFR and the scFv domains have a binding specificity for HER3 simultaneously.

14. The bispecific tetravalent antibody of any one of claims 1 to 13, wherein the IgG1-connector connection is resistant to protease activity.

15. The bispecific tetravalent antibody of any one of claims 1 to 5, wherein the IgG1 heavy chain, connector, and scFv domain have an amino acid comprising SEQ ID NO: 56, 66, 76, 86, 98, 108, 118, and 136.

16. The bispecific tetravalent antibody of any one of claims 1 to 5, wherein at least one of the kappa light chains comprises an amino acid sequence comprising SEQ ID NO: 3, 11, 19, 27, 35, 43, 51, 61, 71, 81, 92, 103, 113, 123, and 131.

17. The bispecific tetravalent antibody any one of claims 1 to 7, wherein the IgG moiety has a binding specificity for HER3, and the scFv domains have a binding specificity for EGFR;

wherein the IgG1 heavy chain, connector, and scFv domain have an amino acid sequence of SEQ ID NO 56, and the kappa light chain has an amino acid sequence of SEQ ID NO: 51.

18. The bispecific tetravalent antibody of any one of claims 1 to 7, wherein the IgG moiety has a binding specificity for HER3, and the scFv domains have a binding specificity for EGFR;

wherein the IgG1 heavy chain, connector, and scFv domain have an amino acid sequence of SEQ ID NO 76, and the kappa light chain has an amino acid sequence of SEQID NO 71.

19. The bispecific tetravalent antibody of any one of claims 1 to 18, wherein the antibody inhibits cancer cell growth.

20. The bispecific tetravalent antibody of any one of claims 10, 13, 17 or 18, wherein the antibody binds to EGRF and HERS with a Kd less than 50nM.

21. The bispecific tetravalent antibody of any one of claims 10, 13, 17 or 18, wherein the antibody simultaneously binds to EGRF with a Kd less than 50nM and binds to HER3 with a Kd less than 50nM.

22. The bispecific tetravalent antibody any one of claims 1 to 5, wherein at least one of the kappa light chains comprises an amino acid sequence selected from SEQ ID NO 3, 11-19, 27, 35, 43, 51, 61, 71, 81, 92, 103, 113, 123, and 131.

23. An isolated nucleic acid encoding the antibody of any one of claims 1 to 5, the IgG1 heavy chain comprising an amino acid sequences selected from SEQ ID NO 7, 15-23, 31, 39, 47, and 127, the kappa light chain of claim 22, the variable light chain comprising an amino acid sequence selected from SEQ ID NO 4, 12, 20, 28, 36, 44, 52, 62, 72, 82, 93, 104, 114, 124, and 132, or the variable heavy chain comprising an amino acid sequence selected from SEQ ID NO 8, 16, 24, 32, 40, 48, 57, 67, 77, 87, 99, 109, 119, 128, and 137.

24. An expression vector comprising the isolated nucleic acid of claim 23.

25. The expression vector of claim 24, wherein the vector is expressible in a cell.

26. A host cell comprising the nucleic acid of claim 23.

27. A host cell comprising the expression vector of claim 25.

28. The host cell of claim 27, wherein the host cell is a prokaryotic cell or a eukaryotic cell.

29. An immunoconjugate comprising the antibody of any one of claims 1 to 22 and a cytotoxic agent.

30. A pharmaceutical composition, comprising the bispecific tetravalent antibody of any one of claims 1 to 22 and a pharmaceutically acceptable carrier.

31. The pharmaceutical composition of claim 30, further comprising radioisotope, radionuclide, a toxin, a therapeutic agent, a chemotherapeutic agent or a combination thereof.

32. A pharmaceutical composition, comprising the immunoconjugate of claim 29 and a pharmaceutically acceptable carrier.

33. Use of the bispecific tetravalent antibody of any one of claims 1 to 22, the expression vector of claim 24 or 25, or the pharmaceutical composition of any one of claims 30 to 32 in the preparation of a medicament for the treatment of a subject with a cancer.

34. The use of Claim 33, wherein the cancer comprises cells expressing at least two members of EGFR family.

35. The use of Claim 33 or 34, wherein the cancer comprises breast cancer, colorectal cancer, pancreatic cancer, head and neck cancer, melanoma, ovarian cancer, prostate cancer, non-small lung cell cancer, glioma, esophageal cancer, nasopharyngeal cancer, anal cancer, rectal cancer, gastric cancer, bladder cancer, cervical cancer, or brain cancer.

36. The use of any one of claims 33-35, further comprising that an effective amount of a therapeutic agent is to be co-administered.

37. The use of Claim 36, wherein the therapeutic agent comprises an antibody, a chemotherapy agent, an enzyme, or a combination thereof.

38. The use of Claim 36, wherein the therapeutic agent comprises an anti-estrogen agent, a receptor tyrosine inhibitor, or a combination thereof.

39. The use of Claim 36, wherein the therapeutic agent comprises capecitabine, cisplatin, trastuzumab, fulvestrant, tamoxifen, letrozole, exemestane, anastrozole, aminoglutethimide, testolactone, vorozole, formestane, fadrozole, letrozole, erlotinib, lafatinib, dasatinib, gefitinib, imatinib, pazopinib, lapatinib, sunitinib, nilotinib, sorafenib, nab-palitaxel, a derivative or a combination thereof.

40. The use of Claim 36, wherein the therapeutic agent comprises a check point inhibitor.

41. The use of Claim 36, wherein the therapeutic agent comprises PD1, PDL1, CTLA4, 4-1BB, OX40, GITR, TIM3, LAG3, TIGIT, CD40, CD27, HVEM, BTLA, VISTA, B7H4, a derivative or a combination thereof.

42. The use of any one of claims 33-41 , wherein the subject is a human.

43. Use of an effective amount of the bispecific tetravalent antibody of any one of claims 1 to 22, the expression vector of claim 24 or 25, or the pharmaceutical composition of any one of claims 30 to 32 in the preparation of a medicament to inhibit a biological activity of a HER receptor in a subject.

44. A solution comprising an effective concentration of the bispecific tetravalent antibody of any one of claims 1 to 22, wherein the solution is blood plasma.

45. The bispecific tetravalent antibody of any one of claims 1 to 22 wherein the first member of the EGER family comprises HER3 or a fragment or a derivative thereof and the second member of the EGFR family comprises EGFR or a fragment or a derivative thereof.

46. The bispecific tetravalent antibody of any one of claims 1 to 22 wherein the first member of the EGFR family comprises EGFR or a fragment or a derivative thereof and the second member of the EGFR family comprises HER3 or a fragment or a derivative thereof.