

head and neck cancers. Apart from the induction of treatment resistance, some side effects of EGFR-targeting antibodies have been observed. One example is the development of a skin rash, associated with EGFR inhibition or anti-EGFR biologic treatment. When extreme, such rashes can lead to a reduction in treatment cycles and/or premature termination of treatment.

Various modes of activation of signaling of the EGF receptor family have been identified. Among these are ligand dependent and ligand independent activation of signaling. Over-expressed HER2 is able to generate oncogenic signaling through the HER2/HER3 heterodimer even in the absence of the HER3 ligand (Junttila, Akita et al. 2009). HER2 activity can be inhibited by HER2 specific antibodies. Such HER2 specific antibodies are for instance used in the treatment of HER2 positive (HER2+) tumors. A problem with such treatments is that often tumors escape the HER2 specific treatment and continue to grow even in the presence of the inhibiting antibody. It has been observed that HER2 positive tumors, such as breast, ovarian, cervical and gastric tumors can escape treatment by the selective outgrowth of a subpopulation of tumor cells that exhibit upregulated HER3 expression (Ocana, Vera-Badillo et al. 2013) and/or HER3 ligand expression (Wilson, Fridlyand et al. 2012). Also activating mutations in the HER3 receptor have been identified.

Thus, in spite of promising results with antibody treatments that specifically target EGF receptor family members, it has been observed that not all tumors respond or respond sufficiently. The present invention provides combinations of binding moieties and methods for producing them that target various members of the EGF receptor family. Combinations of the invention show good efficacy. The combinations can be produced in a cost effective and efficient way.

SUMMARY

In one aspect, the present disclosure provides a composition comprising two or more binding moieties,

wherein each binding moiety comprises a variable domain that binds to an extracellular part of EGFR; and

wherein a first of said binding moieties comprises a variable domain that binds to an extracellular part of HER2 and a second of said binding moieties comprises a variable domain that binds to an extracellular part of HER3.

Preferably, at least one of the two or more binding moieties is an antibody.

In a preferred example, at least two of the two or more binding moieties are antibodies. The antibodies are preferably multispecific antibodies, preferably bispecific antibodies. Preferably at least one, and more preferably at least two of

the antibodies are IgG antibodies. In a preferred example of the disclosure, the composition comprising two bispecific antibodies.

5 A multispecific antibody as described herein preferably comprises heavy chains with a CH3 heterodimerization domain. In one example the CH3 heterodimerization domain of the first and/or the second multispecific antibody is engineered to facilitate heterodimerization of the heavy chain of the EGFR variable domain with the respective heavy chains of the HER2 and HER3 variable domains.

10 The disclosure also provides a composition as described herein for use in the treatment of cancer. In examples the cancer is a solid epithelial cancer. Preferably the composition is used for a cancer that expresses EGFR, HER2 and/or HER3. The composition is used for preferably for pancreatic cancer, colorectal cancer, head & neck cancer, epithelial ovarian cancer, epithelial fallopian tube cancer, epithelial
15 peritoneal cancer, bladder cancer, or prostate cancer. In examples the cancer treated by use of the composition is advanced cancer. The composition is used for preferably for metastatic cancer. The composition is used for preferably for metastatic pancreatic cancer, metastatic colorectal cancer, metastatic head & neck cancer, metastatic epithelial ovarian cancer, metastatic epithelial fallopian tube
20 cancer, metastatic epithelial peritoneal cancer, metastatic bladder cancer, or metastatic prostate cancer. In examples, the composition is used for preferably for the cancer that is gastric cancer, lung cancer, breast cancer or esophagus cancer. Preferably, the composition is used for metastatic gastric cancer, metastatic lung cancer, metastatic breast cancer or metastatic esophagus cancer.

25 In one aspect, the present disclosure refers to a composition disclosed herein for use in treatment.

30 In one aspect, the present disclosure refers to a composition disclosed herein for use in the treatment of cancer, preferably gastric cancer, lung cancer or esophagus cancer.

35 In one aspect, the present disclosure refers to the use of a composition disclosed herein in the manufacture of a medicament for the treatment of cancer, wherein the cancer is preferably gastric cancer, lung cancer or esophagus cancer.

In one aspect, the present disclosure refers to a pharmaceutical composition comprising a composition disclosed herein.

40 In one aspect, the present disclosure refers to two or more binding moieties that each comprise a variable domain that binds to an extracellular part of EGFR; wherein a first of said binding moieties comprises a variable domain that binds to an extracellular part of HER2 and a second of said binding moieties comprises a variable domain that binds to an extracellular part of HER3 for use in the
45 treatment of cancer, preferably gastric cancer, lung cancer or esophagus cancer.

In one aspect, the present disclosure refers to the use of two or more binding moieties that each comprise a variable domain that binds to an extracellular part of EGFR in the manufacture of a medicament for the treatment of cancer; wherein a first of said binding moieties comprises a variable domain that binds to an extracellular part of HER2 and a second of said binding moieties comprises a variable domain that binds to an extracellular part of HER3; and wherein the cancer is preferably gastric cancer, lung cancer or esophagus cancer.

The disclosure further provides a product containing two or more binding moieties that each comprise a variable domain that binds to an extracellular part of EGFR; wherein a first of said binding moieties comprises a variable domain that binds to an extracellular part of HER2 and a second of said binding moieties comprises a variable domain that binds to an extracellular part of HER3 as a combined preparation for simultaneous, separate or sequential use in treating cancer.

In one aspect, the present disclosure refers to a product containing two or more binding moieties, wherein each of said binding moieties comprises a variable domain that binds to an extracellular part of EGFR; and wherein a first of said binding moieties comprises a variable domain that binds to an extracellular part of HER2 and a second of said binding moieties comprises a variable domain that binds to an extracellular part of HER3 as a combined preparation for simultaneous, separate or sequential use in treating cancer preferably gastric cancer, lung cancer or esophagus cancer.

In one aspect, the present disclosure refers to the use of a product containing two or more binding moieties in the manufacture of a combined preparation for simultaneous, separate or sequential use in treating cancer; wherein each of said binding moieties comprises a variable domain that binds to an extracellular part of EGFR; wherein a first of said binding moieties comprises a variable domain that binds to an extracellular part of HER2 and a second of said binding moieties comprises a variable domain that binds to an extracellular part of HER3; and wherein the cancer is preferably gastric cancer, lung cancer or esophagus cancer.

In one aspect, the present disclosure refers to the use of two or more binding moieties in the manufacture of a medicament for the treatment of a subject that has cancer or is at risk of recurrence or relapse of cancer, wherein each of said binding moieties comprises a variable domain that binds to an extracellular part of EGFR; and wherein a first of said binding moieties comprises a variable domain that binds to an extracellular part of HER2 and a second of said binding moieties comprises a variable domain that binds to an extracellular part of HER3.

The disclosure further provides a method for producing a composition according to the disclosure, which method comprises:

- providing a cell comprising
 - a nucleic acid that encodes a polypeptide comprising a heavy chain that together with a common light chain forms a variable domain that binds to an extracellular part of EGFR;
 - a nucleic acid that encodes a polypeptide comprising a heavy chain that together with said common light chain forms a variable domain that binds to an extracellular part of HER2;

- a nucleic acid that encodes a polypeptide comprising a heavy chain that together with said common light chain forms a variable domain that binds to an extracellular part of HER3; and

5 - a nucleic acid that encodes a polypeptide comprising said common light chain;

wherein two or more of said nucleic acids may be physically linked or not and wherein each of said nucleic acids further comprises an expression control sequence to allow expression of the encoded heavy and light chains in said cell and wherein the method further comprises culturing said cell to allow expression of said heavy
10 and light chains and, optionally collecting said two or more binding moieties.

In one aspect, the present disclosure refers to a method for producing a composition disclosed herein, which method comprises:

providing a cell comprising
15 - a nucleic acid that encodes a polypeptide comprising a heavy chain that is capable of pairing with a common light chain to form a variable domain that binds to an extracellular part of EGFR;

 - a nucleic acid that encodes a polypeptide comprising a heavy chain that is capable of pairing with said common light chain forms a variable domain that
20 binds to an extracellular part of HER2;

 - a nucleic acid that encodes a polypeptide comprising a heavy chain that is capable of pairing with said common light chain forms a variable domain that binds to an extracellular part of HER3; and

 - a nucleic acid that encodes a polypeptide comprising said common light
25 chain;

wherein, optionally two or more of said nucleic acids may be physically linked, and wherein each of said nucleic acids further comprises an expression control sequence to allow expression of the encoded heavy and light chains in said cell and;

30 culturing said cell to allow expression of said heavy and light chains; and, optionally,

recovering said two or more binding moieties.

In one aspect, the present disclosure further provides a cell comprising

- a nucleic acid that encodes a polypeptide comprising a heavy chain that together with a common light chain forms a variable domain that binds to an extracellular part of EGFR;

5 - a nucleic acid that encodes a polypeptide comprising a heavy chain that together with said common light chain forms a variable domain that binds to an extracellular part of HER2;

 - a nucleic acid that encodes a polypeptide comprising a heavy chain that together with said common light chain forms a variable domain that binds to an
10 extracellular part of HER3; and

- a nucleic acid that encodes a polypeptide comprising said common light chain;

 wherein two or more of said nucleic acids may be physically linked or not and wherein each of said nucleic acids further comprises an expression control
15 sequence to allow expression of the encoded heavy and light chains in said cell.

In a further example the disclosure provides a container comprising nucleic acid comprising

 - a nucleic acid that encodes a polypeptide comprising a heavy chain that
20 together with a common light chain forms a variable domain that binds to an extracellular part of EGFR;

- a nucleic acid that encodes a polypeptide comprising a heavy chain that together with a common light chain forms a variable domain that binds to an extracellular part of HER2;

25 - a nucleic acid that encodes a polypeptide comprising a heavy chain that together with a common light chain forms a variable domain that binds to an extracellular part of HER3; and

- a nucleic acid that encodes a polypeptide comprising said common light chain;

30 wherein, optionally, two or more of said nucleic acids may be physically linked or not and wherein each of said nucleic further comprises an expression control sequence to allow expression of the encoded heavy and light chains in a cell.

In one aspect, the present disclosure refers to a container comprising nucleic acid comprising

- a nucleic acid that encodes a polypeptide comprising a heavy chain that is capable of pairing with a common light chain forms a variable domain that binds to an extracellular part of EGFR;

- a nucleic acid that encodes a polypeptide comprising a heavy chain that is capable of pairing with a common light chain forms a variable domain that binds to an extracellular part of HER2;

- a nucleic acid that encodes a polypeptide comprising a heavy chain that is capable of pairing with a common light chain forms a variable domain that binds to an extracellular part of HER3; and

- a nucleic acid that encodes a polypeptide comprising said common light chain; wherein, optionally two or more of said nucleic acids may be physically linked, and wherein each of said nucleic acid further comprises an expression control sequence to allow expression of the encoded heavy and light chains in a cell.

The disclosure further provides a composition comprising a binding moiety that specifically binds an extracellular part of EGFR and an extracellular part of HER2.

The disclosure also provides composition comprising a binding moiety that specifically binds an extracellular part of EGFR and an extracellular part of HER3.

The binding moiety is preferably an antibody, preferably an IgG antibody, more preferably a multi-specific antibody.

DETAILED DESCRIPTION OF THE INVENTION

The term EGFR as used herein refers to the protein that in humans is encoded by the epidermal growth factor receptor gene (EGFR). The protein is known under a number of aliases among which: Erb-B2 Receptor Tyrosine Kinase 1; Proto-Oncogene C-ErbB-1; ERBB1; and HER1. A database accession number for the human EGFR protein and the gene encoding it is (GenBank NM_005228.3). The accession number is primarily given to provide a further method of identification of EGFR protein as a target, the actual sequence of the EGFR protein bound by an antibody may vary, for instance because of a mutation in the encoding gene such as those occurring in some cancers or the like. Where reference herein is made to EGFR, the reference refers to human EGFR unless otherwise stated. The EGFR variable domain may, due to sequence and tertiary structure similarity between human and other mammalian orthologues of EGFR, also bind such an orthologue but not necessarily so. The variable domain that binds EGFR, may bind EGFR and a variety of variants thereof such as those expressed on some EGFR positive tumors.

The EGFR binding variable domain of an antibody or binding moiety of the invention preferably binds domain I or domain III of EGFR. The structure of the EGFR protein has been described among others in Ferguson (2008: Annu Rev Biophys. 2008; 37: 353–373. doi: 10.1146/annurev.biophys.37.032807.125829). The domains of human EGFR are described in Figure 1 of the above mentioned Ferguson reference. The EGFR binding variable domain of embodiments of the inventions disclosed herein preferably binds domain III of EGFR. The antibody preferably inhibits EGF induced proliferation of BxPC-3 (ATCC CRL-1687) or BxPC-3-luc2 cells (Perkin Elmer 125058).

The term HER2 as used herein refers to the protein that in humans is encoded by the ERBB-2 gene. Alternative names for the gene or protein include CD340; ErbB-2; HER-2/neu; MLN 19; NEU; NGL; TKR1. The ERBB-2 gene is frequently called HER2 (from human epidermal growth factor receptor 2). Where reference is made herein to HER2, the reference refers to human HER2. An

CLAIMS

1. A composition comprising two or more binding moieties,
wherein each of said binding moieties comprises a variable domain that binds to an extracellular part of EGFR; and
wherein a first of said binding moieties comprises a variable domain that binds to an extracellular part of HER2 and a second of said binding moieties comprises a variable domain that binds to an extracellular part of HER3.
2. The composition of claim 1, wherein at least one and preferably at least two of the two or more binding moieties is an antibody.
3. The composition of claim 1 or claim 2, wherein at least one and preferably at least two of the two or more binding moieties is an IgG.
4. The composition of claim 2 or claim 3, wherein the CH3-regions of the heavy chains of a first and/or a second antibody are engineered to facilitate heterodimerization of a heavy chain with an EGFR binding variable domain with a heavy chain with an HER2 binding variable domain and/or an EGFR binding variable domain with a heavy chain with an HER3 binding variable domain.
5. The composition of any one of claims 2 to 4, wherein at least one, and preferably at least two of the two or more antibodies is a bispecific antibody.
6. The composition of any one of claims 2 to 5, wherein the variable domains that bind to an extracellular part of EGFR of the first and second antibody comprise substantially the same heavy chain variable region.
7. The composition of any one of claims 2 to 6, wherein the variable domain that binds to an extracellular part of EGFR binds domain I or domain III of EGFR, preferably domain III.
8. The composition of any one of claims 2 to 7, wherein the variable domain that binds to an extracellular part of HER2 binds domain I or domain IV of HER2, preferably domain IV.
9. The composition of any one of claims 2 to 8, wherein the variable domain that binds to an extracellular part of HER3 binds domain III of HER3.
10. The composition of any one of claims 6 to 9, wherein the variable domain that binds to an extracellular part of EGFR binds domain I or domain III of EGFR, preferably domain III; wherein the variable domain that binds to an extracellular part of HER2 binds domain I or domain IV of HER2, preferably domain IV; and

wherein the variable domain that binds to an extracellular part of HER3 binds domain III of HER3.

11. The composition of claim 9 or claim 10, wherein the variable domain that binds to an extracellular part of HER3 binds at least to R426 of domain III of HER3.

12. The composition of any one of claims 9 to 11, wherein the affinity (KD) of the variable domain that binds to an extracellular part of HER3, for binding to an HER3 positive SK-BR-3 cell (ATCC® HTB-30™), is lower than or equal to 2.0 nM, preferably from 2.0 to 0.1 nM.

13. The composition of any one of claims 1 to 12, wherein the binding of the variable domain that binds EGFR to EGFR blocks the binding of EGF to EGFR and/or wherein the binding of the variable domain that binds HER3 to HER3 blocks the binding of neuregulin 1 (NRG) to HER3.

14. The composition of any one of claims 1-13, wherein the variable domain that binds to an extracellular part of EGFR comprises a heavy chain variable region comprising a CDR1 sequence NYAMN, a CDR2 sequence WINANTGDPTYAQGFTG and a CDR3 sequence ERFLEWLHFDY or a variant thereof comprising a substitution, deletion and/or insertion of 1, 2, or 3 amino acids in the CDRs.

15. The composition of any one of claims 1-14, wherein the variable domain that binds to an extracellular part of HER2 comprises a heavy chain variable region comprising a CDR1 sequence SYGMH, a CDR2 sequence VISYDGSNKYYADSVKG and a CDR3 sequence DYYRRTARAGFDY or a variant thereof comprising a substitution, deletion and/or insertion of 1, 2, or 3 amino acids in the CDRs.

16. The composition of any one of claims 1-15, wherein the variable domain that binds to an extracellular part of HER3 comprises a heavy chain variable region comprising a CDR1 sequence GYYMH, a CDR2 sequence WINPNSGGTNYAQKFQG and a CDR3 sequence DHGSRHFWSYWGFDY or a variant thereof comprising a substitution, deletion and/or insertion of 1, 2, or 3 amino acids in the CDRs.

17. The composition of any one of claims 1-16
 - wherein the variable domain that binds to an extracellular part of EGFR comprises a heavy chain variable region comprising a CDR1 sequence NYAMN, a CDR2 sequence WINANTGDPTYAQGFTG and a CDR3 sequence ERFLEWLHFDY or a variant thereof comprising a substitution, deletion and/or insertion of 1, 2, or 3 amino acids in the CDRs;

- wherein the variable domain that binds to an extracellular part of HER2 comprises a heavy chain variable region comprising a CDR1 sequence SYGMH, a CDR2 sequence VISYDGSNKYYADSVKG and a CDR3 sequence DYYRRTARAGFDY or a variant thereof comprising a substitution, deletion and/or insertion of 1, 2, or 3 amino acids in the CDRs; and

- wherein the variable domain that binds to an extracellular part of HER3 comprises a heavy chain variable region comprising a CDR1 sequence GYYMH, a CDR2 sequence WINPNSGGTNYAQKFQG and a CDR3 sequence DHGSRHFWSYWGFY or a variant thereof comprising a substitution, deletion and/or insertion of 1, 2, or 3 amino acids in the CDRs.

18. A composition of any one of claims 1 to 17 for use in treatment.

19. A composition according to claim 18 for use in the treatment of cancer, preferably gastric cancer, lung cancer or esophagus cancer.

20. Use of a composition of any one of claims 1 to 17 in the manufacture of a medicament for the treatment of cancer, wherein the cancer is preferably gastric cancer, lung cancer or esophagus cancer.

21. A pharmaceutical composition comprising a composition according to any one of claims 1 to 17.

22. Two or more binding moieties that each comprise a variable domain that binds to an extracellular part of EGFR; wherein a first of said binding moieties comprises a variable domain that binds to an extracellular part of HER2 and a second of said binding moieties comprises a variable domain that binds to an extracellular part of HER3 for use in the treatment of cancer, preferably gastric cancer, lung cancer or esophagus cancer.

23. Use of two or more binding moieties that each comprise a variable domain that binds to an extracellular part of EGFR in the manufacture of a medicament for the treatment of cancer; wherein a first of said binding moieties comprises a variable domain that binds to an extracellular part of HER2 and a second of said binding moieties comprises a variable domain that binds to an extracellular part of HER3; and wherein the cancer is preferably gastric cancer, lung cancer or esophagus cancer.

24. A product containing two or more binding moieties, wherein each of said binding moieties comprises a variable domain that binds to an extracellular part of EGFR; and wherein a first of said binding moieties comprises a variable domain that binds to an extracellular part of HER2 and a second of said binding moieties comprises a variable domain that binds to an extracellular part of HER3 as a

combined preparation for simultaneous, separate or sequential use in treating cancer preferably gastric cancer, lung cancer or esophagus cancer.

25. Use of a product containing two or more binding moieties in the manufacture of a combined preparation for simultaneous, separate or sequential use in treating cancer; wherein each of said binding moieties comprises a variable domain that binds to an extracellular part of EGFR; wherein a first of said binding moieties comprises a variable domain that binds to an extracellular part of HER2 and a second of said binding moieties comprises a variable domain that binds to an extracellular part of HER3; and wherein the cancer is preferably gastric cancer, lung cancer or esophagus cancer.

26. The composition, pharmaceutical composition, binding moieties or product for use or the use of any one of claims 18-25, wherein the cancer comprises cells with an EGFR-mutation that renders the cell resistant to treatment with a tyrosine kinase inhibitor (TKI).

27. The composition, pharmaceutical composition, binding moieties or product for use or the use of any one of claims 18-26, wherein the cancer comprises cells with an EGFR R521K polymorphism.

28. The composition, binding moieties or product for use or the use of any one of claims 1-27, wherein the cancer is gastric cancer.

29. Use of two or more binding moieties in the manufacture of a medicament for the treatment of a subject that has cancer or is at risk of recurrence or relapse of cancer, wherein each of said binding moieties comprises a variable domain that binds to an extracellular part of EGFR; and wherein a first of said binding moieties comprises a variable domain that binds to an extracellular part of HER2 and a second of said binding moieties comprises a variable domain that binds to an extracellular part of HER3.

30. A method for producing a composition according to any one of claims 1-19, which method comprises:

providing a cell comprising

- a nucleic acid that encodes a polypeptide comprising a heavy chain that is capable of pairing with a common light chain to form a variable domain that binds to an extracellular part of EGFR;

- a nucleic acid that encodes a polypeptide comprising a heavy chain that is capable of pairing with said common light chain forms a variable domain that binds to an extracellular part of HER2;

- a nucleic acid that encodes a polypeptide comprising a heavy chain that is capable of pairing with said common light chain forms a variable domain that binds to an extracellular part of HER3; and

- a nucleic acid that encodes a polypeptide comprising said common light chain;

wherein, optionally two or more of said nucleic acids may be physically linked, and wherein each of said nucleic acids further comprises an expression control sequence to allow expression of the encoded heavy and light chains in said cell and;

culturing said cell to allow expression of said heavy and light chains; and, optionally,

recovering said two or more binding moieties.

31. The method of claim 30 comprising providing a plurality of cells with said nucleic acids and selecting from said collection a cell with a desired ratio of expression of the heavy and light chains.

32. The method of claim 30 or claim 31, wherein said two or more binding moieties are antibodies, preferably bispecific antibodies.

33. The method of any one of claims 30 to 32, wherein the cells produce essentially equimolar amounts of the two or more binding moieties.

34. The method of any one of claims 30-33, wherein the cells produce more of a first binding moiety than of a second of said two or more binding moieties.

35. A cell comprising

- a nucleic acid that encodes a polypeptide comprising a heavy chain that together with a common light chain forms a variable domain that binds to an extracellular part of EGFR;

- a nucleic acid that encodes a polypeptide comprising a heavy chain that together with said common light chain forms a variable domain that binds to an extracellular part of HER2;

- a nucleic acid that encodes a polypeptide comprising a heavy chain that together with said common light chain forms a variable domain that binds to an extracellular part of HER3; and

- a nucleic acid that encodes a polypeptide comprising said common light chain;

wherein two or more of said nucleic acids may be physically linked or not and wherein each of said nucleic acids further comprises an expression control sequence to allow expression of the encoded heavy and light chains in said cell.

36. A container comprising nucleic acid comprising
- a nucleic acid that encodes a polypeptide comprising a heavy chain that is capable of pairing with a common light chain forms a variable domain that binds to an extracellular part of EGFR;
 - a nucleic acid that encodes a polypeptide comprising a heavy chain that is capable of pairing with a common light chain forms a variable domain that binds to an extracellular part of HER2;
 - a nucleic acid that encodes a polypeptide comprising a heavy chain that is capable of pairing with a common light chain forms a variable domain that binds to an extracellular part of HER3; and
 - a nucleic acid that encodes a polypeptide comprising said common light chain;
- wherein, optionally two or more of said nucleic acids may be physically linked, and wherein each of said nucleic further comprises an expression control sequence to allow expression of the encoded heavy and light chains in a cell.