While EGF and TGFα do not bind HER2, EGF stimulates EGFR and HER2 to form a heterodimer, which activates EGFR and results in transphosphorylation of HER2 in the heterodimer. Dimerization and/or transphosphorylation appears to activate the HER2 tyrosine kinase. See Earp *et al., supra*. Likewise, when HER3 is co-expressed with HER2, an active signaling complex is formed and antibodies directed against HER2 are capable of disrupting this complex (Sliwkowski *et al., J. Biol. Chem.*, 269(20):14661-14665 (1994)). Additionally, the affinity of HER3 for heregulin (HRG) is increased to a higher affinity state when co-expressed with HER2. See also, Levi *et al., Journal of Neuroscience* 15: 1329-1340 (1995); Morrissey *et al., Proc. Natl. Acad. Sci. USA* 92: 1431-1435 (1995); and Lewis *et al., Cancer Res.*, 56:1457-1465 (1996) with respect to the HER2-HER3 protein complex. HER4, like HER3, forms an active signaling complex with HER2 (Carraway and Cantley, *Cell* 78:5-8 (1994)).

Therapeutics that target the HER pathway are presently in use in treating diseases such as breast cancer, non-small cell lung cancer, colorectal cancer, head and neck cancer and pancreatic cancer. While these therapeutics have had some success, there remain issues related to native and induced resistance and toxicity. Arteaga CL. *J Clin Oncol* 21:289–91s (2003); Hoshi S, *et al., Gan To Kagaku Ryoho* 31:1209–13 (2004); Viloria-Petit AM, and Kerbel RS. *Int J Radiat Oncol Biol Phys* 58:914–26 (2004); Bianco R,, et al., *Endocr Relat Cancer* 12:S159–71 (2005); Engelman JA, and Janne PA., *Clin Cancer Res* 14:2895–9 (2008); Davoli A, *et al., Cancer Chemother Pharmacol.* 65(4):611-23 (2010); Pohlmann PR, *et al., Clin Cancer Res.* 15(24):7479-7491 (2009). In particular, therapeutics that target HER1 (EGFR) are often associated with undesirable side effects, such as significant levels of skin toxicity. Robert, *et al. Lancet Oncology* 6:491-500 (2005).

Accordingly, a need exists to develop improved therapeutics that target the HER pathway.

SUMMARY ~~OF THE INVENTION~~

The ~~invention~~disclosure provides for multispecific antibodies comprising an antigen-binding domain that specifically binds to at least two HER receptors selected from the group consisting of (a) EGFR and HER2, (b) EGFR and HER3, and (c) EGFR and HER4. The antibody inhibits a biological activity of at least one of the HER receptors. In particular embodiments, the multispecific antibody specifically binds to its target HER receptors and does not specifically bind to the non-target HER receptors. Accordingly, in one embodiment, the antibody specifically binds to EGFR and HER3 but does not specifically bind to HER2 or HER4. In another embodiment, the antibody specifically binds to EGFR and HER2 but does not specifically bind to HER3 or HER4. In another embodiment, the antibody specifically

binds to EGFR and HER4 but does not specifically bind to HER2 or HER3. The ~~invention~~disclosure also provides for monospecific antibodies that specifically bind to a target HER receptor.

One ~~aspect~~embodiment of the ~~invention~~disclosure provides for multispecific antibodies that are capable of specifically binding to EGFR and another HER receptor that are less toxic than traditional EGFR antagonists, such as cetuximab. In one embodiment, the toxicity is dermatological toxicity. In one embodiment, the multispecific HER antibody comprises an antigen-binding domain that specifically binds to EGFR and HER3.

In one ~~aspect~~embodiment, the ~~invention~~disclosure provides a multispecific antibody comprising an antigen-binding domain that specifically binds to EGFR and HER3. In one embodiment, the multispecific antibody is less toxic than EGFR antagonists. In one embodiment, the multispecific antibody inhibits a biological activity of at least one of EGFR and HER3. In one embodiment, the antibody inhibits EGF binding to EGFR. In another embodiment, the antibody inhibits TGF-α induced EGFR phosphorylation. In some embodiments, the antibody inhibits tumor cell growth. In one embodiment, the multispecific antibody specifically binds to EGFR and HER3 but does not specifically bind to HER2 or HER4.

In one embodiment, the multispecific antibody comprises an antigen-binding domain that specifically binds to EGFR and HER3 with a Kd of less than 10^{-6} M. In one embodiment the multispecific antibody comprises an antigen-binding domain that specifically binds EGFR with a Kd of less than 10^{-6} M and specifically binds HER3 with a Kd of less than 10^{-7} M.

In one aspect~~embodiment~~, there is provided a multispecific antibody comprising an antigen-binding domain that specifically binds to EGFR and HER3, wherein the antibody comprises (a) HVR-H1 comprising the amino acid sequence of LSGDWIH (SEQ ID NO: 48); (b) HVR-H2 comprising the amino acid sequence of VGEISAAGGYTD (SEQ ID NO: 51); and (c) HVR-H3 comprising the amino acid sequence of ARESRVSFEAAMDY (SEQ ID NO: 53); and (d) HVR-L1 comprising the amino acid sequence of NIATDVA (SEQ ID NO: 55); (e) HVR-L2 comprising the amino acid sequence of SASF (SEQ ID NO: 56); and (f) HVR-L3 comprising the amino acid sequence of SEPEPYT (SEQ ID NO: 57).

In one embodiment, the multispecific antibody comprising an antigen-binding domain that specifically binds to EGFR and HER3 comprises (a) a heavy chain variable domain having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 30; (b) a light chain variable domain having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 29; or (c) a heavy chain variable domain sequence as in (a) and a light chain variable domain sequence as in (b). In one embodiment, the multispecific antibody comprising an antigen-binding domain that specifically binds to EGFR and HER3 comprises a heavy chain variable domain sequence of SEQ ID NO: 30. In one embodiment,

the multispecific antibody comprising an antigen-binding domain that specifically binds to EGFR and HER3 comprises a light chain variable domain sequence of SEQ ID NO: 29. In another embodiment, the multispecific antibody comprising an antigen-binding domain that specifically binds to EGFR and HER3 comprises a heavy chain variable domain sequence of SEQ ID NO: 30 and a light chain variable domain sequence of SEQ ID NO: 29.

In another aspect, there is provided a multispecific antibody comprising a heavy chain variable domain sequence of SEQ ID NO: 30 and a light chain variable domain sequence of SEQ ID NO: 29, wherein the antibody specifically binds to EGFR and HER3.

In some embodiments, the multispecific antibody comprising an antigen-binding domain that specifically binds to EGFR and HER3 is a full length IgG1 antibody.

In one aspect, there is provided an isolated nucleic acid encoding the antibody as defined above.

In another aspect, there is provided a host cell comprising the nucleic acid as defined above.

In another aspect, there is provided a method of producing an antibody comprising culturing the host cell as defined above so that the antibody is produced.

One ~~aspect~~embodiment of the ~~invention~~disclosure provides for an isolated nucleic acid encoding the multispecific HER antibodies. Another ~~aspect~~embodiment provides for a host cell comprising the nucleic acid encoding the multispecific HER antibodies. Yet another ~~aspect~~embodiment provides for a method of producing a multispecific HER antibody comprising culturing the host cell comprising the nucleic acid encoding the multispecific HER antibody so that the antibody is produced.

In one aspect, there is provided an immunoconjugate comprising the antibody as defined above and a cytotoxic agent.

In another aspect, there is provided a pharmaceutical formulation comprising the antibody as defined above and a pharmaceutically acceptable carrier.

One ~~aspect~~embodiment of the ~~invention~~disclosure provides for an immunoconjugate comprising a multispecific HER antibody and a cytotoxic agent. Another ~~aspect~~embodiment provides a pharmaceutical formulation comprising a multispecific HER antibody and a pharmaceutically acceptable carrier.

One ~~aspect~~embodiment of the ~~invention~~disclosure provides for a method of treating an individual having cancer comprising administering to the individual an effective amount of a multispecific HER antibody. In one embodiment, the multispecific HER antibody comprises an antigen-binding domain that specifically binds to EGFR and HER3. In one embodiment, the cancer treated by the multispecific HER antibody comprises cells that express EGFR and HER3. In one embodiment, the cancer treated by the multispecific HER antibody is breast cancer, colorectal cancer, pancreatic cancer, head and neck cancer, melanoma, ovarian cancer, prostate cancer, or non-small lung cell cancer.

Another ~~aspect~~embodiment of the ~~invention~~disclosure provides for a method of inhibiting a biological activity of a HER receptor in an individual comprising administering to the individual an effective amount of a multispecific HER antibody. In one embodiment, the multispecific HER antibody comprises an antigen-binding domain that specifically binds to EGFR and HER3.

In one aspect, there is provided the antibody as defined above for use as a medicament.

One ~~aspect~~embodiment of the ~~invention~~disclosure provides for a multispecific HER antibody for use as a medicament. Another ~~aspect~~embodiment provides for a multispecific HER antibody for use in treating cancer, such as breast cancer, colorectal cancer, pancreatic cancer, head and neck cancer, melanoma, ovarian cancer, prostate cancer, or non-small lung cell cancer. Another ~~aspect~~embodiment provides for a multispecific HER antibody for use in inhibiting a biological activity of a HER

receptor. In one embodiment, the multispecific HER antibody comprises an antigen-binding domain that specifically binds to EGFR and HER3.

In one aspect, there is provided the use of the antibody as defined above in the manufacture of a medicament.

In another aspect, there is provided the use of the antibody as defined above in the manufacture of a medicament for treatment of cancer.

Another ~~aspect~~embodiment of the ~~invention~~disclosure provides for a multispecific HER antibody for use in the manufacture of a medicament. The medicament can be used, in one embodiment, to treat cancer, such as breast cancer, colorectal cancer, pancreatic cancer, head and neck cancer, melanoma, ovarian cancer, prostate cancer, or non-small lung cell cancer. In one embodiment, the medicament is for inhibiting a biological activity of a HER receptor. In one embodiment, the multispecific HER antibody comprises an antigen-binding domain that specifically binds to EGFR and HER3. In one embodiment, the multispecific HER antibody is less toxic than traditional EGFR antagonists, such as cetuximab.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows inhibition of tumor growth by anti-EGFR antibody D1.5 in an A431 xenograft model.

Figure 2 shows the binding specificity of clones with dual specificity.

Figure 3 shows the inhibition of TGF-α-induced EGFR phosphorylation using selected antibodies having dual specificity (anti-EGFR/HER3 and anti-EGFR/HER2 antibodies).

Figure 4 shows that anti-EGFR/HER3 antibodies block heregulin binding to HER3-ECD-Fc.

Figure 5 shows the inhibition of HRG-induced receptor phosphorylation in MCF7 cells by anti-EGFR/HER3 bispecific antibodies.

Figure 6 shows the inhibition of cellular proliferation of MDA-175 cells by anti-EGFR/HER3 antibodies.

Figure 7 is an image showing the D1.5-100 hot spots mapped on 4D5-Fv structure.

Figure 8 outlines a strategy for affinity maturation of D1.5-100 EGFR-HER3 antibody using shotgun libraries.

Figure 9 shows that two selected affinity matured antibodies (DL7 and DL11) are specific for both EGFR and HER3.

Figure 10: A) Shows the comparison of the inhibitory function of affinity matured antibodies DL7 and DL11 and parental antibody D1.5 on EGFR. B) Shows the comparison of the inhibitory function of affinity matured antibodies DL7 and DL11, and monospecific anti-HER3 antibody DL3.6 on HER3 transactivation.

CLAIMS

1. A multispecific antibody comprising an antigen-binding domain that specifically binds to EGFR and HER3, wherein the antibody comprises

(a) HVR-H1 comprising the amino acid sequence of LSGDWIH (SEQ ID NO: 48);

(b) HVR-H2 comprising the amino acid sequence of VGEISAAGGYTD (SEQ ID NO: 51); and

(c) HVR-H3 comprising the amino acid sequence of ARESRVSFEAAMDY (SEQ ID NO: 53); and

(d) HVR-L1 comprising the amino acid sequence of NIATDVA (SEQ ID NO: 55);

(e) HVR-L2 comprising the amino acid sequence of SASF (SEQ ID NO: 56); and

(f) HVR-L3 comprising the amino acid sequence of SEPEPYT (SEQ ID NO: 57).

2. The antibody of claim 1, comprising

(a) a heavy chain variable domain having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 30; and

(b) a light chain variable domain having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 29.; ~~or~~

~~(c) a heavy chain variable domain as in (a) and a light chain variable domain as in (b).~~

3 The antibody of claim 2, comprising a heavy chain variable domain of SEQ ID NO: 30.

4. The antibody of claim 2, comprising a light chain variable domain sequence of SEQ ID NO: 29.

5. A multispecific antibody comprising a heavy chain variable domain sequence of SEQ ID NO: 30 and a light chain variable domain sequence of SEQ ID NO: 29, wherein the antibody specifically binds to EGFR and HER3.

6. The antibody of any of claims 1-5 which is a bispecific antibody.

7. The antibody of any of claims 1-6, wherein the antibody is a full length IgG1 antibody.

8. The antibody of any of claim 1-7, wherein the antibody exhibits ADCC activity.

20. The antibody of any of claims 1-19, wherein the antibody binds to domain III of HER3.

21. The antibody of any of claims 1-20, wherein the antibody binds to domain III of EGFR and domain III of HER3.

22. The antibody of any of claims 1-21, wherein the antibody binds to EGFR and HER3 with a Kd of less than 10^{-6} M.

23. The antibody of any of claims 1-22, wherein the antibody binds to EGFR with a Kd of less than 10^{-6} M and binds to HER3 with a Kd of less than 10^{-7} M.

24. Isolated nucleic acid encoding the antibody of any of claims 1-23.

25. A host cell comprising the nucleic acid of claim 24.

26. A method of producing an antibody comprising culturing the host cell of claim 25 so that the antibody is produced.

27. An immunoconjugate comprising the antibody of any of claims 1-23 and a cytotoxic agent.

28. A pharmaceutical formulation comprising the antibody of any of claims 1-26, 1-23 and a pharmaceutically acceptable carrier.

29. The antibody of any of claims 1-23 for use as a medicament.

30. Use of the antibody of any of claims 1-23 in the manufacture of a medicament.

31. The use of the antibody of any of claims 1-23 in the manufacture of a medicament for treatment of cancer.

32. The use of claim 31, wherein the cancer comprises cells that express EGFR and HER3.

33. The use of claim 32, wherein the cancer is selected from the group consisting of breast cancer, colorectal cancer, pancreatic cancer, head and neck cancer, melanoma, ovarian cancer, prostate cancer, and non-small lung cell cancer.