

Anti-HER2 Antibodies

This application claims priority from SG10201801219V filed 13 February 2018, the contents and elements of which are herein incorporated by reference for all purposes.

Field of the Invention

The present invention relates to antibodies that bind to the HER2 protein.

Background to the Invention

Breast cancer is the most common cause of cancer deaths in females with more than 400,000 deaths per year globally. In Singapore, it is the most frequent cancer in women; affecting more than 8,000 women in Singapore from 2007-2011, representing about 30% of cancer cases in Singaporean females and being the leading cause of cancer deaths in Singaporean women (Singapore Cancer Registry, Interim Annual Registry Report, Trends in Cancer Incidence in Singapore).

A turning point in breast cancer treatment has been reached with the introduction of the monoclonal antibody, trastuzumab (Herceptin®) targeting the Epidermal Growth Factor Receptor 2 (HER2/HER-2) positive cancers, i.e. ~20-30% of breast cancers and the most aggressive ones. HER2 is a member of the epidermal growth factor receptor family including epidermal growth factor receptor (EGFR/HER1), HER3 and HER4. Either homo- or hetero-dimerization of these receptor tyrosine kinases results in the phosphorylation of intracellular domain residues, triggering a downstream signalling cascade including the RAS/Raf and Akt pathways. Dimerization of HER2 is known to result in increased cell survival, proliferation and resistance to apoptosis.

Trastuzumab is a humanised antibody approved for the treatment of HER2-positive breast cancer by FDA in 1998. Trastuzumab binds to domain IV of HER2, and while the mechanism of action of the antibody is still not well understood, several effects had been observed, including cell cycle arrest, immune-mediated cytotoxicity and inhibition of angiogenesis. However, response rates to trastuzumab treatment are still low, ranging from 15-30%. While the use of trastuzumab in combination with chemotherapy prolongs response duration, a majority of responders develop resistance within a year.

The mechanism of resistance has been studied intensively, but has not been validated clinically. Possible causes of resistance include reduced trastuzumab binding, e.g., the masking of trastuzumab binding sites by MUC4 glycoprotein upon association with HER2, signalling through alternative HER2 independent pathways, e.g., Insulin-like growth factor-I receptor (IGF-IR), and constitutive HER2 signalling as a result of mutations in downstream signalling molecules such as PTEN and PI3K.

In addition, there is evidence for an important role of HER3/HER2 heterodimers in breast cancer development, and the inability of trastuzumab to inhibit such heterodimer formation could be a contributing factor in the resistance to treatment.

To address this, pertuzumab (Perjeta®), a second generation HER2 humanised monoclonal antibody was developed. It binds to HER2 domain II, an epitope which hinders the receptor's dimerization. A phase III clinical study comparing the use of pertuzumab in combination with trastuzumab, and an anti-mitotic drug docetaxel (Taxotere®) in HER2-positive patients, showed significant improvements in overall survival with the new regimen, prompting the FDA to approve the combination therapy for treatment of HER2-positive metastatic breast cancer patients in 2012.

The prevalence of HER2-related cancers and the high risk of resistance to current therapies highlights the importance of developing new therapeutics to treat such cancers.

Summary

The present disclosure is concerned with antibodies, or antigen binding fragments, that bind to HER2. Heavy and light chain polypeptides are also disclosed. The antibodies, antigen binding fragments and polypeptides may be provided in isolated and/or purified form and may be formulated into compositions suitable for use in research, therapy and diagnosis.

In one aspect, the present disclosure refers to an antibody or antigen binding fragment which is capable of binding to HER2, optionally isolated, wherein the antibody or antigen binding fragment comprises: (i) a light chain variable region incorporating the following CDRs:

LC-CDR1: GLSSGSVSTGHYAS (SEQ ID NO:15)
 LC-CDR2: NTNTRSS (SEQ ID NO:16)
 LC-CDR3: VLYVGDGIWV (SEQ ID NO:17); and a heavy chain variable region

incorporating the following CDRs:

HC-CDR1: SYYIH (SEQ ID NO:18)
 HC-CDR2: IINPGNGDTNYAQRFGG (SEQ ID NO:19)

HC-CDR3: EIASYSGSYDY (SEQ ID NO:20).

5 In another aspect, the present disclosure refers to a chimeric antigen receptor (CAR) comprising an antibody or antigen binding fragment as disclosed.

In another aspect, the present disclosure refers to an *in vitro* complex, optionally isolated, comprising an antibody or antigen binding fragment as disclosed, or a CAR according as disclosed, bound to HER2.

10 In another aspect, the present disclosure refers to a nucleic acid, optionally isolated, encoding an antibody or antigen binding fragment as disclosed, or a CAR as disclosed.

In another aspect, the present disclosure refers to a vector comprising the nucleic acid as disclosed.

15 In another aspect, the present disclosure refers to a cell comprising the nucleic acid as disclosed, or the vector as disclosed.

20 In another aspect, the present disclosure refers to a method for making an antibody or antigen binding fragment as disclosed herein, or a CAR as disclosed herein, comprising culturing a cell as disclosed under conditions suitable for the expression of the antibody, antigen binding fragment or CAR from the nucleic acid or vector.

In another aspect, the present disclosure refers to a composition comprising an antibody or antigen binding fragment as disclosed, or a cell as disclosed herein, and at least one pharmaceutically-acceptable carrier.

5 In another aspect, the present disclosure refers to an antibody or antigen binding fragment as disclosed herein, a cell as disclosed herein, or a composition as disclosed, for use in therapy, or in a method of medical treatment.

10 In another aspect, the present disclosure refers to a use of an antibody or antigen binding fragment as disclosed, a cell as disclosed or a composition as disclosed herein, in the manufacture of a medicament for use in the treatment of cancer.

In another aspect, the present disclosure refers to a method comprising contacting, *in vitro*, a sample containing, or suspected to contain, HER2 with an antibody or antigen binding fragment as disclosed herein, and detecting the formation of a complex comprising the antibody or antigen binding fragment and HER2.

15 In yet another aspect, the present disclosure refers to a method of diagnosing a HER2-positive cancer in a subject, the method comprising contacting, *in vitro*, a sample from the subject with an antibody or antigen binding

fragment as disclosed, and detecting the formation of a complex comprising the antibody or antigen binding fragment and HER2.

In one example of the present disclosure an antibody, or antigen binding fragment, is provided, the amino acid sequence of the antibody or antigen binding fragment may comprise the amino acid sequences i) to iii), or the amino acid sequences iv) to vi), or preferably the amino acid sequences i) to vi):

- i) LC-CDR1: GLSSGSVSTX₁X₂YX₃S (SEQ ID NO:9);
- ii) LC-CDR2: X₄TNX₅RSS (SEQ ID NO:10);
- iii) LC-CDR3: X₆LYX₇GX₈GIX₉V (SEQ ID NO:11);
- iv) HC-CDR1: X₁₀X₁₁X₁₂X₁₃X₁₄X₁₅X₁₆ (SEQ ID NO:12);
- v) HC-CDR2: X₁₇X₁₈X₁₉X₂₀X₂₁X₂₂GX₂₃TX₂₄YX₂₅X₂₆X₂₇X₂₈X₂₉X₃₀ (SEQ ID NO:13);
- vi) HC-CDR3: X₃₁X₃₂X₃₃X₃₄X₃₅SX₃₆X₃₇YX₃₈X₃₉X₄₀X₄₁X₄₂X₄₃ (SEQ ID NO:14);

or a variant thereof in which one or two or three amino acids in one or more of the sequences (i) to (vi) are replaced with another amino acid, where X₁ = G or S; X₂ = H or Y; X₃ = A or P; X₄ = N, S or T; X₅ = T, S or I; X₆ = V or M; X₇ = V or M; X₈ = D or S; X₉ = W or S; X₁₀ = S or absent; X₁₁ = S or absent; X₁₂ = S or G; X₁₃ = Y or N; X₁₄ = Y or W; X₁₅ = I or W; X₁₆ = H, G or S; X₁₇ = I or absent; X₁₈ = I, S or E; X₁₉ = N or I; X₂₀ = P, Y or N; X₂₁ = G, Y or H; X₂₂ = N or S; X₂₃ = D or S; X₂₄ = N or Y; X₂₅ = A or N; X₂₆ = Q or P; X₂₇ = R or S; X₂₈ = F or L; X₂₉ = Q or K; X₃₀ = G or S; X₃₁ = E or absent; X₃₂ = I, Y or M; X₃₃ = A or G; X₃₄ = S, P, I or A; X₃₅ = Y, D or N; X₃₆ = G or S; X₃₇ = G or S; X₃₈ = L or absent; X₃₉ = V or Y; X₄₀ = A, D or G; X₄₁ = F, M or Y; X₄₂ = D or absent; and X₄₃ = I, V or absent.

In some examples LC-CDR1 is one of GLSSGSVSTGHYAS (SEQ ID NO:15), GLSSGSVSTGYYP (SEQ ID NO:21), or GLSSGSVSTSYYP (SEQ ID NO:27).

5 In some examples LC-CDR2 is one of NTNTRSS (SEQ ID NO:16), STNSRSS (SEQ ID NO:22), TTNIRSS (SEQ ID NO:28), or STNTRSS (SEQ ID NO:33).

In some examples LC-CDR3 is one of VLYVGDGIWV (SEQ ID NO:17), VLYMGSGISV (SEQ ID NO:23); MLYMGSGIWV (SEQ ID NO:29), or VLYMGSGIWV (SEQ ID NO:34).

10 In some examples HC-CDR1 is one of SYYIH (SEQ ID NO:18), SSSYYWG (SEQ ID NO:24), GYYWS (SEQ ID NO:30), or SSNWWS (SEQ ID NO:35).

In some examples HC-CDR2 is one of IINPGNGDTNYAQRFG (SEQ ID NO:19), SIYYSGSTYYNPSLKS (SEQ ID NO:25), EINHSGSTNYNPSLKS (SEQ ID NO:31), or EIYHSGSTNYNPSLKS (SEQ ID NO:36).

In some examples HC-CDR3 is one of EIASYSGSYDDY (SEQ ID NO:20), YAPDSSGYLVAFDI (SEQ ID NO:26), MGINSGGYLYGMDV (SEQ ID NO:32), or MGANSGGYLYGMDV (SEQ ID NO:37).

5 In some examples the antibody, or antigen binding fragment, may comprise at least one light chain variable region incorporating the following CDRs:

Claims:

1. An antibody or antigen binding fragment which is capable of binding to HER2, optionally isolated, wherein the antibody or antigen binding fragment comprises:
 - a light chain variable region incorporating the following CDRs:
 - LC-CDR1: GLSSGSVSTGHYAS (SEQ ID NO:15)
 - LC-CDR2: NTNTRSS (SEQ ID NO:16)
 - LC-CDR3: VLYVGDIWV (SEQ ID NO:17); and
 - a heavy chain variable region incorporating the following CDRs:
 - HC-CDR1: SYYIH (SEQ ID NO:18)
 - HC-CDR2: IINPGNGDTNYAQRFGG (SEQ ID NO:19)
 - HC-CDR3: EIASYSGSYDDY (SEQ ID NO:20).
2. The antibody or antigen binding fragment according to claim 1, wherein the antibody or antigen binding fragment comprises:
 - a light chain variable region having at least 85% sequence identity to the light chain variable region sequence of SEQ ID NO:1; and
 - a heavy chain variable region having at least 85% sequence identity to the heavy chain variable region sequence of SEQ ID NO:5.
3. The antibody or antigen binding fragment according to claim 1 or claim 2, wherein the antibody or antigen binding fragment binds specifically to human, rhesus macaque or murine HER2.
4. The antibody or antigen binding fragment according to any one of claim 1 to 3, conjugated to a drug moiety or a detectable moiety.
5. The antibody or antigen binding fragment according to claim 4, wherein the drug moiety comprises an anti-cancer drug moiety.
6. A chimeric antigen receptor (CAR) comprising an antibody or antigen binding fragment according to any one of claims 1 to 3.
7. An *in vitro* complex, optionally isolated, comprising an antibody or antigen binding fragment according to any one of claims 1 to 5, or a CAR according to claim 6, bound to HER2.
8. A nucleic acid, optionally isolated, encoding an antibody or antigen binding fragment according to any one of claims 1 to 5, or a CAR according to claim 6.
9. A vector comprising the nucleic acid according to claim 8.

10. A cell comprising the nucleic acid according to claim 8, or the vector according to claim 9.
11. A method for making an antibody or antigen binding fragment according to any one of claims 1 to 5, or a CAR according to claim 6, comprising culturing a cell according to claim 10 under conditions suitable for the expression of the antibody, antigen binding fragment or CAR from the nucleic acid or vector.
12. A composition comprising an antibody or antigen binding fragment according to any one of claims 1 to 5, or a cell according to claim 10, and at least one pharmaceutically-acceptable carrier.
13. An antibody or antigen binding fragment according to any one of claims 1 to 5, a cell according to claim 10, or a composition according to claim 12, for use in therapy, or in a method of medical treatment.
14. Use of an antibody or antigen binding fragment according to any one of claims 1 to 5, a cell according to claim 10, or a composition according to claim 12, in the manufacture of a medicament for use in the treatment of cancer.
15. The use according to claim 14, wherein the cancer is a HER2-positive cancer.
16. The use according to claim 15, wherein the cancer comprises a HER2-positive tumour cell.
17. A method comprising contacting, *in vitro*, a sample containing, or suspected to contain, HER2 with an antibody or antigen binding fragment according to any one of claims 1 to 5, and detecting the formation of a complex comprising the antibody or antigen binding fragment and HER2.
18. A method of diagnosing a HER2-positive cancer in a subject, the method comprising contacting, *in vitro*, a sample from the subject with an antibody or antigen binding fragment according to any one of claims 1 to 5, and detecting the formation of a complex comprising the antibody or antigen binding fragment and HER2.