phosphotidylinositol-3 kinase binding site and a PDZ domain binding motif (Plowman G D, wt al, PNAS 90:1746-50 (1993); Zimonjic D B, et al, Oncogene 10:1235-7(1995); Culouscou J M, et al, J. Biol. Chem. 268:18407-10(1993)). The protein binds to and is activated by neuregulins-2 and -3, heparin-binding EGF-like growth factor and betacellulin. Ligand binding induces a variety of cellular responses including mitogenesis and differentiation. Multiple proteolytic events allow for the release of a cytoplasmic fragment and an extracellular fragment. Mutations in this gene have been associated with cancer. Alternatively spliced variants which encode different protein isoforms have been described; however, not all variants have been fully characterized.

Anti-HER4 antibodies for use in anti-cancer therapy are known e.g. from US 5,811,098, US 7,332,579 or Hollmén M, et al, Oncogene. 28 (2009) 1309-19 (anti-ErbB-4 antibody mAb 1479).

So far it was not possible to select antigen binding proteins, in particular antibodies,that specifically bind to the beta-hairpin of HER3 as this beta-hairpin of HER3 represents a hidden epitopes, which is not accessible in the equilibrium state of HER3 (see Figure 1).

Summary ~~of the Invention~~

We now have found a method using the beta-hairpin of HER3 (and HER4 for counterscreenig) functionally presented in a 3-dimensional orientation within SlyD scaffolds (see e.g Figure 2, and the polypeptides of SEQ ID NOs. 13, and 17 to 24) to obtain such antibodies.

The ~~invention~~disclosure provides, in one aspect, a method for selecting an antigen binding protein, in particular an antibody, that binds to human HER3 (and that does not crossreact with human HER4),

wherein the antigen binding protein, in particular the antibody, binds within an amino acid sequence of PQPLVYNKLTFQLEPNPHT (SEQ ID NO:1) of human HER3;

wherein

a) at least one polypeptide selected from the group consisting of:

SEQ ID NO: 13 TtSlyD-FKBP-Her3,

SEQ ID NO: 17 TtSlyDcas-Her3,
SEQ ID NO: 18 TtSlyDcys-Her3,
SEQ ID NO: 19 TgSlyDser-Her3, and
SEQ ID NO: 20 TgSlyDcys-Her3,

which comprises the amino acid sequence of SEQ ID NO:1;

and

b) at least one polypeptide selected from the group consisting of:

SEQ ID NO: 21 TtSlyDcas-Her4,
SEQ ID NO: 22 TtSlyDcys-Her4,
SEQ ID NO: 23 TgSlyDser-Her4, and
SEQ ID NO: 24 TgSlyDcys-Her4,

which comprises the amino acid sequence of SEQ ID NO:2;

are used to select antigen binding proteins, in particular antibodies, which show binding to the at least one polypeptide under a) and which shows no binding to the at least one polypeptide under b)

and thereby selecting an antigen binding protein, in particular anantibody that binds within an amino acid sequence of PQPLVYNKLTFQLEPNPHT (SEQ ID NO:1) of human HER3 and that does not crossreact with human HER4.

The ~~invention~~disclosure provides, in another aspect, an antigen binding protein, in particular an antibody, obtained by such selection method.

The ~~invention~~disclosure provides an isolated ~~an~~ antigen binding protein, in particular an antibody, that binds to human HER3 (and that does not crossreact with human HER4), wherein the antigen binding protein, in particular the antibody, binds within an amino acid sequence of PQPLVYNKLTFQLEPNPHT (SEQ ID NO:1) of human HER3.

The ~~invention~~disclosure further provides, in another aspect, an isolated antigen binding protein that binds to human HER3 (and that does not crossreact with human HER4),

a) wherein the antigen binding protein binds to a polypeptide of

SEQ ID NO: 18 TtSlyDcys-Her3,

and

b) wherein the antigen binding protein does not crossreact with a polypeptide of

SEQ ID NO: 22 TtSlyDcys-Her4.

The ~~invention~~disclosure further provides, in another aspect, an isolated antigen binding protein that binds to human HER3 (and that does not crossreact with human HER4),

a) wherein the antigen binding protein binds within an amino acid sequence of PQPLVYNKLTFQLEPNPHT (SEQ ID NO:1) which is comprised in a polypeptide of SEQ ID NO: 18 (TtSlyDcas-Her3), and

b) wherein the antigen binding protein does not crossreact with an amino acid sequence of PQTFVYNPTTFQLEHNFNA (SEQ ID NO:2) which is comprised in a polypeptide of SEQ ID NO: 22 (TtSlyDcas-Her4).

The ~~invention~~disclosure further provides an isolated antibody that binds to human HER3 (and that does not crossreact with human HER4),

a) wherein the antibody binds to a polypeptide of

SEQ ID NO: 18 TtSlyDcys-Her3,

and

b) wherein the antibody does not crossreact with a polypeptide of

SEQ ID NO: 22 TtSlyDcys-Her4.

The ~~invention~~disclosure further provides an isolated antibody that binds to human HER3 (and that does not crossreact with human HER4),

a) wherein the antibody binds within an amino acid sequence of PQPLVYNKLTFQLEPNPHT (SEQ ID NO:1) which is comprised in a polypeptide of SEQ ID NO: 18 (TtSlyDcas-Her3), and

b) wherein the antibody does not crossreact with an amino acid sequence of PQTFVYNPTTFQLEHNFNA (SEQ ID NO:2) which is comprised in a polypeptide of SEQ ID NO: 22 (TtSlyDcas-Her4).

The ~~invention~~disclosure provides, in another aspect, an isolated antibody that binds to human HER3 (and that does not crossreact with human HER4), wherein the antibody binds to the amino acid sequence SEQ ID NO:1 in activated HER3.

The ~~invention~~disclosure provides an isolated antibody that binds to human HER3 (and that does not crossreact with human HER4), wherein the antibody

binds to the amino acid sequence SEQ ID NO:1 in activated HER3; and

inhibits the heterodimerisation of HER3/HER2 heterodimers.

The ~~invention~~disclosure provides an isolated antibody that binds to human HER3 (and that does not crossreact with human HER4), wherein the antibody

a) binds to the amino acid sequence of SEQ ID NO:1; and/or

b) binds to the amino acid sequence SEQ ID NO:1 in activated HER3; and/or

c) binds within an amino acid sequence of PQPLVYNKLTFQLEPNPHT (SEQ ID NO:1) which is comprised in a polypeptide selected from the group consisting of:

SEQ ID NO: 13	TtSlyD-FKBP-Her3,
SEQ ID NO: 17	TtSlyDcas-Her3,
SEQ ID NO: 18	TtSlyDcys-Her3,
SEQ ID NO: 19	TgSlyDser-Her3, and
SEQ ID NO: 20	TgSlyDcys-Her3;

and/or

d) binds to the ß-hairpin region of HER3; and/or

e) inhibits the heterodimerisation of HER3/HER2 heterodimers; and/or

f) has a ratio of the association constant (Ka) of binding to Thermus thermophilus SlyD FKBP-Her3 (SEQ ID NO:13) and the association constant (Ka) of binding to HER3-ECD (SEQ ID NO:4) of 1.5 or higher

0 minutes after incubation with the antibody in a FACS assay with HER3 expressing T47D cells; and/ or

q) shows approximately complete internalization of HER3 in the presence of Heregulin after 4 h after incubation with the antibody in a FACS assay with HER3 expressing T47D cells.

The disclosure provides, in another aspect, an isolated antibody that binds to human HER3, wherein the antibody has one or more of the following properties:

a) the antibody binds to the amino acid sequence SEQ ID NO:1 in activated HER3; and/or

b) the antibody binds within an amino acid sequence of PQPLVYNKLTFQLEPNPHT (SEQ ID NO:1) which is comprised in a polypeptide selected from the group consisting of:

SEQ ID NO: 13 TtSlyD-FKBP-Her3,
SEQ ID NO: 17 TtSlyDcas-Her3,
SEQ ID NO: 18 TtSlyDcys-Her3,
SEQ ID NO: 19 TgSlyDser-Her3, and
SEQ ID NO: 20 TgSlyDcys-Her3;
and/or

c) the antibody inhibits the heterodimerisation of HER3/HER2 heterodimers; and/or

d) the antibody has a ratio of the association constant (Ka) of binding to Thermus thermophilus SlyD FKBP-Her3 (SEQ ID NO:13) and the association constant (Ka) of binding to HER3-ECD (SEQ ID NO:4) of 1.5 or higher (Ka (Thermus thermophilus SlyD FKBP-Her3)/ (Ka (HER3-ECD)), when measured in a Surface Plasmon Resonance assay; and/or

e) the antibody has a ratio of the Molar Ratio MR of binding to Thermus thermophilus SlyD FKBP-Her3 (SEQ ID NO:13) and the Molar Ratio MR of binding to HER3-ECD (SEQ ID NO:4) of 2.0 or higher (MR (Thermus thermophilus SlyD FKBP-Her3)/ (MR (HER3-ECD)), when measured in a Surface Plasmon Resonance assay

f)the antibody has no crossreactivity to the amino acid sequence of SEQ ID NO:2; and/or

g) the antibody has no crossreactivity to the amino acid sequence of PQTFVYNPTTFQLEHNFNA (SEQ ID NO:2) which is comprised in a polypeptide selected from the group consisting of:

SEQ ID NO: 21 TtSlyDcas-Her4,
SEQ ID NO: 22 TtSlyDcys-Her4,
SEQ ID NO: 23 TgSlyDser-Her4, and
SEQ ID NO: 24 TgSlyDcys-Her4;

h) the antibody does not compete for binding to HER3 with Heregulin; and/or

i) the antibody induces binding of Heregulin to HER3; and/or

j) the antibody shows an at least two fold higher binding level in the presence of Heregulin when compared to the binding level in the absence of Heregulin, as detected 0 minutes after incubation with the antibody in a FACS assay with HER3 expressing T47D cells; and/ or

the antibody shows approximately complete internalization of HER3 in the presence of Heregulin after 4 h after incubation with the antibody in a FACS assay with HER3 expressing T47D cells.

In one embodiment such anti-HER3 antibody is a monoclonal antibody.

In one embodiment such anti-HER3 antibody is a human, humanized, or chimeric antibody.

In one embodiment such anti-HER3 antibody is an antibody fragment that binds human HER3.

The disclosure provides, in another aspect, ~~In one embodiment the invention provides~~ an anti-HER3 antibody comprising

i) (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:25;
(b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:26;
(c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:27;
(d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:28;
(e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:29; and
(f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:30;

ii) or a humanized variant of the HVRs of the antibody under i) (a), (b), (d) and/or (e).

The disclosure provides, in another aspect, ~~In one embodiment the invention provides~~ an anti-HER3 antibody comprising

i) (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:33;
(b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:34;
(c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:35;
(d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:36;
(e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:37; and
(f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:38;

ii) or a humanized variant of the HVRs of the antibody under i) (a), (b), (d) and/or (e).

The disclosure provides, in another aspect, ~~In one embodiment the invention provides~~ an anti-HER3 antibody comprising

i) (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:41;
(b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:42;
(c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:43;
(d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:44;
(e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:45; and
(f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:46;

ii) or a humanized variant of the HVRs of the antibody under i) (a), (b), (d) and/or (e).

The disclosure provides, in another aspect, ~~In one embodiment the invention provides~~ an anti-HER3 antibody comprising

i) (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:49;
(b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:50;
(c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:51;
(d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:52;
(e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:53; and
(f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:54;

ii) or a humanized variant of the HVRs of the antibody under i) (a), (b), (d) and/or (e).

In one embodiment such anti-HER3 antibody comprises

i) comprises a VH sequence of SEQ ID NO:31 and a VL sequence of SEQ ID NO:32;

ii) or humanized variant of the VH and VL of the antibody under i).

In one embodiment such anti-HER3 antibody comprises

i) comprises a VH sequence of SEQ ID NO:39 and a VL sequence of SEQ ID NO:40;

ii) or humanized variant of the VH and VL of the antibody under i).

In one embodiment such anti-HER3 antibody comprises

i) comprises a VH sequence of SEQ ID NO:47 and a VL sequence of SEQ ID NO:48;

ii) or humanized variant of the VH and VL of the antibody under i).

In one embodiment such anti-HER3 antibody comprises

i) comprises a VH sequence of SEQ ID NO:56 and a VL sequence of SEQ ID NO:57;

ii) or humanized variant of the VH and VL of the antibody under i).

In one embodiment such anti-HER3 antibody is a full length IgG1 antibody or IgG4 antibody.

In one embodiment such anti-HER3 antibody is a Fab fragment.

The ~~invention~~disclosure further provides, in another aspect, an isolated nucleic acid encoding such anti-HER3 antibody.

The ~~invention~~disclosure further provides, in another aspect, a host cell comprising such nucleic acid.

The ~~invention~~disclosure further provides a method of producing an antibody comprising culturing such host cell so that the antibody is produced.

In on embodiment such method further comprises recovering the antibody from the host cell. Hence, the disclosure provides, in another aspect, a method of producing an antibody comprising culturing the host cell as described herein so that the antibody is produced, and recovering said antibody from said cell culture or the cell culture supernatant.

The ~~invention~~disclosure further provides, in another aspect, an immunoconjugate comprising such anti-HER3 antibody and a cytotoxic agent.

The ~~invention~~disclosure further provides, in another aspect, a pharmaceutical formulation comprising such anti-HER3 antibody, or the immunoconjugate comprising the anti-HER3 antibody and a cytotoxic agent, and a pharmaceutically acceptable carrier.

The ~~invention~~disclosure further provides, in another aspect, the anti-HER3 antibody described herein, or the immunoconjugate comprising the anti-HER3 antibody and a cytotoxic agent, for use as a medicament. The ~~invention~~disclosure further provides, in another aspect, the anti-HER3 antibody described herein, or the immunoconjugate comprising the anti-HER3 antibody and a cytotoxic agent, for use in treating cancer. The ~~invention~~disclosure further provides, in another aspect, the anti-HER3 antibody described herein for use in inhibition of HER3/HER2 dimerization.

The disclosure further provides, in another aspect, ~~U~~use of such anti-HER3 antibody, or an immunoconjugate comprising the anti-HER3 antibody and a cytotoxic agent, in the manufacture of a medicament. Such use wherein the medicament is for treatment of cancer.Such use wherein the medicament is for the inhibition of HER3/HER2 dimerization.

The ~~invention~~disclosure further provides a method of treating an individual having cancer comprising administering to the individual an effective amount of the anti-HER3 antibody described herein, or an immunoconjugate comprising the anti-HER3 antibody and a cytotoxic agent.

The ~~invention~~disclosure further provides a method of inducing apoptosis in a cancer cell in an individual sufferning from cancer comprising administering to the individual an effective amount of an immunoconjugate comprising the anti-HER3 antibody described herein and a cytotoxic agent, thereby inducing apoptosis in a cancer cell in the individual.

The disclosure further provides, in another aspect,~~One embodiment of the invention is~~ a polypeptide selected from the group consisting of:

i) SEQ ID NO: 12 TtSlyD-FKBP-Her3,
ii) SEQ ID NO: 16 TtSlyDcas-Her3,
iii) SEQ ID NO: 17 TtSlyDcys-Her3,
iv) SEQ ID NO: 18 TgSlyDser-Her3, and
v) SEQ ID NO: 19 TgSlyDcys-Her3,

which polypeptide comprises the amino acid sequence of SEQ ID NO:1.

The ~~invention~~disclosure further provides the use of one of such polypeptides selected from the group consisting of:

i) SEQ ID NO: 12 TtSlyD-FKBP-Her3,
ii) SEQ ID NO: 16 TtSlyDcas-Her3,
iii) SEQ ID NO: 17 TtSlyDcys-Her3,
iv) SEQ ID NO: 18 TgSlyDser-Her3, and
v) SEQ ID NO: 19 TgSlyDcys-Her3,

for eliciting an immune response against SEQ ID NO:1 in an experimental animal.

The ~~invention~~disclosure further provides a method for producing an antibody specifically binding to the ß-hairpin of HER3 with the amino acid sequence of SEQ ID NO:1 comprising the following steps:

a) administering to an experimental animal a polypeptide selected from the group consisting of:

i) SEQ ID NO: 12 TtSlyD-FKBP-Her3,

ii) SEQ ID NO: 16 TtSlyDcas-Her3,

iii) SEQ ID NO: 17 TtSlyDcys-Her3,

iv) SEQ ID NO: 18 TgSlyDser-Her3, and

v) SEQ ID NO: 19 TgSlyDcys-Her3,

for at least one time, whereby the polypeptide comprises the ß-hairpin of HER3 with the amino acid sequence of SEQ ID NO:1,

b) recovering from the experimental animal three to ten days after the last administration of the polypeptide B-cells that produce the antibody specifically binding to the ß-hairpin of HER3 with the amino acid sequence of SEQ ID NO:1, and

c) cultivating a cell comprising a nucleic acid encoding the antibody specifically binding to the the ß-hairpin of HER3 with the amino acid sequence of SEQ ID NO:1 and recovering the antibody from the cell or the cultivation medium and thereby producing an antibody specifically binding to a target antigen.

The ~~invention~~disclosure further provides the use of a polypeptide selected from the group consisting of:

i) SEQ ID NO: 12 TtSlyD-FKBP-Her3,

ii) SEQ ID NO: 16 TtSlyDcas-Her3,

iii) SEQ ID NO: 17 TtSlyDcys-Her3,

iv) SEQ ID NO: 18 TgSlyDser-Her3, and

v) SEQ ID NO: 19 TgSlyDcys-Her3,

for epitope mapping, whereby the polypeptide comprises the epitope in the the ß-hairpin of HER3 with the amino acid sequence of SEQ ID NO:1.

Using the beta-hairpin of HER3 functionally presented in a 3-dimensional orientation within SlyD scaffolds (see e.g Figure 2, and the polypeptides of SEQ ID NOs. 13, and 17 to 24) the anti-HER3 antigen binding proteins, in particular antibodies, described herein binding to this beta-hairpin could be selected. It was found that the antigen binding proteins, in particular antibodies, according to the ~~invention~~disclosure have highly valuable properties such as internalization of HER3 in HER3 expressing cancer cells, , or very specific pharmacokinetic properties (such as faster association rates and higher Molar Ratios of the binding to a Thermus thermophilus SlyD FKBP-Her3 scaffold (which presents the HER3 ß hairpin in a 3 dimensionals functional struature and mimics the the open HER conformation for the ß-hairpin of HER3) when compared to the binding to HER3-ECD in its closed conformation (i.e. the absence of Heregulin)

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 Schematic overview of "closed" and "open" HER3 conformation and the influence of the Neuregulin family ligands (like e.g. Heregulin abbreviated here as HR) on the conformation change.

Figure 2 3D-structure of the beta-hairpin of HER3 functionally presented in a 3-dimensional orientation within a SlyD scaffold of Thermus thermophiles.

Figure 3 SDS-PAGE analysis of Ni-NTA purification of TtSlyD-FKBP-Her3. E1 and E2 show the purified fractions 12 and 13.SN: *E.coli* lysate supernatant before purification.

Figure 4 SEC elution profile of a Ni-NTA purified fraction of Thermus thermophilus SlyD-FKBP-Her-3.

Figure 5 A) Testing of specificity and reactivity in IHC of the selected clones. As shown all clones are specific for the detection of HER3 and show no cross reactivity with the other members of the HER family (HER1, HER2, and HER4).-Antibodies M-08-11, 17-02 and 17-07.

B) Testing of specificity and reactivity in IHC of the selected clones. As shown all clones are specific for the detection of HER3 and show no cross reactivity with the other members of the HER