

However, despite these multiple layers of testing for HER2 positive tumours, not all tumours identified as HER2 positive are responsive to current HER2 specific therapies. Therefore, there remains a need in the art for treatments for patients with HER2 positive tumours, as well as means for identifying patients likely to benefit from such therapies. In addition, as mentioned above, acquired resistance to known therapies for HER2, such as trastuzumab, means that there is a need in the art for therapies suitable for treating such resistant HER2 positive cancers.

Antigen-binding Fc fragments (also referred to as Fcab™ [Fc fragment with Antigen Binding]) comprising e.g. a modified IgG1 Fc domain which binds to HER2 with high affinity are described in WO 2009/132876 A1 and WO 2009/000006 A1. Such Fcabs have been shown to have favourable properties, such as half-lives of approximately 60 hours in mice.

Summary

In one aspect, the present invention is directed to the use of a specific binding member which binds to Human Epidermal Growth Factor Receptor 2 (HER2) in the manufacture of a medicament for treating cancer in a patient, wherein said cancer has an average HER2 gene copy number of greater than or equal to 10 per tumour cell, and wherein the specific binding member is: (a) a specific binding member comprising a HER2 antigen binding site engineered into a structural loop region of a CH3 domain of the specific binding member and containing the amino acid sequences FFTYW (SEQ ID NO: 12), and DRRRWTA (SEQ ID NO: 14); or (b) a specific binding member which competes with a specific binding member according to (a) for binding to HER2. In another aspect, the present invention refers to a method of identifying a cancer in a patient which is susceptible to treatment with: (a) a specific binding member comprising a HER2 antigen binding site engineered into a structural loop region of a CH3 domain of the specific binding member and containing the amino acid sequences FFTYW (SEQ ID NO: 12), and DRRRWTA (SEQ ID NO: 14); or (b) a specific binding member which competes with a specific binding member according to (a) for binding to HER2; the method comprising: (i) determining the average gene copy number of the HER2 gene per tumour cell in a tumour sample obtained from the patient, wherein an average gene copy number of greater than or equal to 10 per tumour cell indicates that the cancer is susceptible to treatment with the specific binding member. In still another aspect, the present invention refers to a method of predicting the response of a cancer to treatment with: (a) a specific binding member comprising a HER2 antigen binding site engineered into a structural loop region of a CH3 domain of the specific binding member and containing the

amino acid sequences FFTYW (SEQ ID NO: 12), and DRRRWTA (SEQ ID NO: 14); or (b) a specific binding member which competes with a specific binding member according to (a) for binding to HER2; the method comprising: (i) determining the average gene copy number of the HER2 gene per tumour cell in a tumour sample obtained from a patient, wherein a gene copy number of greater than or equal to 10 per tumour cell indicates that the cancer is susceptible to treatment with the specific binding member, and wherein a gene copy number of less than 10 per tumour cell indicates that the cancer is not susceptible to treatment with the specific binding member. In another aspect, the present invention refers to a specific binding member for use according to the present invention, wherein the cancer is refractory to treatment with trastuzumab and/or trastuzumab plus pertuzumab.

Statements and detailed description of the invention

The present inventors have discovered that a specific binding member comprising a HER2 antigen binding site engineered into a structural loop region of a constant domain, e.g. CH3 domain, of the specific binding member can be used to treat cancers with a HER2 gene copy number of greater than or equal to 10 per tumour cell. The present inventors have also discovered that such specific binding members can be used to treat cancers with a high HER2 mRNA levels. In particular, such specific binding members can be used to treat cancers resistant to treatment with known HER2 specific treatments, such as trastuzumab monotherapy, or treatment with trastuzumab in combination with pertuzumab.

As explained above, HER2 is known to be expressed in a number of different cancers. More data is available in breast cancer than in other cancers known to express HER2, as this is perhaps the most well studied HER2 positive cancer. Most data is available for tumours having an IHC score of 3+. Hoff *et al.* (2002) determined that 8% of all breast cancer patients had gene copy number greater than or equal to 10. In the largest adjuvant breast cancer trial of trastuzumab (the HERA trial), gene copy number of the tumours was assessed in 2071 (61%) patients by FISH. Of those patients, a gene copy number of greater than or equal to 10 was identified in about 65% of the patients. This represents about 13% of all breast cancer patients (Dowsett *et al.*, 2009). While the number of copies of the HER2 gene has been studied in some clinical trials, little information is available on the corresponding level of HER2 mRNA in these cancers. However, it is known that an increase in gene copy number in a cell often results in greater expression of that gene, and hence in

Claims

1. ~~A Use of a specific binding member which binds to Human Epidermal Growth Factor Receptor 2 (HER2) in the manufacture of a medicament for use in a method of treating~~ cancer in a patient, wherein said cancer has an average HER2 gene copy number of greater than or equal to 10 per tumour cell, and wherein the specific binding member is:

(a) a specific binding member comprising a HER2 antigen binding site engineered into a structural loop region of a CH3 domain of the specific binding member and containing the amino acid sequences FFYIW (SEQ ID NO: 12), and DRRRWTA (SEQ ID NO: 14); or

(b) a specific binding member which competes with a specific binding member according to (a) for binding to HER2.

2. ~~A specific binding member for~~ The use according to claim 1, wherein a tumour sample obtained from said patient has been determined to have an average HER2 gene copy number of greater than or equal to 10 per tumour cell.

3. ~~The A specific binding member for~~ use according to claim 1 or 2, wherein the cancer has, or has been determined to have, a high HER2 mRNA level, wherein a high HER2 mRNA level corresponds to a HER2 cDNA copy number of greater than or equal to 200 in a tumour sample obtained from said patient relative to the cDNA copy number of a reference gene in said sample, wherein the cDNA copy number of HER2 and the reference gene in the sample is determined by RT-PCR followed by quantitative PCR.

4. ~~The A specific binding member for~~ use according to claim 1 or 2, wherein the cancer has, or has been determined to have, a high HER2 mRNA level, wherein a high HER2 mRNA level corresponds to a HER2 cDNA copy number of greater than or equal to 200 and less than or equal to 820 in a tumour sample obtained from said patient relative to the cDNA copy number of a reference gene in said sample, wherein the cDNA copy number of HER2 and the reference gene in the sample is determined by RT-PCR followed by quantitative PCR.

5. ~~The A specific binding member for~~ use according to claim 1, wherein the method comprises:

(i) determining the average gene copy number of the HER2 gene per tumour cell in a tumour sample obtained from the patient; and

(ii) treating said patient with said specific binding member if the average HER2 gene copy number is greater than or equal to 10 per tumour cell.

6. ~~The A-specific binding member for~~ use according to any one of claims 1 to 5, wherein the HER2 gene copy number is greater than or equal to 11, greater than or equal to 12, greater than or equal to 13, greater than or equal to 14, greater than or equal to 15, greater than or equal to 16, greater than or equal to 17 or greater than or equal to 18.
7. ~~The A-specific binding member for~~ use according to claim 6, wherein the HER2 gene copy number is greater than or equal to 18.
8. ~~The A-specific binding member for~~ use according to any one of claims 1 to 7, wherein the cancer is gastric cancer, breast cancer, colorectal cancer, ovarian cancer, pancreatic cancer, lung cancer, stomach cancer, or endometrial cancer.
9. ~~The A-specific binding member for~~ use according to claim 8, wherein the cancer is gastric cancer, breast cancer, or colorectal cancer.
10. ~~The A-specific binding member for~~ use according to claim 8, wherein the cancer is gastric cancer.
11. ~~The A-specific binding member for~~ use according to claim 8, wherein the cancer is breast cancer.
12. ~~The A-specific binding member for~~ use according to any one of claims 1 to 11, wherein the patient has exhibited an inadequate response to trastuzumab and/or trastuzumab plus pertuzumab.
13. ~~The A-specific binding member for~~ use according to any one of claims 1 to 12, wherein the cancer is refractory to treatment with trastuzumab or trastuzumab plus pertuzumab.
14. ~~The A-specific binding member for~~ use according to any one of claims 1 to 13, wherein the specific binding member of claim 1, part (b), comprises a HER2 antigen binding site engineered into a structural loop region of a CH3 domain of the specific binding member.
15. ~~The A-specific binding member for~~ use according to claim 14, wherein the specific binding member comprises a HER2 antigen binding site engineered into structural loop regions AB and EF of a CH3 domain of the specific binding member.

16. ~~The A-specific binding member for use according to any one of claims 1 to 15,~~
wherein the specific binding member of claim 1, part (a), and/or claim 1, part (b) is, or comprises, an antigen-binding Fc fragment.
17. ~~The A-specific binding member for use according to any one of claims 1 to 16,~~
wherein the specific binding member of claim 1, part (a), and/or claim 1, part (b), comprises the CH3 domain of SEQ ID NO: 11 or the CH3 domain of SEQ ID NO: 11 minus one or two C-terminal amino acids.
18. ~~The A-specific binding member for use according to claim 17,~~ wherein the specific binding member of claim 1, part (a), and/or claim 1, part (b), further comprises the CH2 domain of SEQ ID NO: 10.
19. ~~The A-specific binding member for use according to any one of claims 1 to 18,~~
wherein the specific binding member of claim 1, part (a), is a dimer of a polypeptide of SEQ ID NO: 8, or a dimer of a polypeptide of SEQ ID NO: 8 minus one or two C-terminal amino acids.
20. ~~The A-specific binding member for use according to any one of claims 1 to 19,~~
wherein the specific binding member of claim 1, part (b) competes with a specific binding member according to claim 1 part (a) for binding to HER2, as determined by surface plasmon resonance (SPR), a competitive enzyme-linked immunosorbent assay (ELISA), fluorescence activated cell sorting (FACS), or competition immunocytochemistry.
21. ~~The A-specific binding member for use according any one of claims 1 to 20,~~ wherein the specific binding member of claim 1, part (b), binds to same epitope on HER2 as a specific binding member according to claim 1 part (a), and wherein the specific binding member according to claim 1 part (a) is a dimer of a polypeptide of SEQ ID NO: 8, or a dimer of a polypeptide of SEQ ID NO: 8 minus one or two C-terminal amino acids.
22. ~~The A-specific binding member for use according to any one of claims 2 or 5 to 21,~~
wherein the HER2 gene copy number is determined using fluorescence *in situ* hybridization (FISH), chromogenic *in situ* hybridisation (CISH), or quantitative polymerase chain reaction (qPCR).
23. ~~The A-specific binding member for use according to claim 22,~~ wherein the HER2 gene copy number is determined using FISH.

24. ~~The~~A specific binding member for use according to any one of claims 3 to 19, wherein the method comprises determining the HER2 mRNA level in a tumour sample obtained from the patient, wherein a high HER2 mRNA level corresponds to a HER2 cDNA copy number of greater than or equal to 200 in the tumour sample relative to the cDNA copy number of a reference gene in said sample, and wherein a high HER2 mRNA level indicates that the average HER2 gene copy number per tumour cell is greater than or equal to 10.

25. ~~The~~A specific binding member for use according to claim 24, wherein the cDNA copy number of HER2 and the reference gene is determined using reverse transcription PCR followed by quantitative PCR.

26. ~~— A method of treating cancer in a patient, wherein said cancer has an average HER2 gene copy number of greater than or equal to 10 per tumour cell, and wherein the method comprises administering to the patient a therapeutically effective amount of:~~

~~a) a specific binding member comprising a HER2 antigen binding site engineered into a structural loop region of a CH3 domain of the specific binding member and containing the amino acid sequences FFTYW (SEQ ID NO: 12), and DRRRWTA (SEQ ID NO: 14); or~~

~~(b) a specific binding member which competes with a specific binding member according to (a) for binding to HER2.~~

27. ~~— A method according to claim 26, wherein a tumour sample obtained from said patient has been determined to have an average HER2 gene copy number of greater than or equal to 10 per tumour cell.~~

28. ~~— A method according to claim 26 or 27, wherein the cancer has, or has been determined to have, a high HER2 mRNA level, wherein a high HER2 mRNA level corresponds to a HER2 cDNA copy number of greater than or equal to 200 in a tumour sample obtained from said patient relative to the cDNA copy number of a reference gene in said sample, wherein the cDNA copy number of HER2 and the reference gene in the sample is determined by RT-PCR followed by quantitative PCR.~~

29. ~~— A method according to claim 26 or 27, wherein the cancer has, or has been determined to have, a high HER2 mRNA level, wherein a high HER2 mRNA level corresponds to a HER2 cDNA copy number of greater than or equal to 200 and less than or equal to 820 in a tumour sample obtained from said patient relative to the cDNA copy number of a reference gene in said sample, wherein the cDNA copy number of HER2 and the reference gene in the sample is determined by RT-PCR followed by quantitative PCR.~~

~~30.~~ A method according to claim 26, wherein the method comprises:

- ~~(i) determining the average gene copy number of the HER2 gene per tumour cell in a tumour sample obtained from the patient; and~~
- ~~(ii) administering a therapeutically effective amount of the specific binding member to the patient if the average HER2 gene copy number is greater than or equal to 10 per tumour cell.~~

~~31~~26. A method of identifying a cancer in a patient which is susceptible to treatment with:

- (a) a specific binding member comprising a HER2 antigen binding site engineered into a structural loop region of a CH3 domain of the specific binding member and containing the amino acid sequences FFTYW (SEQ ID NO: 12), and DRRRWTA (SEQ ID NO: 14); or
- (b) a specific binding member which competes with a specific binding member according to (a) for binding to HER2; the method comprising:
 - (i) determining the average gene copy number of the HER2 gene per tumour cell in a tumour sample obtained from the patient, wherein an average gene copy number of greater than or equal to 10 per tumour cell indicates that the cancer is susceptible to treatment with the specific binding member.

~~32~~27. A method according to claim ~~31~~26, wherein the method further comprises:

- (ii) selecting said patient for treatment with the specific binding member if the average HER2 gene copy number is greater than or equal to 10 per tumour cell.

~~33~~28. A method of predicting the response of a cancer to treatment with:

- (a) a specific binding member comprising a HER2 antigen binding site engineered into a structural loop region of a CH3 domain of the specific binding member and containing the amino acid sequences FFTYW (SEQ ID NO: 12), and DRRRWTA (SEQ ID NO: 14); or
- (b) a specific binding member which competes with a specific binding member according to (a) for binding to HER2; the method comprising:
 - (i) determining the average gene copy number of the HER2 gene per tumour cell in a tumour sample obtained from a patient, wherein a gene copy number of greater than or equal to 10 per tumour cell indicates that the cancer is susceptible to treatment with the specific binding member, and wherein a gene copy number of less than 10 per tumour cell indicates that the cancer is not susceptible to treatment with the specific binding member.

~~34~~29. A method according to claim ~~33~~28, wherein the method further comprises:

- (ii) selecting said cancer for treatment with the specific binding member if the HER2

gene copy number is greater than or equal to 10 per tumour cell.

3530. A method according to any one of claims 26 to ~~3429~~, wherein the HER2 gene copy number is greater than or equal to 11, greater than or equal to 12, greater than or equal to 13, greater than or equal to 14, greater than or equal to 15, greater than or equal to 16, greater than or equal to 17, or greater than or equal to 18.

3631. A method according to claim 3530, wherein the HER2 gene copy number is greater than or equal to 18.

3732. A method according to any one of claims 26 to 3631, wherein the cancer is gastric cancer, breast cancer, colorectal cancer, ovarian cancer, pancreatic cancer, lung cancer, stomach cancer, or endometrial cancer.

3833. A method according to claim 3732, wherein the cancer is gastric cancer, breast cancer, or colorectal cancer.

3934. A method according to claim 3833, wherein the cancer is gastric cancer.

4035. A method according to claim 3833, wherein the cancer is breast cancer.

4136. A method according to any one of claims 26 to 4035, wherein the patient has exhibited an inadequate response to trastuzumab and/or trastuzumab plus pertuzumab.

4237. A specific binding member for use according to any one of claims 26 to 4136, wherein the cancer is refractory to treatment with trastuzumab and/or trastuzumab plus pertuzumab.

4338. A method according to any one of claims 26 to 4237, wherein the specific binding member of claim 26, ~~part (b), claim 3126, part (b), or claim 3328, part (b)~~, comprises a HER2 antigen binding site engineered into a structural loop region of a CH3 domain of the specific binding member.

4439. A method according to claim 4338, wherein the specific binding member comprises a HER2 antigen binding site engineered into structural loop regions AB and EF of a CH3 domain of the specific binding member.

4540. A method according to any one of claims 26 to 4439, wherein the specific binding member of claim 26, ~~part (a) and/or (b)~~, ~~claim 3126~~, part (a) and/or (b), or claim 3328, part (a) and/or (b), is, or comprises, an antigen-binding Fc fragment.

4641. A method according to any one of claims 26 to 4540, wherein the specific binding member of claim 26, ~~part (a) and/or (b)~~, ~~claim 3126~~, part (a) and/or (b), or claim 3328, part (a) and/or (b), comprises the CH3 domain of SEQ ID NO: 11, or the CH3 domain of SEQ ID NO: 11 minus one or two C-terminal amino acids.

4742. A method according to claim 4641, wherein the specific binding member of claim 26, ~~part (a) and/or (b)~~, ~~claim 3126~~, part (a) and/or (b), or claim 3328, part (a) and/or (b), further comprises the CH2 domain of SEQ ID NO: 10.

4843. A method according to any one of claims 26 to 4742, wherein the specific binding member of claim 26, ~~part (a)~~, ~~claim 3126~~, part (a), or claim 3328, part (a), is a dimer of a polypeptide of SEQ ID NO: 8, or a dimer of a polypeptide of SEQ ID NO: 8 minus one or two C-terminal amino acids.

4944. A method according to any one of claims 26 to 4843, wherein the specific binding member of claim 26, ~~part (b)~~, ~~claim 3126~~, part (b), or claim 3328, part (b), competes with a specific binding member according to claim 26, ~~part (a)~~, ~~claim 2926~~, part (a), or claim 3328, part (a), respectively, for binding to HER2 as determined by surface plasmon resonance (SPR), or a competitive enzyme-linked immunosorbent assay (ELISA), fluorescence activated cell sorting (FACS), or competition immunocytochemistry.

5045. A method according any one of claims 26 to 4944, wherein the specific binding member of claim 26, ~~part (b)~~, ~~claim 3126~~, part (b), or claim 3328, part (b), binds to same epitope on HER2 as a specific binding member according to claim 26, ~~part (a)~~, ~~claim 29~~, ~~part (a)~~, or claim 3328, part (a), respectively, and wherein the specific binding member according to claim 26, ~~part (a)~~, ~~claim 2926~~, part (a), or claim 3328, part (a), is a dimer of a polypeptide of SEQ ID NO: 8, or a dimer of a polypeptide of SEQ ID NO: 8 minus one or two C-terminal amino acids.

5146. A method according to any one of claims 26 to 5045, wherein the HER2 gene copy number is determined using fluorescence *in situ* hybridization (FISH), chromogenic *in situ* hybridisation (CISH), or quantitative polymerase chain reaction (qPCR).

- | 5247. A method according to claim 5146, wherein the HER2 gene copy number is determined using FISH.
- | 5348. A method according to any one of claims 26 to 5247, wherein the method comprises determining the HER2 mRNA level in a tumour sample obtained from the patient, wherein a high HER2 mRNA level corresponds to a HER2 cDNA copy number of greater than or equal to 200 in the tumour sample relative to the cDNA copy number of a reference gene in said sample, and wherein a high HER2 mRNA level indicates that the average HER2 gene copy number per tumour cell is greater than or equal to 10.
- | 5449. A method according to claim 5348, wherein the cDNA copy number of HER2 and the reference gene is determined using reverse transcription PCR followed by quantitative PCR.